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Dettol Brand Efforts during Covid-19

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Abstract:

The present article aimed to highlight the efforts made by Reckitt Benckiser Private Limited Company during the lockdown period. The rising demand in sanitization in Covid-19 poses an opportunity for Reckitt Benckiser to sell more Dettol. The RB has adopted various marketing strategies to sustain the label during the pandemic situation Covid-19. New labels entered the market and created a competition to establish the trusted brand Dettol. Dabur, HUL like big players, Non-branded local brands also entered into the market. Two objectives were set for the study as to understand and evaluate the efforts of Dettol during the Covid-19 pandemic situation and to identify successful outcomes during the pandemic situation. Analysis based on secondary sources e-newspaper, television commercials, published research articles, websites. The outcome reveals that during the pandemic situation, the percentage of television advertisement insertion of Dettol liquid soap was more as compared to Dettol toilet soap. 25% television ad with emotional appeal inserted for Dettol toilet soap. The Product was stretched to 'Disinfectant Spray' to exploit the favorable situation. Partnership with Tik Tok, Social website alertness, financial performance went well. Antiseptic liquid market share is largely covered by Dettol compared to its competitors. Reckitt Benckiser adopted an effective brand management process during Covid-19 through various campaigns viz. Back to School campaign, *Swachh Banega India and Maa Maane Dettol Ka Dhula. This way market got the lesson how to manage the brand in difficult situations in Covid-10.*

Introduction

Reckitt Benckiser India Ltd (RBIL) is a fully owned subsidiary of Reckitt Benckiser Private Limited Company., well renowned in India in household cleaning. An Organization operating in 60 countries, its sales in 180 countries and has had net revenues of more than $5.5 billion. Reckitt Benckiser India Ltd (RBIL) manufactures and markets a wide range of products in Personal care, Pest control, Shoe care, Antiseptics, Surface care, Fabric care,

other categories. Amongst its many well-known brands are Dettol, Mortein, Harpic, Cherry Blossom, Lizol, Disprin, Robin powder, Colin, etc. Most of these brands are either number 1 or number 2 in their respective categories in India.

India's most trusted brand 'Dettol' is marketed as protection from germs. The rising demand in sanitization in Covid-19 poses an opportunity for Reckitt Benckiser to sell more Dettol. The RB has adopted various marketing strategies to sustain the label during the pandemic situation Covid-19. However, new labels entered the market and created a competition to establish the trusted brand Dettol. Dabur, HUL like big players, Non-branded local brands entered into the market. Thus, the market share was diluted and Dettol has the opportunity to exploit the situation but was a critical situation to protect its brand away from the competition. Therefore, interest is created to understand and analyze the efforts of Dettol to protect its market share during the pandemic situation.

Research Methodology

The present study aimed with the two objectives to understand and evaluate the efforts of Dettol during the Covid-19 pandemic situation and to identify successful outcomes during the pandemic situation. Research conducted during the lockdown period to December 2020, Descriptive study purely based on secondary sources e-newspaper, television commercials, published research articles, websites. It is analyzed through the information collected through news form and data presented with discussion and findings and conclusion.

Analysis and Discussion

Reckitt Benckiser (RB) promoted its brand Dettol in various ways like Increase Television Commercial Advertisement Insertion, Launch Dettol Spray, Launch New Range of Products with Mothers, Handwash Challenge with Tik Tok, create awareness to maintain personal hygiene during Pandemic Situation. RB used a social website platform but criticized its exaggerated claim of commercials that Dettol kills coronavirus.

RB has made 25943 insertions in the television ad during the pandemic of these Dettol Liquid soaps (13524) insertions are more compared to toilet soaps (12419). During the Covid-19 pandemic situation, various brands did a total of 46,250 insertions of an ad on television. Of these 28% insertions is of Olx, 25% of Amazon.in & 25% of Dettol Toilet Soap & 22% of Facebook.

An Organization launched a new product '***Dettol Disinfectant spray'*** which is a one-stop solution for the germ-free home during the pandemic situation. Brand Dettol enhanced by product stretching strategy.

To make the brand strong RB's emotional appeal, "Dettol with Moms", mothers emotional & sensitive approach well worked in ad promotion during sensitive & insecure situations of covid-19.

To spread the government's message on handwashing company did a partnership with Tik Tok. The Campaign Handwash Challenge aimed to reach many people & their goal was 100 billion views at the point of a brand in India. The campaign's videos were viewed by nearly 125 billion times So, this effort was successful.

Another ad campaign used education appeal on personal hygiene to prevent and spread of Covid-19, germ protection. Reckitt Benckiser took the initiative in delivering the social message on personal hygiene. There are chances to forget the message if it is exposed one or two times so increased television commercial frequency.

Sometimes controversy also indirectly helps to show the presence of a brand. When Facebook user Andy Freeman posted an image of Dettol disinfectant spray, saying it can kill the nCoV 2019 The Dettol spray bottle label information text was it can kill cold viruses (human coronavirus and RSV) and not the nCoV 2019. This post has been read by more than a thousand times. But the authority clarified the issue in media by saying: "As this is an emerging outbreak, we do not yet have access to the new virus (2019-nCoV) for testing. Our products have been tested against other coronaviruses such as MERS-CoV and SARS-CoV and have been found to kill the virus. Although 2019-nCoV is a new strain, the virus is very similar to other coronaviruses." The firm added it will continue to work to understand the virus and test Dettol's effectiveness against it. "We are working with our partners to ensure we have the latest understanding of the virus, route of transmission and will test our product range as soon as possible," it stated.

During the lockdown, the organization took all possible measures to step up production activities. RB's offices in Makati, in the Metropolitan Manila region, were converted overnight into accommodation for more than 200 factory staff, complete with showers and canteen. The factory was still fighting to meet a Dettol demand. Every day, every week they were increasing capacity, increasing fulfillment rates. They were still under pressure to deliver. Globally the company eventually housed 1,000 workers. They put in accommodation, they arranged transport, and they arranged everything. In this position, they were making decisions which are for life or death," There were customer complaints

on the shortage & scarcity of hand sanitizer & soaps. The Demand for Dettol was rising during the pandemic situation but Reckitt Benckiser was unable to meet rising demand due to some unavoidable & unpredictable situations e.g. CEO (important decision-maker) was locked in London flat & important production center was located in Woohan (China) center of Hot-Spot of Corona Virus in the world. This was a very adverse & problematic situation due to adverse conditions.

The performance of the Dettol brand in the Hygiene sector is more. i.e. 38% & 8% in Portfolio, 21% in both Home & Health sector, 4% Food sector and 8%.Pharma sector. There was strong consumer demand, particularly in March & April. The sale of Hygiene products increased by 12.8% and Dettol Handwash was increased by 13.6%.

The demand for Dettol increased by 62% around the world due to Covid-19. In the year 2020, the Net revenue is 6,911 £m & the gross profit is 4,212 £m. In the year 2019, net revenue was 6,240 £m & gross profit is 3,757 £m. The net income is 1,087 £m in the year 2020 which is 124 £m in the year 2019.

In 2019 during the Covid-19 pandemic situations, Dettol launched a new product Disinfectant Spray, and maintain its Dettol label through product development.

The Market share of Dettol was 83%, 10% Savlon, and 7% others. It reveals even though market share and sales increased during the covid situation but the company was unable to restrict potential competition.

According to FMCG review of Nielsen, In January & February 2020, the three most selling brands in the hand sanitizer segment (i.e. Dettol, Savlon, Lifebuoy) alone had a market share of 85% while others including existing players & smaller brands had only 15% share of the market collectively. The sale for the top three brands decreased to 39% in March 2020 as there was a sudden increase in demand for hand sanitizers but limited due to lockdown. Hence, smaller players' entry was easier.

RB introduced various campaigns as *Back to school campaign, Dettol-Banega Swachh India, Maa Maane Dettol Ka Dhula*" which was launched in the year 2014 & featured Amitabh Bachchan. RB was partnered with NDTV & Facebook to launch "*Dettol-Banega Swachh India*"- a 5-year ambitious program that addresses the rising need for hygiene & sanitization. To create awareness and importance of hygiene & sanitation, "Maa *Maane Dettol Ka Dhula*". The brand has promoted not just Dettol Original but also its variants. It makes seasonal campaigns too under this tagline which is also remembered for the longest time.

Dettol starts to awaken customers not to consume its cleaning products, after the comments of President Donald Trump. Who suggested the possibility of injecting disinfectants to protect people from coronavirus. Reckitt Benckiser (RBGLY), a British company, warned that human consumption of disinfectant products is dangerous. It issued the statement "recent speculation and social media activity." "As a global leader in health and hygiene products, we must be clear that under no circumstance should our disinfectant products be administered into the human body (through injection, ingestion or any other route)," It shows that cognizance of media and social websites responses make the people recall.

Findings

1. During the pandemic situation, the percentage of television advertisement insertion of Dettol liquid soap is more as compared to Dettol toilet soap.
2. Total 46,250 insertions in television ads out of which 25% ads inserted by Dettol toilet soap.
3. Dettol brand enhanced by product stretching strategy through 'Disinfectant Spray' to exploit the favorable situation.
4. Emotional appeal in television ads became effective during the pandemic situation
5 A Platform of Tik Tok helps to increase large coverage during the lockdown.
7. Reckitt Benckiser took the advantage of social media raised controversy but immediately the responsible authority has given the statement & clarified the issue and controlled the situation.
8. Demand for Dettol was rising during the pandemic situation but Reckitt Benckiser was unable to meet rising demand due to some unavoidable situation.
9. The performance of Dettol in the Hygiene product category is more i.e. 38% compared to other categories like health, home & very least in food
10. The performance of the Dettol Brand was better than its forecast during the pandemic situation.
12. Dettol remained a trusted brand during the pandemic situation.
14. Financial performance of the brand is increased in the year 2020 as compared to the year 2019.
16. Antiseptic liquid market covered by Dettol brand compared to its competitors
17. Reckitt Benckiser was unable to meet the rising demand for Dettol sanitizer hence, Local brands jumped in the market race to fulfill the gap between increased demand & less amount supply.

18. Reckitt Benckiser adopted an effective brand management process during Covid-19 through various campaigns viz. Back to School campaign, ***Swachh Banega India and Maa Maane Dettol Ka Dhula***

Conclusion

Brand Dettol is a trusted brand in antiseptic antibacterial agents for the safety of an entire family. Brand Dettol has come up with many products other than antiseptic liquid-like hand sanitizers, soaps, surface cleaners, Disinfectant Spray, etc. for the fulfillment of various needs of customers. In the pandemic situation of Covid-19 make compulsion for wide usage of a cleansing agent as disinfectant & sanitizers. Dettol brand got an opportunity to exploit the situation. For getting benefit from this opportunity Reckitt Benckiser took many efforts regarding Dettol brand management & increases the performance of a brand. They updated their tv commercial insertions, introduced 'Disinfectant Spray'. Thus, Reckitt Benckiser has exploited the opportunity posed during the pandemic situation due to its trusted label & continuous promotional efforts. Two challenges they faced during lockdown one is meeting rising demand and effective distribution as their production and distribution center is located in lockdown area and its marketing CEO also locked in London hotel due to pandemic lockdown so managed with digital communication.

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A Study of Online Buying Behavior of Consumers toward Standardized Products

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Abstract:
In today globalized era e-commerce becomes common to all. Techno savvy people adopt e-commerce as it provides various facilities as it save time, save efforts such as waiting in a queue etc. But exactly what kind of products prefer by online buyers is become a matter of issues. As young generation attracts towards that e-commerce, uniquequality of may be one issue or standardized product having low consumers involvement may be prefer by consumers. Researcher selects this paper to find out factors that affect the choice of online products by online buyers. The paper may help to segment market on the basis of types of products to be preferred. Instrument is executed on 796 samples in Satara district, to find out influencing factors. Result of the research indicates that the product generally not available in local and nearby market, consumers like to shop online The Standardized products mostly having low consumers involvement are shopped on-line.

Key Words*: Online Buying, Standardized Product, Unique Product, E-Commerce*

Introduction:

Globalization brings quick and rapid access of all things around the world. In today's modern era person do not have much time to visit at various showrooms and made shopping. This problem was addressed by E-shopping. It is the concept of electronic shopping means to shop online using internet from anytime, anywhere.

E-shopping means act of purchasing product or services over the internet. Online shopping has grown popularly over the years, mainly because people find it convenient and easy to bargain from the comfort of their place, home or office. An important benefit e-shopping provide is that unlike traditional shopping there is no need to wait in long lines or search from store to store. It is just search ofan advertise by Google.

E-commerce, which stands for electronic commerce, refers to the exchange of products and services over an electronic network, such as the phone or the Internet. It refers to a website that accepts credit card payments and sells goods or services directly from the site utilising a shopping cart or shopping basket system online. It entails carrying out business using electronic

media and information technology, including electronic data interchange (EDI). In simple words, electronic commerce involves buying and selling of goods and services over the World Wide Web. Customers can purchase anything at anytime right from a car or a cake sitting comfortably at their location and gift it to someone sitting miles apart just by click of a mouse.

Literature Review:-

A plethora of search scholar studied on the online shopping among national and international level. Adoption of online shopping still observed on nascent stage. The researchers seem to take different perspectives and focus on different factors in different ways.

Researcher has attempted contextual review of articles published in international, national and regional research journals.

Most of these studies have attempted to identify factors influencing or contributing to online shopping attitude and behavior, few of them also focus their attention on demographic difference in online shopping behavior. The researcher seems to take different perspective by different ways viz. (Haq, 2010) Author opine that the perception of online shoppers is independent of their age and gender but dependent of their qualification & gender and income & gender. Further more (Ahasanul Haque, 2006), reported that gender and family income had significant relationship with overall attitude. (Almousa, 2011)revealed that in 18-25 years age groups, both males and females, use the internet heavily and more adapted to internet shopping. Although this age group does not have higher incomes of their own and is not expected to earn income yet in the Saudi culture, rather, they are mostly dependent on others until they graduate and then participate in the job market.

On contrary (Srikanth Beldona, 2011) didn't observed any significant difference between male and female online buyers. This result is also supported by (Nabil Tamimi, 2004) that gender and frequency of online shopping are independent of each other. Also (Yet Mee Lim, 2010) did not find any statistical significant gender differences in online behaviours and Attitudes.

Again on contrary (Jooyoung Park, 2009), opine that as compare to male females were search more information by visiting more product pages in the online shopping process. Author agrees that female are more interested in clothing and males are more interested in electronics goods category. He also opines that females are more likely to read the reviews on products or services and seeks the help on an assistant's agent for online shopping. Specifically, females consulted

customer reviews and used an assistant agent more often when shopping for experience goods than when shopping for search goods. On the other hands, males showed no significant differences in information search across product categories. This implies that the influence of product characteristics on consumers' information search differs between males and females. A consistent result is also observed by (Arpita Khare, 2011) The male and female students differed in their attitude toward online shopping, utilitarian motives, and purchase intention. Men are likely to perceive online shopping Web sites as convenient, flexible, enabling product, price comparisons, and easy to operate.

(Acilar, 2012), reported that male students have more positive attitudes toward online shopping than female students , consistent result revealed by (Ms. Asmatara Khan, 2012) Among the entire population of internet users, men more than women are inclined to trying the internet for varied reasons.

1.2 Research Problem :

India's economic growth has accelerated significantly from last two decades and it has inflated the spending power of its citizens. With rising incomes, household consumption has increased and a new Indian middle class has emerged. The world is changing very fast. Technosavy people don't have time to west on shopping. Their trends towards adopting new technologies of shopping were increasing.

Estimate of internet users 'universe' includes those accessing internet on their mobile phone. Users are also profiled as consumers of a variety of product and services. E-shopping now a days provides variety of produce viz. FMCG product, Wearable, Household and kitchen durables, Automobiles, Electronics, Mobiles, and various services.

Changing Attitude towards Online Shopping

Despite the proliferation of "Awareness, Future Demand Emphasis for Developing Markets & Present Problems" malls, individuals still prefer to shop online. Modern consumers are more sensible and able to access the market's options. With the internet, consumers are made aware. Every day, more people are using the internet, which draws in customers who can shop online. It was never anticipated that Indians would use e-commerce in this manner. Ticketing, trip bookings and even books and movies appear fine to buy online. Knowing that in India sizes vary

from brand to brand and quality is inconsistent, even for some of electronic items, how is it that there are people buy these items online? In India there are few segments of people who have not yet tried purchasing over internet.

Hypotheses of the Study:

Standardized product can be defined as the product which produces with the process of setting generally uniform characteristics for a particular good or service. Product standardization among the goods provided by different businesses operating in technology-based industries can be useful for consumers since it permits competition among the various suppliers.[1]

A standardized product means a good quality product and branded product, as online buying lacks in physical teach and feel approach. Generally standardized products are more prefer by the consumers hence following parameters are sought to judge the consumers behavior.

1. The Standardized products mostly having low consumers involvement are shopped on-line.

Objectives of the Study:

1. To find out factors influencing purchase decision regarding e-shopping.

Research Methodology:-

The study is conducted in Satara District State of Maharashtra, India. Study limited to the buyers from various locations in Satara district. Samples are selected from 11 taluka places in Satara district vary in numbers. Structured schedules are the instrument for data collection.

Data has processed using MS-Excel and analyzed using SPSS Package. Descriptive analysis, inferential statistics and multivariate statistical tools brought into use.

Data Analysis:

Nature of product prefers to buy online

Standardized Product

Following table shows the agreement of samples towards buying online nature of as a standardized product. Six parameters were asked to option on five point likert type scale. 1 for

1. [1] Read more: http://www.businessdictionary.com/definition/product-standardization.html -#ixzz36h7wLrwY

strongly disagree and 5 for strongly agree. The options were analyzed using mean, SD and ranks calculated on mean score as follows.

Table 1
Standardized Product

(n=796)

Sr.	Standardized Product	Mean	SD	Rank
1	I like to buy popular brands via online shopping.	4.58	0.686	1
2	A popular brand means good quality products.	4.38	0.632	2
3	Internet shopping provides a better qualityproduct.	4.03	0.925	8
4	I would like to pay more for branded product.	3.95	0.995	6
5	It is important for me to buy products/services with popular brand names	4.2	0.797	3
6	If I buy products/services from a web-retailer, I would prefer to buy popular brand name	4.15	0.777	4

(Source: Field Data)

Table 1 presented above reveals that the samples prefer to purchase standardized product online. Customer involvement is low in case of standardized and branded products; hence samples had given highest preference i.e. 1st rank to buy popular brands with 4.58 mean. A quality is important parameter which makes brand popular is next preferred by samples with 4.38 mean and 2nd ranks. Samples had given 3rd rank to "It is important for me to buy products/services with popular brand names" with 4.2 mean. Remaining parameter having their mean values more than 3.96 it means that samples are agreed to buy standardized or branded products online.

Unique Product

Following table shows the agreement of samples towards buying online nature of as a unique product. Four parameters were asked to option on five point likert type scale. 1 for strongly disagree and 5 for strongly agree. The options were analyzed using mean, SD and ranks calculated on mean score as follows.

Table 2
Unique Product

(n=796)

Sr.	Unique Product	Mean	SD	Rank
1	I prefer to buy unique product via online shopping	4.3	0.844	1
2	The product generally not available in local and nearby market, I prefer to shop online.	4.24	0.73	2
3	New arrivals / products are quickly available online	4.16	0.816	3
4	I like to introduce new style	3.82	0.951	4

(Source: Field Data)

Table shows that unique product offered by online retailer attracts most of the samples as that parameter secured 1st rank with 4.30 mean. "The products generally not available in local and nearby market, respondent prefer to shop online," this parameter secured 2nd rank to with 4.24 mean. Respondent believes that new arrivals/ products are quickly available online as it scored 3rd rank with 4.16 mean and parameter 'I am one who tends to introduce new style' secure low rank with 3.82 mean.

Inferential Analysis:

H0: All types of products are shopped online.

H1: The Standardized products mostly having low consumers involvement are shopped on-line.

Samples were asked to rate their opinion on statements representing standardized products and unique products. Six statements were representing standardized products and four statements were representing unique products. The opinions were sought on five point scale. The mean score has calculated and the series of mean score of opinions of standardized products and unique products have put to test for test of significance.

Description of type of product shopped online

The nature of product shopped online has been assessed as follows. Two type of products were ask to opine on one is standardized product and another is unique product.

Table: 3
Description of type of product shopped online
(n=796)

Sr.	Particulars	Mean	N	S.D.	SE Mean
1	Standardized Product	4.210	796	.522	.0185
2	Unique Product	4.13	796	.599	.021

Source: (Field data processed)

Above table shows that the opinion of samples means score for standardized product is 4.2 with standard deviation 0.522 and that of mean score for unique product is 4.13 with standard deviation of 0.599. It has observed that the figures of mean score and standard deviation are almost same.

Type of product shopped online test of significance

Following table shows test of significance regarding opinion of samples towards shopping standardized products and unique products. Paired sample 't' test has used to test the significance.

Table: 4
Type of product shopped online test of significance
(n=796)

		Paired Differences					t	df	Sig. (2-tailed)
Sr	Particulars	Mean	SD	SE Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
1	Standardized Product and Unique Product	0.0887	.6605	.0234	.04281	.1347	3.792	795	.000

Source: (Field data processed)

Above table shows the value of calculated paired 't' is 3.792 with a 'p' value 0.000 the test is significant hence **null hypothesis is rejected and alternative hypothesis is accepted**. The alternative hypothesis is The Standardized products mostly having low consumers involvement

are shopped on-line. In this test the merely opinions of samples towards standardized products has compared with opinions of same samples towards unique products.

Looking at the mean difference value is 0.088 with a standard deviation 0.66 shows proximity of the opinions of samples towards statements related with standardized product and unique product.

Findings:-

1. Customer involvement is low in case of standardized and branded products; hence samples had given highest preference i.e. 1st rank to buy popular brands with 4.58 mean. A quality is important parameter which makes brand popular is next preferred by samples with 4.38 mean and 2nd ranks. Samples had given 3rd rank to "It is important for me to buy products/services with well-known brand names" with 4.2 mean. (Refer Table No. 1)
2. Unique product offered by online retailer attracts most of the samples as that parameter secured 1st rank with 4.30 mean. "The product generally not available in local and nearby market, Samples like to shop online," this parameter secured 2nd rank to with 4.24 mean. Samples believes that new arrivals / products are quickly available online as it scored 3rd rank with 4.16
3. It is opined that the opinion of samples means score for standardized product is 4.2 with standard deviation 0.522 and that of mean score for unique product is 4.13 with standard deviation of 0.599. It has observed that the figures of mean score and standard deviation are almost same.
4. It is observed that the value of calculated paired 't' is 3.792 with a 'p' value 0.000 the test is significant hence **null hypothesis is rejected and alternative hypothesis is accepted**. The alternative hypothesis is The Standardized products mostly having low consumers involvement are shopped on-line.

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RESEARCH ARTICLE

BUYER'S PERCEPTION OF E-VEHICLE IN SATARA

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ABSTRACT

The paper aims to understand the awareness of customers and government efforts to promote e-vehicle and also to understand customers' product perception. This study is conducted in Satara city with 125 samples which consist of both existing customers and potential customers. A stratified disproportionate sampling technique is adopted to select the sample. The Schedule is designed to collect the feedback from the sample. The nature of the research study is descriptive. The study identified and evaluated the consumer perception of various factors about the electric bike. The result reveals that Government is taking rigorous efforts through FAME Amendment and PLI Scheme for the Auto sector. Satara customers are well aware of e-vehicle. There is a growth in both e-bikes and e-cars in Maharashtra. There is a combination of both positive and negative perceptions about e-vehicle. Most of the respondents consider the cost and the mileage in purchasing a bike, so there is ample potential for an electric bike in two-wheeler sectors. But their battery performance, speed, and appearance are the major factors that are affecting the sales of electric bikes.

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INTRODUCTION

In a thrust toward incentivizing new-age technologies and fulfilling the policy taken at COP26 to reduce its carbon emissions to zero by the year 2070, India is aggressively promoting the adoption of Electric Vehicles (EVs). India aims to switch 30 percent of private cars, 70 percent of commercial vehicles, and 80 percent of two and three-wheelers to Electronic Vehicles by the year 2030. For this, both Central and state governments are offering various incentives to buyers and manufacturers. Electric scooter sales touched double digits in the recent period. They could already reach one million sales, had it not been for the Russia-Ukraine war, which has led to a shortage of semiconductors and other materials. According to the source of published news, Last year, 0.2 million electric scooters were sold. Analysts estimate that the two key players Ather Energy and Ola Electric will jointly sell between 0.25 million to 0.26 million scooters this year. By 2023 the expectation based on the capacity built up by manufacturers is that sales could range between 1.5.2 million, making a further dent in the overall two-wheeler market. If that happens, electric scooters will cross another milestone, they will account for 10% of the total 18-20 million per annum two-wheeler market, which includes motorbikes. It could also mark a shift in the domestic market, where 70% of two-wheeler sales come from motorcycles, in favor of electric scooters in the coming years. Apart from anything else, there are only a few electric bikes and these have just entered the market. The government has its estimate. It believes that 80% of the bikes will be electric by 2030. We estimate that about 0.7-0.8 million electric scooters will be sold in 2022.

Currently, there are 35-36 players but it expects consolidation in the next two to three years. The market will grow gradually. The Price of electric scooters is also likely to go up when the government eventually withdraws the subsidy, which is currently helping to keep its price low. Perception matters a lot when anyone in the business world. Needless to say, companies that enjoy favorable customer opinions are often the ones that regularly break the ceiling and achieve remarkable success year in and year out. On the other hand, businesses with poor customer perceived value find it hard to maximize their effort, struggle to realize the true potential, and in most cases, also get consigned to obscurity. So, one should always strive to meet the expectations of customers and want to be seen in a positive light, and maintain a desired level of perception. Consumer perception is vital for any business for many reasons. Perception builds trust, Perception propels sales, Perception creates reputation, and Perception drives key metrics Perception generates word of mouth. Customers often form an opinion about a product based on many factors and not all will be in your control. If you have the right customer experience strategy, you can easily work on most things that shape or break the perception and bring the desired improvement to the result. Similarly, there are so many aspects such as price, quality; positioning, etc. that can decide how your business is seen by others in the market.

Review of Literature

(Ashok, 2019)This article highlight the importance of E-Vehicles and the efforts of the government in implementing policies to promote E-Vehicles to reduce the dependence on oil, decrease greenhouse gasses and improve air quality.

The study was conducted in Bangalore city. The study analyses the awareness levels of customers on government initiatives for E-transportation in India. (Sanguesa Julio A, 2021)This paper reviews the advances of EVs regarding battery technology trends, charging methods, as well as new research challenges and open opportunities. (Hannan M A, 2014)This paper highlights existing technologies are more or less capable to perform HEV well; however, the reliability and the intelligent systems are still not up to the mark and also highlighted many factors, challenges, and problems with sustainable next-generation hybrid vehicles.(Garling Anita, 2001) Author outlines a two-phase strategy for the marketing of Electric Vehicles (EVs) based on a discussion of current and expected future characteristics of EVs and a review of research on early adopters.

RESEARCH METHODOLOGY

The research study is conducted in Satara city from Nov 2021 to Jan 2022 with the objectives of understanding the awareness of customers and government efforts to promote e-vehicle and knowing the product perception of existing and potential customers and to know the price perception and identifying the influencing factor in buying. An unequal proportionate stratified sampling technique is used to collect feedback from a total of 125 samples.

A Schedule is used to collect the opinion of respondents. Both actual buyers and potential buyers are selected as sample units. Collected data analyzed with the help of descriptive analysis and presented with tabulation and described through data analysis and discussion. The nature of the study is descriptive. Results are presented in the form of findings and classified data and tabulation presented in the annexure.

Data Analysis and Presentation: A Researcher has analyzed the collected data and discussed it as follows. Published data talks about the growth of e-vehicles in Maharashtra in cars and two-wheelers. It shows as follows. The above figures depict that E-cars and two-wheelers are increasing in Maharashtra. Of these two-wheelers, growth is higher compared to e-Cars in Maharashtra.

Table 1. E- Vehicles (cars and 2-wheelers) Growth in Maharashtra

Sr.	Year	Cars	2-wheelers	Registered
1	2019-20	183	5479	7400
2	2020-21	1128	6875	9415
3	2021-22	2633	19396	23786

Source: https://timesofindia.indiatimes.com/city/mumbai/e-vehicles-in-maharashtra-up-153-in-1-year-more-than-double-in-mumbai/articleshow/88555385.cms

It reveals that acceptance of e-vehicles are increasing day by day in Maharashtra. The government's efforts in implanting e- vehicles policies are getting success to some extent. It is a very good time for electric vehicles in Mumbai and Maharashtra. While the state saw a phenomenal 153% rise in new e-vehicle registrations in the first nine months of the financial year 2021-2022, the city recorded a growth of 112% in the same period, the latest transport statistics show. Compared to 9,415 e-vehicles registered in 2020-21, the number of registrations skyrocketed to 23,786 in just nine months of 2021-22 (April 1 to December 27 this year).

Government Efforts: India is aggressively promoting the adoption of Electric Vehicles (EVs). For this, both Central and state governments are offering various incentives to buyers and manufacturers.

PLI Scheme for Auto Sector: In September this year, the Union Cabinet approved a Rs 26,058 crore production-linked incentive (PLI) scheme to accelerate domestic manufacturing of electric and fuel cell vehicles and drones in India. As per the government's estimate, the scheme would attract Rs 42,500 crore in fresh investment in the automobile and auto components industry over five years. The government has allocated Rs 25,938 crore for the automobile sector and the remaining Rs 120 crore for the drone sector

FAME II Amendment: FAME-II (Faster Adoption and Manufacturing of Electric Vehicles-II) scheme. Under this, the government significantly reduced the price gap between petrol-powered two-wheelers and electricians by increasing the subsidy rate for electric two-wheelers from Rs 10,000/kWh, to Rs 15,000/kWh, while also capping the incentives at 40 percent of the cost of vehicles as against 20 percent earlier. Government official plans were afoot for 1500 new EV charging stations across the Mumbai region. The government also wants to ensure that 10% of new vehicle registrations by 2025 are electric vehicles.

Perception of Customers in Satara City: The Researcher collected the opinion of customers in Satara city to examine their perception of customers. After analysis of the collected data, it shows that 60% of respondents have bought an electric bike and 40% are potential buyers. Of these respondents, 75.2% of respondents are aware of electric bikes, and very few i.e. 24% are unaware. This percentage is due to existing customers' samples being more than potential.43.2% of the sample perceive that the price of a vehicle is high, 26.4% perceive low, and 5.6% only perceive very low. There is mixed opinion about the price of Electric Bike. There is a need to plan an effective convincing price aspect for Electric Bike. 36.8% perceives mileage of vehicle is 'Very Good', 24.8% perceive 'Good', 28% perceive 'Bad'& 10.4% perceive 'Very Bad'. It reveals that the perception of customers towards mileage of the electric bike is satisfactory as they perceive (61.6%) 'Good' rest i.e.39.4% perceives 'Bad'. 40.8%sampleperceive the speed of the vehicle high, 11.2% perceive very high, 22.4% perceive low & 25.6% perceive very low. Thus, the perception of samples towards the speed of the electric bike is satisfactory.

Respondent (53.4%) preferred Electric Bike price range between 50,000-90,000 & rest i.e.46.6% preferred the price range between90000-110000. Zero Emissions and Environment & Tax Benefits are two important criteria that make respondents purchase Electric Bike.(66.66%)samples are dissatisfied with their post-purchase experience & (33.33%) customers showed satisfaction towards post-purchase experience. It reveals that 66.66% are dissatisfied with the post-purchase experience**.** There is a need to identify the reasons for their dissatisfaction. There is further scope to study customer satisfaction towards Electric Bike. (60%) sample perceives the high cost of maintenance and (40%) perceives low-cost maintenance of e-bikes. There is a need to identify the reasons for their high-cost maintenance. There is further scope to study customers' high-cost experience with Electric Bike.54.66% samples said e-bike speed is 'average '& (26.66%) samples said 'good' speed & (18.66%) said 'poor'. There is a need to improve the speed of the Electric Bike. When respondents talk about mileage coverage per charge, (52%) samples said they cover 90-110 km mileage per charge & (30.67%) cover110-130km,(12%) said 70-90km & (5.33%) very few customers said mileage covered per charge is above130km.It reveals that the majority of sample cover distance is 90-110km per charge. A very few (5.33%) customers said the distance covered per charge is above 130km. So there is a need to study more on their power-saving storage of batteries to cover long distances per charge.

RESULTS

Results of a study found after analyzing the data where 60% samples are existing users of Electric Bike & 40% are willing to buy i.e. potential customers. There is a mixed non-proportionate group of samples used for the study. The results are based on their opinion. The majority of respondents are aware of electric bikes. There is mixed opinion on the perception of price. Perception of about mileage of the electric bike is satisfactory as they perceive (61.6%) good & rest i.e.39.4% perceive bad. The perception of customers towards the speed of the electric bike is satisfactory. Respondents are more convinced to buy Electric Bike for low running features than other features. The preferred price of a vehicle ranges from 50000 to 90000. It shows respondents are not ready to spend more. Zero Emissions and Environment & Tax Benefits are two important criteria that make

respondents purchase Electric Bike. All sources viz. The Campaign, advertising, pamphlet, & others are used by the customer to know the Electric Bike. Respondents gave more preference to advertising the product. Actual customers are more preferred the campaign & Potential customers prefer the pamphlet. The majority of respondents are dissatisfied with the post-purchase experience**.** There is a need to identify the reasons for their dissatisfaction. It is well said that dissatisfied customers are always more dangerous than satisfied customers. There is further scope to study customer satisfaction towards Electric Bike. The majority perceive the maintenance cost of the vehicle as high**.** There is a need to identify the reasons for their high-cost maintenance. The majority perceive the speed of the vehicle to be average as per their post-experience**.** It reveals that customers get attracted by vehicle speed. So to attract more customers need to improve the speed of the vehicle. The majority of samples covered distance is 90-110km per charge and very few covered above 130km. Therefore, there is a need to examine power-saving storage i.e. battery to cover long distance per charge. Respondent thinks appearance plays a major role while making purchasing decisions so the Electric Bikes need to be made more attractive.

CONCLUSION

To be conclude that the maximum number of respondents are not aware of Electric bikes. Thus, it requires various promotional activities to increase the awareness level & thereby increases the sales.

The study also identified and evaluated the consumer perception of various factors about the electric bike. The result of this study shows that there is a both positive and negative perception about e-vehicle. Here most of the respondents consider the cost and the mileage while purchasing a bike, majority of customers cover distance is 90-110km per charge so there is ample potential for the electric bike. But their battery performance, speed, and appearance are the major factors that are affecting the sales of electric bikes. The study explains the perceptions prevailing in the minds of customers and highlights the areas to improve the e-bike in near future.

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Appendices

Table No. 1 Distribution of Respondents As Per Their Type of Customer

Sr. No	Type of customer	Frequency	Percentage (%)
1	Actual customer	75	60
2	Potential customer	50	40
	Total	125	100

(Source:-field data)

Table No: 2 Awareness of Electric Bike

Sr. No	Parameters	Frequency	Percentage (%)
1	Yes	94	75.2
2	No	31	24.8
	Total	125	100

(Source:-field data)

Table No: 3 Respondent Perceptions Towards Price Of Electric Bike

Sr.	Perception	Types of customer				Total	
		Actual customer		Potential customer			
		Freq	%	Freq	%	Freq	%
1.	Very High	19	25.33	12	24	31	24.8
2.	High	29	38.67	25	50	54	43.2
3.	Low	22	29.33	11	22	33	26.4
4.	Very low	05	6.67	02	4	07	5.6
Total		75	100	50	100	125	100

(Source:-field data)

Table 4 Criteria Used to Prefer Electric Bike

Sr.	Parameters	Weighted Average	Rank
1	Insurance of registration cost	123	3
2	Petrol Consumption	123	3
3	Tax Benefits	124	2
4	Zero Emissions And Environment	125	1

(Source:-field data)

Table No 5. Perceptions towards Speed of Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Very High	08	10.66	06	12	14	11.2
2.	High	29	38.66	22	44	51	40.8
3.	Low	9	12.02	19	38	28	22.4
4.	Verylow	29	38.66	03	6	32	25.6
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No. 6 Perception of Customer Towards Sources Used To Know Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Campaign	22	29.33	07	14	29	23.2
2.	Advertising	32	42.67	22	44	54	43.2
3.	Pamphlet	10	13.33	14	28	24	19.2
4.	Other	11	14.67	7	14	18	14.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table 7. Perception Of Customer Towards Mileage Of Electric Bike

Sr.	**Opinion**	**Types of customer**				**Total**	
		Actual customer		**Potential customer**			
		Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
1.	Very Good	39	52	7	14	46	36.8
2.	Good	10	13.33	21	42	31	24.8
3.	Bad	16	21.34	19	38	35	28
4.	Very Bad	10	13.33	03	6	13	10.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 8 Respondent Perceptions Towards Feature To Convinced To Buy Of Electric Bike

Sr.	**Opinion**	**Types of customer**				**Total**	
		Actual customer		**Potential customer**			
		Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
1.	Low weight	11	14.67	09	18	20	16
2.	Low running	39	52	25	50	64	51.2
3.	Registration onnot required	19	25.33	14	28	33	26.4
4.	Others	06	8	02	4	08	6.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 9 Respondent Perception towards Price Range Preferred

Sr.	Price Range	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	50000- 70000	04	5.33	09	18	13	10.2
2.	70000- 90000	29	38.67	25	50	54	43.2
3.	90000- 110000	34	45.33	14	28	48	38.4
4.	110000-&above	08	10.67	02	4	10	8.2
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 10 Respondent Perceptions Towards Over All Post Purchase Experience

Sr no.	**Opinion**	**Frequency**	**Percentage (%)**
1.	Strongly dissatisfied	21	28
2.	Dissatisfied	29	38.66
3.	Neutral	18	24
4.	Satisfied	07	9.33
5.	Strongly satisfied	0	0
Total		75	100

(Source:-field data)

Table No. 11 Perception Towards Over All Post Mileage Experience

Sr no.	**Opinion**	**Frequency**	**Percentage (%)**
1.	Good	20	26.66
2.	Average	41	54.66
3.	Poor	14	18.66
Total		75	100

(Source:-field data)

Table No. 12 Perception Towards Over All Mileage Covered Per Charge

Sr.	Opinion	Frequency	Percentage (%)
1.	70-90 Km	09	12
2.	90-110 Km	39	52
3.	110-130 Km	23	30.67
4.	130-Above	04	5.33
Total		75	100

(Source:-field data)

Table No. 13. Perception of Maintenance Cost of Electric Bike

Sr.	Opinion	Frequency	Percentage (%)
1.	Low Cost	30	40
2.	High Cost	45	60
Total		75	100

(Source:-field data)

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Study of Factors affecting the National Anonymously: Dark Web

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Abstract: ***This paper talks about how the dark web is utilized to genuine purposes just as to hide the noxious exercises or criminalism. The Dark Web is at the focal point of the discussion about whether online namelessness ought to be kept up despite the criminal behavior that it empowers. This paper will limit its degree by concentrating exclusively on the national contemplations of the Dark Web, and not those issues that dig into the domain of local law implementation. Drug dealing, firearms, fake products, unlawful erotic entertainment, and so forth these are issues that this paper characterizes as falling into the domain.***

Keywords: Dark web, security, social media, cyber attacks

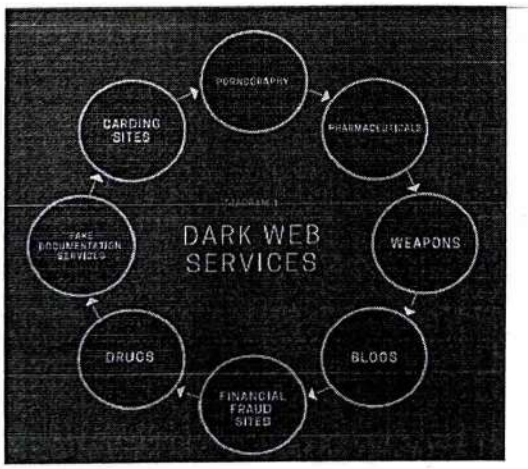
I. INTRODUCTION

The dark web frames a little piece of the profound web, the piece of the Web not recorded by web indexes, albeit here and there the term profound web is generally used to imply explicitly to the dark web. The dark Web is portion of reflective Web which has been intentionally enclosed, is blocked over ordinary Web programs. While having the network has associated the whole world readily available and has upset how tasks occur all through the world, some may contend that the web has brought more damage than anything else

Cyber attack targets are usually aimless: unselective, however in modern times, targeted attacks that too without any purpose are made. It is believed that such attacks are made for a specific purpose. In these present times, it is thus problematic to avoid or even stop all the cyber attacks, even when we have taken security measures. They are often same that they have a tendency to area unit in a very defense solely state of affairs. So as to beat this example, it is essential to foresee the cyber attacks and to involve applicable security methods ahead. It is essential to apply risk intelligence that permits this. In common, several aggressors share data and tools accessible for attacks in special societies on the dark web. This is the reason, it is supposed that there is huge volume of threat intelligence on web.

Thus by means of consuming the intelligence, we are able to observe cyberattacks ahead and develop a active defense.

Dark web excavation is a quickly developing area for research. Web mining systems are also utilized to distinguish as well as avoid dread pressures brought about by pirate terrorists everywhere throughout the world. Nowadays, these attacks ahead and develop a active defense .



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Oral Cancer Detection Using Image Processing and Deep Neural Networks.

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Abstract-*The paper proposes accomplice revolutionary deep convolution neural community (DCNN) mixed with texture map for detection cancerous areas and staining the ROI for the duration of an unmarried version mechanically. The projected DCNN version carries 2 cooperative branches, specially accomplice better department to carry out carcinoma detection, and a decrease department to carry out linguistics segmentation and ROI marking. With the better department the community version extracts the cancerous areas, and additionally the decrease department makes the cancerous areas extra preciseness. To shape the alternatives inside the cancerous extra regular, the community version extracts the texture photos from the enter image. A window is then carried out to cipher the same old deviation values of the texture image. Finally, the excellent deviation values are accustomed assemble a texture map, that's partitioned into more than one patches and used due to the fact the laptop documents to the deep convolution community version. The tactic projected via way of means of this paper is called texture-map-primarily based totally department-collaborative community.*

Keywords— Deep Neural Network, Image Processing, Oral Cancer, Texture Map.

1.INTRODUCTION

Oral Cancer is especially denoted as class of head and neck cancer includes major sub regions of the lip covering mouth cavity, and tubular cavity (National Institutes of Health, 2018; WHO, 2017), consisting of concerning eighty fifth of the class. Right off the bat, carcinoma could be a life-threat sickness because of the very fact that its precursor symptoms and warning signs might not be ascertained by the patients routinely as a result of that this sickness could chop-chop progress into malignant neoclassic disease stage at intervals brief amount Oral cavity cancers also are better-known to own a high repetition rate compared to different cancers. Therefore, AN in-depth exploration of either its staging or its grading is important for its prognostic treatment. quite ninetieth of cancers that occur within the remoras square measure squalors cell carcinomas (SCC). This cancer cluster is characterized by animal tissue squalors tissue differentiation and aggressive growth disrupting the basement membrane of the inner cheek region. Commonly, clinical procedures for prognosis and treatment square measure evaluated on Tumor-Node-Metastasis (TNM) staging. However, a five-year survival report supported oral cancer reveals a prognosis rate of roughly thirty fifth to five hundredth guaranteeing quantitative microscopic anatomy grading of tumors, that comes with the in-depth study of assorted pathological aspects associated with SCC, as a additional advantageous

Method than growth staging for increasing malady survival rate. Hence, from a pathologist's point of read, providing precise histopathological identification within the context of multi-class grading is vital. This provides a principle to combat the problem by incorporating deep learning based malady identification or prediction strategies with clinical prospective that square measure hot analysis Oral SCC is

Morphologically classified into traditional, Well-differentiated, Moderately differentiated and poorly differentiated categories supported Brooder's system of microscopic anatomy grading. The cellular morphometry highlight the growth displays a terribly minute microscopic anatomy distinction separating the 3 categories that square measure very exhausting to capture by the human eye. it's remained elusive thanks to its extremely similar microscopic anatomy options that even pathologists realize troublesome to classify. Although most oral SCCs square measure moderately differentiated, all of them have totally different distribute characteristics and implicate different prognosis, repetition rate and survival, and treatment management.. Therefore, with the expansion of care standards everywhere the world, it's necessary for AN overhaul of pathology, which might involve additional fast and accurate identification.

2. LITERATURE SURVEY

Oral cancer is that the commonest form of head and neck cancer worldwide, with associate degree calculable 377,713 new cases and 177,757 deaths in 2020 [1]. Surgery is that the usual primary treatment and customarily yields high treatment success, with overall survival rates reaching 75–90% within the early stages [2, 3]. However, over hour of the cases are diagnosed at a sophisticated stage and progress with high morbidity and mortality [2,4]. Considering the terrible incidence and mortality rates, carcinoma screening has been a very important [illegible] aid programs, as

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Secure Desktop Computing In the Cloud

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bstract—

omputation that employees perform on their desktop nd the management of the desktop computing ıfrastructure to the cloud, the need for securing such loud-hosted user computing tasks and environments :ome paramount. In this paper, we present Venia, a ecu cloud-based desktop computing platform esigned to protect against both external and internal ıreats. Accessible to end-users through a thin Remote esktop Protocol (RDP) client Venia isolates end-ser's applications and data into containers and ubjects the interactions with and among the ontainers to security policies. Following a principle of :ast privilege, Venia security policies control user's ccess to containers, network and file system ıteraction of the containers, cross-container data haring and also enables collection of detailed logs for uditing purpose. Venia has been deployed to a 3rd arty test environment where it demonstrated that nd-users can perform the tasks they need on a daily asis, without introducing greater risk to the overall rganization, and its currently undergoing security and erformance evaluation by an independent evaluation .am.

. INTRODUCTION

he next step within the trend of moving backend ervices and supporting computing infrastructure) the cloud, is to maneuver end-user computing nd its supporting infrastructure to the cloud dditionally. Cloud computing provides economy of :ale, eliminates the headache of computer code nd hardware management and maintenance, and ermits on-demand scaling and pay as you utilize ıting. Properly architected, moving end-user ɔmputation to the cloud will offer a security profit. conscientious cloud seller can offer stronger erimeter protection, specialised employees, and stablished tools, techniques and procedures for andling security incidents than a typical enterprise ill generally deploy. However, sharing machine resources within the cloud presents a brand new set of security challenges for ensurinorganization and even worse, users from completely different organizations cannot breach security to attain malicious objectives.

2. Related Work

Secure Desktop computing in the cloud

Current solutions for desktop computing within the cloud square measure
based off of a Virtual Desktop Infrastructure (VDI) approach.
VDI could be a variety of virtualization wherever entire desktop solutions
are hosted within the cloud, so accessed employing a skinny consumer,
usually with RDP. One such technology is Horizon seven by VMWare. in hand with these solutions is their wholesale exporting of the desktop atmosphere to the cloud. While helping to modify the digital geographic point and providing a centralized management over resource and network access, these
solutions still maintain the appliance primarily based security problems inherent in a very ancient desktop.

3. Design Goals And Approach

The main style goals for Venia were:

• Role-specific UCEs: UCEs for individual users ought to consist of role-specific application bundles, whenever a job defines that desktop applications and resources area unit required to perform a particular job connected operate. A single user might have multiple roles, presumably requiring use of applications from multiple operative systems (e.g., Linux and Windows) in a very single role, and resources will be limited among completely different roles.

• Enterprise-specific security management and auditing:

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Secure Cloud Computing

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ABSTRACT- It is nothing but way to deliver computing source in Cloud Computing. It ranges from data storage and processing to software i.e., customer relationship management systems, which is available instantly and on demand.

Manipulation, configuration and accessing is refered by cloud Computing h/w & s/w source monitor. Online data storage, infrastructure and application is offered by it. In this research paper the researcher has discussed about the introduction part of the cloud computing, types of cloud computing. A research has also focus on Benefits limitation and future working of the cloud computing. In overall conceptual are discussion of cloud computing with the research has also focuses in future development.

1. INTRODUCTION

A social media is the depending with the cloud computing. cloud computing simple line meaning is transport of the computing with the service.

Types of Cloud Computing are as follows:

- Infrastructure as a service (IaaS)
- Platform as a service (PaaS)
- Software as a service (SaaS)

Cloud computing can interacts with networks it's rules and regulations which is joined to its role. So, to start with the, we have already listen the asset of knowing to code for cloud computing.

1.1. What are Cloud Computing Security?

Collection of security measures are designed to secure cloud-based infrastructure, data and application. The other name of Cloud Computing security is collection of security measures. The above ways assure user and device verification. In today's era there is high demand to work in Cloud Computing field.

1.1. Use of Cloud Computing:

CC allows to the access file, resources, data, files whenever we are not connected to network the cloud computing is allows to access to their network with the help of internet.

1.2. Types of cloud Computing:

Commonly using the cloud computing use cases:

- IaaS
- PaaS
- SaaS

1] IaaS: IaaS is nothing but the h/w as a service. the access are in many clients and source internet used with online paying System. Iaas is using three types of the cloud first is the public second is private and third one is hybrid cloud. The cloud computing is used for helps to changing clients in the requirements and services.

2] PaaS: it is the purchase online mode for available in this platform. This platform as a service in cloud service provider. this is the developing the developer platform application. there are many languages is available in this like a java, php, perl and so on.

3]SaaS: this application stands for software as a service. This application is also known as the requirement for software. and also, customer with relationship is the management system making a electronic devices.

2. 7 CASES IN CLOUD COMPUTING:

1. Infrastructure as a Service (IaaS)
2. Platform as a Service (PaaS)
3. Software as a Service (SaaS)
4. Hybrid cloud and multi cloud
5. Test and development
6. Reach Robotics
7. Big data analytics.

Latest Cybersecurity Trends

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bstract - *The likelihood of a security breach has never 'en higher due to the variety of new threats that are 'veloping from both inside and outside your network. The mbination of all these risk variables with a significant man component presupposes that everything has been set) and configured correctly to get the best results possible)m each security instrument. In order to address this issue, ganisations often spend more money on security controls, hich increases management visibility and makes it easier for am like Security operation to see better results and provide)od return on investment.*

The author covers the current trends in bersecurity in this research report. Investigations have also cused on cybersecurity trends advantages and potential owth. The conceptual analysis of trends as a whole in the search has also given consideration to future evolution.

ey Words: Cyber Security, Data, Attacks, Threats, yptocurrency, Breach

INTRODUCTION

The cyber security domain growing faster as ith offensive and defensive security service providers mpete to outwit one another. Technology is constantly veloping and improving, and new threats and creative luti s to combat them are constantly emerging. As more ople started working from home recently due to the ndemic, fraudsters discovered new techniques, tactics, d strategies to take over networks and steal data in order exact a ransom.

The newest developments in cyber security are scussed in this overview.

CYBER SECURITY TRENDS

2.1 Machine Learning

Although it is one of the newest technologies in bersecurity, machine learning is playing a bigger and more oactive role. One of the reasons is that machine learning akes cybersecurity easier, more efficient, and less pensive (ML). This system builds patterns and anipulates them, anticipating and responding to active tacks in real-time using sophisticated algorithms that are sed on complex data. To put it another way, applying ML to cybersecurity systems helps them to assess threat patterns and learn the habits of hackers, assisting in the prevention of future attacks and decreasing the amount of time cybersecurity experts must dedicate to repetitive operations.

ML makes cybersecurity easier to use, more efficient, and more affordable all at once. ML creates patterns and uses algorithms to alter them from a complex set of data. It can therefore anticipate dangers in real time and respond to them accordingly. In order to create efficient algorithms, this technology primarily depends on complex and rich data. The information must come from everywhere and must cover as many probable outcomes as is practical. In order to assess attack trends and learn hackers' tactics, cybersecurity systems can now use machine learning (ML)

2.2. Artificial Intelligence (AI)

It is impossible for human being to operate large number of cyber security threats. As a result, organisations are increasingly turning to AI and ML to hone their security infrastructure. AI has played a key role in developing automatic threat detection, face recognition, natural language processing, and security automation systems. AI also enables the much quicker analysis of enormous amounts of danger data. This is advantageous for both huge enterprises dealing with massive amounts of data and small or mid-sized businesses with sometimes under-resourced security teams. While AI has enormous potential for businesses to detect threats more thoroughly, thieves are also leveraging the technology to automate their attacks by using model-stealing and data-poisoning methods.

2.3 Need of Multi-Factor Authentication

Password protection is no longer enough due to the sophistication of modern cyberattacks. Compared to a basic password, a multi-factor authentication (MFA) is much more secure. By simply adding an additional layer of security, multi-factor authentication helps to prevent illegal access to online accounts. MFA makes sure that businesses can better safeguard employee data and manage access. Every time someone signs in, they must additionally provide a verification code that is sent to their registered phone number or through an authentication app. The "gold standard" of authentication is multi-factor authentication (MFA).

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Security Factors affecting Internet of Things

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Abstract: ***Internet of things (IoT) is the following huge thing in the networking field. The vision of IoT is to connect day by day used gadgets that have the capacity of sensing and actuation to the internet. This can or may additionally or might not contain human. In this paper we are able to go through all of the demanding situations of IOT and mainly focus on IOT safety undertaking. IoT entails adding net connectivity to a system of interrelated computing gadgets, mechanical and digital machines, items, animals and/or people. Each "component" is furnished a completely unique identifier and the capability to automatically switch statistics over a community. Allowing devices to connect with the internet opens them as much as some of severe [illegible]erabilities if they're now not nicely included.***
Keywords: Internet of things, security challenges

I. INTRODUCTION

Internet of Things (IoT) is extra than the machine to machine conversation. "IoT is a network of dedicated bodily gadgets (things) that comprise embedded generation to experience or interact with their internal state or external environment. The IoT accommodates an environment that consists of things, conversation, packages and records evaluation. Massive objects are to be connected to internet.

The devices will interact with different devices by way of pervasive computing but there's heterogeneity within the architectures. On pinnacle of this protection is any other massive project in IoT implementation. Primary goal of IoT is to reduce strength consumption and decrease the usage of resources.

A. Ease of Use

IoT discovers application in numerous fields like medicinal drug e.g. looking at heartbeat tempo of patient and tracking the records and with data it's going to decide or ship the data to specialist about it, domestic robotization for instance controlling room temperature, business organizations as an instance quality control, fitness hardware as an example energy to be scorched, smart city regions as an example transport on course sign to daily workers and so forth. Remote sensor systems which are meanings of IoT can show to us a few preparations.

Far off sensor structures is utilized to hit upon the object and transmit the facts, for detecting it need not hassle with an awful lot ca[illegible]lation control but transmitting the detected data desires some correspondence way which may additionally activate protection trouble.

In this paper, we examine of the principle IoT security threats, consisting of clever cars, clever domestic, aircraft, and provides considerations to network standards for the IoT, and advise future studies consideration to receive a at ease IoT offerings.

II. LITERATURE REVIEW

Valeriy G. Semin Russian State Social University Moscow, Russia , Eugeniy R. Khakimullin Academy of State fire service of EMERCOM of Russia Moscow, Russia mentioned Filling the idea of "Internet of factors" with a result of technological content material and implementation of sensible solutions, starting from 2010, is taken into consideration a solid fashion in facts technologies, mostly due to the good sized distribution of wi-fi networks, the emergence of cloud computing, the development of intermachine interaction technology, the transition to IPv6 and software program improvement -configurable networks.

1) Endless sharing of data among "things" plus the customers can increase when inappropriate verification, validation and permission. Presently, there are certainly not any dependable platforms that deliver entry to manipulate and personalized safety policy based entirely on operator's requirements and context across one of a kind styles of "things". The "things" in any IoT network are regularly ignored and overlooked; consequently, they are at risk of outbreaks. Furthermore, [illegible]mum IoT network and the communications make spying easy as the network are wireless. The destiny considerable for implementation of IoT will

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he Impact of Stock Market on Indian Economy

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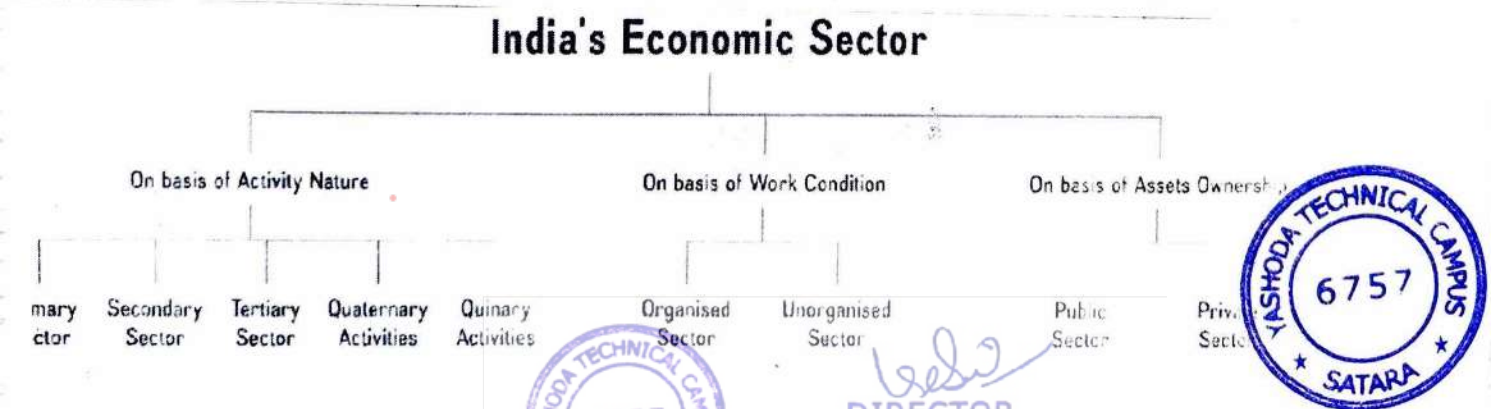
t: The Third Largest within the world of Indian Economy in terms of buying power. it's going to touch new heights in back years. The Global investment Bank , by once North American country and China 2035 India would third largest y of the globe. it'll grow to hour of size of the North American country economy. This booming economy of nowadays iss through several phases before it will bring home the bacon the current milestone of Sept. 11 gross domestic product. ents within the stock exchange will have a profound economic impact on the economy and individual shoppers. vse in share costs has the potential to cause widespread economic disruption. This paper deal s with stock market play po[illegible]t role growth of Indian Economy and additionally the Impact stock exchange on Indian Economy by approach of tual Methodology exploitation to the Journals of Indian stock exchange.

I. INTRODUCTION

exchange, equity market or share market is that the aggregation of consumers and sellers (a loose network of economic ions, not a physical facility or separate entity) of stocks (also known as shares), that represent possession claims on ses: these may embrace securities listed on a public securities market, further as stock that's solely listed in camera .es of the latter include shares of non-public firms that area unit oversubscribed to investors through equity crowd funding ns. Stock exchanges list shares of common equity further as different security varieties, e.g. company bonds and convertible Stock market as argued by several economists is believed to exert Associate in Nursing impact on the economic growth of a for it provides a platform wherever capital can be raised for the institution of latest comes by firms or growth of their ons. As Osho (2014) noted, stock market plays a serious role as Associate in Nursing economic establishment which boosts / in capital formation and allocation, it allows corporations and the government to raise long run capital that allows them to new comes or expand its activities. In support of the preceding argument, Jecheche (2011) sees the stock market to serve the for growing firms to raise capital at lower price and additionally, firms in countries with developed stock markets area unit pendent on bank funding, which can scale back the danger of squeeze. Although stock exchange is seen as a stream for formation, its impact on economic process might not essentially be vital. Mark (2000) quoted economic expert as oral nicatiou oral communication stock exchange isn't merely Associate in Nursing economical thanks to raise capital and e liv[illegible] standards, however may be connected to a casino game or game of chancel. economic expert arguments stem Stocks ssified in numerous ways that. One approach is by the country wherever the company is domiciled. For example, Nestle and is are domiciled in European country, therefore they might be thought about as half of land stock market, though their stock ılso be listed on exchanges in different countries, for example, as yank repository receipts (ADRs) on US stock markets



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Web 3.0 – Future Of The Internet.

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***Abstract*– In an ongoing organized world, the Web has emerged as the most practical method of communication. During the early improvements of the Internet, there was a bit of thought that one day the progress of this Internet web would be a huge blow. In such a short time frame, Web 2.0 and now Web 3.0 have reached exceptional heights in the Internet industry. The split from Web 1.0 to Web 2.0 was advertised in virtually 10 years. However, shortly after Web 2.0, another Web 3.0 advance has increased interest and many inquiries from engineers, customers, and controllers. What is really needed at this stage, what are the driving variables, how unique are they in terms of Web 2.0 and the Semantic Web**

Keyword – Semantic web, web1.0, web 2.0, block chain, Decentralized

I. INTRODUCTION

Web 3.0 is a new era of the World Wide Web, where Web 2.0 innovations are closely linked to the Semantic Web, allowing both humans and machines to access and use data stored on the Web. With Web 3.0, machines actually have to perform tasks that require human insight, significantly reducing time and effort on the Internet. Web 3.0, aimed at making the Internet a better and smarter organization, is the predecessor of the complete Semantic Web and replaces Web 2.0. After quite a long time working on a centralized framework, the Internet will leap forward with the help of blockchain and its decentralized center. Migrating to Web 3.0 addresses the next stage of the Web, with freedom of information, practices, and common activity paths as standard. Web 3.0 innovation is returning privileges to customers by focusing on collaborative collaboration and discouraging collaboration with a unified organization. However, this change is difficult to understand, and of course many high-tech business visionaries and regular web consumers still don't know exactly what Web3 means. Web 2.0 provides important authority for collaborative use of the Internet, allowing individuals to connect to information and contribute to their perspective through wikis, web journaling, personal communication environments, and more. Models: Wikipedia, Blogger, Digg, Technorati, Stumble upon, MySpace, Facebook, Flickr and more. The idea behind the use of the Semantic Web is to capture and decode specific situations and ideas of information. Then, when the customer searches for an answer, Web3.0 provides the end customer with the most reliable and meaningful results. Therefore, this third era of the Internet is the era of evaluating customized connections to machines and websites, just as we are talking to other

II. What is Web 3.0

In the semantic web, the data is analyzed

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Robotics: Social Robot

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tract: Social robots are is also called to 'family robot' .In a social Robots are the study which provides to the communication ut them selves . it is ourselves with people, and with the environment, within the joined to its role. This paper discusses the cept of a social robot. To the Current. Social robots also used in house and health care.
n the world's first social robot designed by MIT robotics professor Cynthia Breazeal, the Jibo robot is often described as world's first family robot". Social Robots are helpful in health care and domestic areas, and in education and language ning's, arts and entertainments. In this aim is to create guideline, an intended design for future developments of social ot. In this research paper the researcher has discussed about the introduction part of the social robots, applications of social ots, research has also focus on Benefits limitation and the future working of the social robots. In overall conceptual ussion of social robots the research has also focuses on future development.
words: Social Robot , Robotics , Human robot Interaction , Learning , Healthcare, Education.

I. INTRODUCTION

ocial robots is a artificial intelligence system that is designed to communicate with humans and other robots. It is the robots ch having an it's own laws. And which contains human interaction. Social robotics is a recently branch of robotics.
ial robots can interacts with humans it's rules and regulations which is joined to it's role. Some artificial social agents are gned with a screen to represent the head or 'face' in a constant change which is communicate with users

What are Social robot?

ike the robots that have became a familiar view in factories and warehouse, which have only,limited contact with humans. Social ots are designed to communicate with us. Nowadays "People don't want to work with robots, because they're going to lose their ."

What is the use of Social Robots in Today's World?

he some of the way to social robot are used to today include was their in taken to tutoring - provide learners with a fun is to onsive in a practice and master was new learning skills and for health care target in .

Use case for a Social Robots

arly in generations of the social robot in were designed for not dependent and similar tasks, such as expanding the ocean floor. viding the produce the process or helping to fulfill warehouse orders. Some of the another ways that social robots are used today ude:

Tutoring: This provides learners with a fun, responsive way to practice and master new learning skills.
Companionship: In the provide to emotional support young , the elderly or disabled.
Customer Engagement: Social robots provides an possible customers with information about products and services, store hours and pricing.

II. LITERATURE REVIEW

Joseph E Michaelis and Bilge Mutlu are the author of "Supporting Interest in Science Learning with a Social Robot"(June 2019) in that experts are studied on basics robots .In Interaction Design and Children (IDC '19) June 12–15, 2019, Bo[illegible], USA . New York, NY, USA. In this research paper education researchers and learning scientists have emphasi[illegible] student learning requires than the , it was knowledge the goes simple acquisition of facts and procedure.
Tony Beldame and James Kennedy are the author of "Social robots for education: A review"research paper [illegible] rights reserved; exclusive licensee American Association for the Advancement of Science. No c[illegible] in the original Government Works. They have been shown to be effective at increasing the cognitive and affective outcomes and have achieved by outcomes equal to those of human tutoring on limited tasks.

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Overview of Social Media

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ct: For young people, social media provides Friends we could not see in person were available online and allowed us 'ant points of connection. On the other hand, fewer opportunities to interact in person with friends and family meant less orld scrutiny on some of the negative effects of social media. Whether it's social media or in person, a good peer group a difference. a platform to help them discover who they are. For very shy or introverted youth, this can be a way to meet people with similar interests. During the pandemic, social media made it possible for people to connect in ways when in- socialization was not possible. Social support and socialization are important influences on coping and resilience.

I. INTRODUCTION

ntana is responsible for increasing mental health problems. This systematic study summarizes the effects of social network mental health. Social media activity like spending time together shown to have a positive effect on mental health domains. ver, due to the cross-sectional design of the sample and methodological limitations, there are considerable differences. The sition of social media effects on mental health needs to be further analysed through qualitative research and vertical cohort s. Man is a social animal who needs the co-operation of others to progress in life. Thus, being socially connected with other : can relieve stress, anxiety and sadness, but a lack of social connectedness can pose serious risks to mental health. Social has recently become a part of people's daily activities. Many of them spend hours every day on Messenger, Instagram, ook and other popular social media. For teenage girls in particular, the more time they spend on social media is directly related much they absorb the idea that being thin is the norm, motivated to try to be thin and/or scrutinize their bodies excessive up of friends who connect over shared interests like art or music, and who are balanced in their outlook on eating and looks, is tive. If you think social media is a negative experience, you may need a break. It's more difficult to permanently disconnect social media—especially for young people. These platforms are powerful tools for connecting and staying up-to-date with s and family Social events too. If you are not on social media then you are depending on your friends to contact you in person. doesn't always happen.

gure below shows news consumption by US adults on social media.

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Embedded system-based intelligent wheelchairs for disabled people

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Abstract - Mobility impairment is a major problem affecting the independence of people with physical disabilities. Therefore, mobility aids need to be improved in order to improve people's living standards. This paper describes the design of smart wheelchairs using embedded systems. This paper describes the design and development of smart wheelchairs using embedded systems. The proposed design of the wheelchair can be controlled via Bluetooth, thus allowing the user to control the wheelchair with less effort. In addition to the virtual joystick control interface, touch commands are provided to the system to enhance user interaction. It helps people with disabilities to carry out their daily indoor activities independently. Experiments will be conducted to verify the functions of the developed smart wheelchair.

1.INTRODUCTION

This paper focuses on the problem of disabled people who want to commute by themselves but cannot drive for natural reasons. This proposed project focuses on Bluetooth control of a wheelchair with automatic movement in directions such as forward, backward, left, right, and diagonal by Bluetooth commands. This model uses an Android app to pass Bluetooth commands to the Raspberry PI 3 via Bluetooth communication with the Bluetooth module. People who become disabled face many problems when moving from one place to another. Most disabled people use conventional wheelchairs. Previously, wheelchairs were manually operated. Operated by hand or by another person if the patient is unable to drive. For this type of wheelchair, the person must have sufficient strength to control it. Otherwise, another person must be present to monitor the movement of the chair. Some face big problems. In this case, a second person is always required [1]. So people working small parts of the body can use it with minimum effort and maximum precision and speed control. The device is loaded with many extra features that make it smart. This wheelchair is therefore designed to overcome the above problems and allow the end-user to perform only safe movements and perform daily necessities.

This paper describes a simple, intelligent, affordable, motor-controlled key device that is easy to use, provides customized commands to the , and allows the wheelchair to move independently. I'm here. A smart phone is used as the robot's brain to give instructions. Bluetooth simplifies his communication from wired to wireless. The IR sensor is also used to detect and notify you when you find an obstacle in your passageway. This design requires the user to control the movement of her wheelchair using Bluetooth commands. These commands are received by the Android application on the user's phone, which is connected to the wheelchair via the Bluetooth module. Commands issued relate to the and RS channels and are received by the module. The purpose of the Bluetooth controlled wheelchair is to listen to the and respond to commands received from the user. This application is just an artificial intelligence application. Here, the system requires training of the user, after which the device will start capturing his commands issued. This is done by attaching comment to the controller via code.

2. Working Methodology

The Smart Wheelchair consists of a wheelchair-controlled Bluetooth module. To set up a system for cheap monitoring, the Raspberry PI 3 UNO allows you to approach the system without viewing the unit. Wheelchair movement can be controlled manually via Bluetooth. Commands are implemented using a Bluetooth mobileapp and sent to the Raspberry PI 3 UNO where the commands are processed. [3,4] After processing, the commands are sent in the form of digital signals to the motor driver IC to control it. wheelchair movement. This system was also developed to control wheelchairs with Android devices. The user can steer the wheelchair by selecting specific directions displayed in the four quadrants of the Android smartphone screen. Raspberry PI 3 UNO will

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Mobility operation in the 5G Network between colorful Access Networks

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Abstract— The 5G network, which aims to manipulate in 2020, is secerning in terms of data transmission speed, quiescence, and capacity of outstations on the network compared with the 4G network. One of the major design generalities for the 5G network is to accommodate colorful multiple access networks with the core network, and to give flawless mobility service. In this paper, we present the conception of Multiple Access Protocol Data Unit(MAPDU) session to control large data transmission in 5G network, and propose a dynamic anchoring mobility operation between different access networks.

Keywords—dynamic anchoring, mobility management, 5G network

1. INTRODUCTION

The 5G network, which aims to manipulate in 2020, differs from the 4G network in terms of data transmission speed, quiescence, and capacity of outstations on the network. In addition, the 5G network is anticipated to play a part not only as an structure for mobile communication services, but also as a base for future diligence

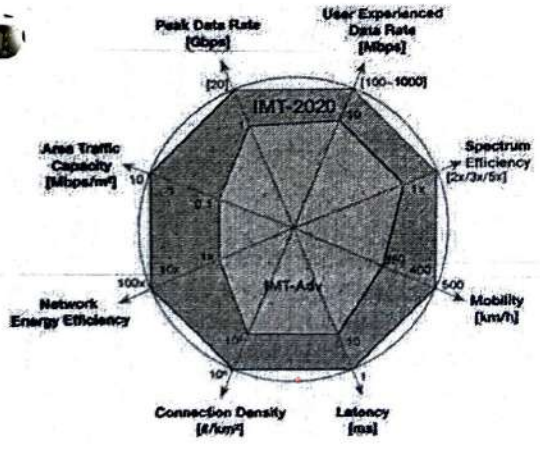

Fig. 1. Enhancement of key capabilities from 4G to 5G

The 5G network aims to achieve data rates of over to 20 Gbps, which is 20 times faster than the 4G network with a outside of 1 Gbps(2). still, the factual data transmission speed that 5G mobile service druggies can witness is aimed at 100 Mbps. This raises enterprises about whether it'll be possible to handle contents that bear large quantities of data, similar as virtual reality or holograms. To break this problem, one of the major design generalities for the 5G network is to accommodate colorful multiple access networks with the core network. This allows druggies to enjoy immersive contents that they hadn't preliminarily endured through the 5G network. For this purpose, there's a need for a control system able of transmitting a large quantum of data by contemporaneously using colorful kinds of access networks constituting the 5G network(6).

Another of the main design generalities for 5G network is to have a distributed control structure to help centralization of data business. The 4G network has a hierarchical structure in which several S- GWs are connected to a P- GW where an IP address is anchored and several base stations are connected to the S- GW. thus, in order to use the Internet service, the data business is concentrated in the P- GW, performing in hamstrung data paths. In the 5G network, the GW that anchoring the IP address is distributed close to the access networks to support a large quantum of data business. In order to support similar distributed structure, mobility control for data business between anchoring GWs is needed. While the stoner terminal moves in the 5G network and coincidently attaches to the 3GPP and the Non3GPP Access networks, and when the data packet transmitted to the 3GPP Access network is path switched to theNon-3GPP Access network, there may arise a problem that order of the packets isn't guaranteed because of the transmission detention difference on the paths in the colorful access networks.

In this paper, we present the conception of Mama- PDU(Multi Access PDU) session to control large data transmission in 5G network, and propose mobility control system to guarantee nonstop data transmission between multiple access networks. The remainder of this paper is organized as follows. In Section 2, affiliated exploration trends on mobility control are explained. Section 3 describes the structure of 5G network, which is being formalized in 3GPP. In Section 4, a dynamic anchoring mobility operation with the End Marker is presented to insure flawless data transmission between 5G and WiFi access networks.

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API Testing Using Postman Tool

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Abstract: ***Postman is one of the greatest API automation and documentation tools available today. Postman began as a simple Chrome browser plugin and has now grown to be a full API testing solution used by 5 million developers and over 100,000 enterprises all over the world. With a $2 billion value, it's a unicorn in its own right, and it's become the go-to platform for creating enterprise APIs.***

As the API economy continues to growing, more challenges are created for developers. The old methods of manually creating and testing APIs no longtime scale as today's software and services can interface with hundreds of APIs within a single application. Development, testing, and delivery teams must work together to make sure that applications work barrier with APIs to provide a business advantage, rather than cause a business obstacle.

Collaboration and operational efficiency are the keys to sharing modern API- powered applications. And this is the space that Postman plays in. In one of our GlobalLogic projects related to a Banking as well as Financial Services Customer Communications Management platform that is CCMP, we need to use Postman and its CI/CD add-on Newman to create, customize and automate Web API tests. This white paper and technical report narrate our passage direct this automation and showcases some learnings and best practices realized along the way. This is our view from the trenches on the capacity and possibilities of the Postman platform for API testing.

Keywords: Connecting to API, Postman Tool

I. INTRODUCTION

API is a Application Programming Interface. They are connection Between two Application. In API There are two types one types is use for without internet connection and another type is used for with internet connection. Those types one is used with internet connection is call 'Web Services'.

Let see What is web services?

For Example: You took a Flipkart of application, Flipkart is a mobile app and as well a web app. You can use Flipkart whenever your internet you can't turn on Flipkart. The data you will using the Flipkart application the data will be connected to your server. web application you use to connect to the data server is also called API. API is divide inti many types but there are two main method currently used First is SOAP Method and Another is REST Method. The SOAP Method is doesn't use much because Longform of SOAP is Simple Object Access Protocol. If you want to connect through SOAP API then you can use xml body and SOAP API is only use only POST API Method. They are in return xml body, because they are heavy, they consuming a more bandwidth and it is a Slow. In REST API we can use xml, plan-text format. Every format gives different method like POST,GET, PUT, DELETE different method are available for each operation. They are lightweight and they are fast comparatively SOAP API. Because of all this reasons most people use REST API service.

II. NEED OF WEB SCRAPING

Postman is an API client for developers that makes it simple to create, distribute, test, and document APIs. Users may construct and save basic and sophisticated HTTP/s queries, as well as view their answers, to do this. As a result, work is more efficient and less tiresome.

III. PROPERTIES OF REST API

1) REST API follows the client -Server Architecture.
2) Stateless
3) Cache
4) Uniform Interface

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Virtual Smart Phones

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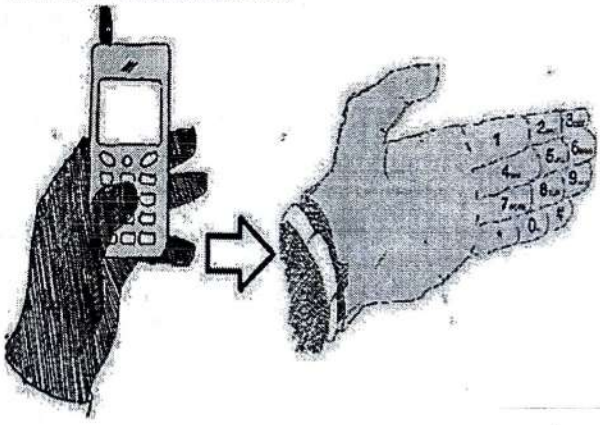
Abstract

For every departure metal money communication may be a manner by that they share/pass their thoughts/fillings to I another. we have a tendency to homosepians chiefly use verbal communication to speak with one another. during this Paper we have a tendency to introduce VSP, a Virtual good Phone that is largely a step to attach each the Physical and virtual world, by employing a little projector, Camera, Speaker, microphone & Cloud Computing Technology over the net within the kind of wearable device. In VSP all the specified element area unit fancied within the wearable device by that use communicate with the assistance of natural hand gesture, Hand movement and net. In VSP user communicate with one another by Virtual mobile with the assistance of bit gesture electromagnetic radiation and cloud computing technology.
VSP can finish the physical dependency of mobile. VSP offer novel interaction methodology to seamlessly communicate with one another in an exceedingly fun and intuitive manner. The user will bit their Palm to form decision and might even be used for looking at movies or pictures on their Palm/wrist. bit gesture is employed for creating and Terminating the decision. VSP uses touch-based interactions as instruction for establishing communication between the various users.

I. INTRODUCTION

The recent advent of novel sensing and show technologies has inspired the event of a spread of multi-touch and gesture primarily based interactive systems. In these systems user could move directly with info victimization bit add natural hand gestures. these days there area unit voluminous approach by that we will hook up with digital world within the controlled surroundings victimization muti-touch and gesture primarily based interaction. sadly, most gestural and multi-touch primarily based interactive systems don't seem to be mobile and little mobile devices fail to supply the intuitive expertise of full-sized gestural systems.
Moreover, info still resides on screens or dedicated projection surfaces. there's no link between our interaction with these digital devices and interaction with the physical world around US. during this paper, we have a tendency to gift VSP-Virtual sensible Phone, a multi-touch and gesture primarily based interaction system. that replace the physical transportable device to the virtual multi-touch & natural gesture primarily based interaction on the user palm by that user communicate with alternative digital devices over the network. VSP primarily turns the human hand as a transportable by that is ready to user hook up with the digital world additionally as alternative peoples like their friends and relatives.



VSP is largely a computer-vision primarily based wearable and gestural info interface that augments the physical world around US with digital info and proposes natural hand gestures because the mechanism to move therewith info.

2. RELATED WORK

Recently, there are an excellent form of multi-touch interaction and mobile device merchandise or analysis prototypes that have created it doable to directly manipulate computer programme parts victimisation bit and natural hand gestures. Most of those systems rely upon the physical bit-based interaction between the user's fingers and physical screen and therefore don't acknowledge and incorporate touch freelance freehanded gestures. VSP Virtual sensible Phone Technology takes a unique approach to computing and tries to form the digital facet of our lives a lot of intuitive, interactive and, above all, a lot of natural. It's plenty of advanced technology squeezed

An Overview of Bluetooth Technology and its Communication Applications

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Abstract

Bluetooth is a new RF short-range wireless technology which is designed for wireless communication between different devices. There is increase in popularity of Bluetooth technology and is being accepted in today's world. There are organizations which are doing research on Bluetooth technology, but very few of their research analysis provide a balanced view of the technology, describing its implications for businesses, pros and cons. In this paper analysis have been done keeping in mind various perspectives of the Bluetooth technology. The analysis starts with a description of the technology in terms of its network infrastructure, hardware and software. Then it is continued by the Error corrections and retransmission. The analysis is done on macro analytical view including the business implications, advantages of this technology, its role in

Keywords: Bluetooth; Bluetooth architecture; Frequency-hopping spread spectrum (FHSS); Logical Link Control and Adaptation.

1. Introduction

We have all experienced the problem which arises when connections are made between peripheral and computer or connection between the electronicdevices. Thus the companies of telecommunications needed to develop an opened, low cost interface to make easier the communication between devices without using cables. Bluetooth is a wireless technology having very short range designed enabling communication between the devices like computers,entertainment systems and other electronic devices without the use of cables and connectors. There is a strong need for a better way for all the electronic devices to communicate with each other, in order to make the aforementioned systems, computers and/or Harald Blaotland (Bluetooth), a Danish king born in AD 908. The technology was developed by an Ericsson-led consortium, including Toshiba, IBM, Nokia and Intel. In early January 2000, the technology was further promoted by the Bluetooth Special Interest Group (SIG) comprised of 1371 member companies.

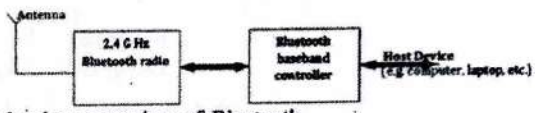
In this paper firstly we will discuss a microanalysis of Bluetooth Technology .The microanalysis of Bluetooth describes the technical details such as

2. Bluetooth Technology: A Microanalysis

Bluetooth is the technology which allows the devices to communicate with each other, synchronize data with each other, and connect to the Internet without the use of cables or wires. To add Bluetooth functionality to a computer or other host device a Bluetooth radio and base band controller can be installed on a device that

links to an integrated on a system board, a Universal Serial Bus (USB) port, or a PC Card. These components are shown in Fig. 1

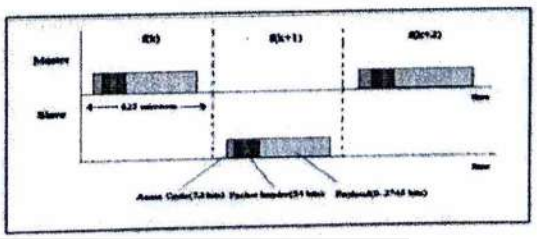
Fig.1 Bluetooth components



A. Technology overview of Bluetooth

In this section the technology specification have been explained .The Bluetooth technology is divided into two specifications: first is the core and second is the profile specifications. How the technology works is explained by the core specification, how to build interoperating devices using the core technologies is explained by the profile specification. Bluetooth air interface works on a antenna power of 0 dBm (1 mW) and be extended up to 20 dBm (100 mW) worldwide. This interface complies with ISM band rules up to 20 dBm in America, Japan, and most European countries. Frequency hopping method is used to spread the energy across the ISM spectrum in 79 hops displaced by 1 MHz, starting from 2.402 GHz and stopping at 2.480 GHz. The Bluetooth Special Interest Group is working to harmonize this 79- channel radio .These 79 channel radio are working globally and has initiated changes within Japan, Spain, and other countries. An electronic conversation determines whether they have data to share or whether one needs to control the other, whenever Bluetooth-capable devices come within range of one another.

Fig.2 Bluetooth Frame



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Green Computing for Internet of Things

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tract— Cloud computing services are used to meet the -growing demand of IoT. Data centers are becoming one the largest energy consumers to provide the ıstructure for the IoT paradigm. The demand for energy increase in the future as more innovation emerges and nol follows new practices that lead to the adoption of n computing. Green computing strategies reduce the gy consumption of IoT devices without compromising ormance. This white paper evaluates many aspects of n computing for IoT computing and analyzes key epts, challenges, and mitigations.

ex Terms – Internet of Things (IoT), Cloud Computing, : Computing, Energy Consumption.

NTRODUCTION

Internet of Things (IoT) brings together intelligent cts integrated into heterogeneous networks to monitor esses and make decisions. This is large-scale sensor data is leveraged using computational resources. Green puting can use resources or do otherwise in an ronmentally friendly way. It involves the development removal of various elements used in computers to ce the environmental impact. Companies are starting to st in omputing equipment made from recyclable rials. The purpose of green computing is to use puting resources and economically viable ways in an ronmentally friendly manner. Figure 1 shows IoT device stics by year. IoT devices are connected to various orks and their growth continues to accelerate as nesses embark on digital transformation. It also ences the spending and revenue of his IoT market in the d. These added devices also pose network security s that need to be addressed accordingly.

ACKGROUND

n computing

n computing is the design and use of resources that are onmentally friendly and sustain computing power out degrading it. Resources used in computers are led after use. Companies making these devices should ess energy and be more biodegradable. The majority of levices are energy efficient sensors, which has led to massive use by industrial players. These sensors also advance IT to use wireless networks efficiently. Data centers provide data storage and processing capabilities for big data. Cloud computing platforms face the challenge of increasing numbers of IoT devices. These IoT devices require low latency and mobility, which is why they employ edge computing for real-time services. Fog computing is a distributed computing paradigm aimed at connecting network devices at different computing layers. It provides IoT devices with low-latency responses that centralized cloud computing architectures cannot provide. Green computing focuses on preserving computing power while reducing energy consumption and being environmentally friendly. Computer CPU manufacturing technology has advanced, making it more energy efficient with each generation. However, as the number of computing devices in use has increased, it has become imperative to meet the demands of green computing. Green computing has been introduced to cloud computing to reduce energy consumption and reduce the use of harmful substances within devices.

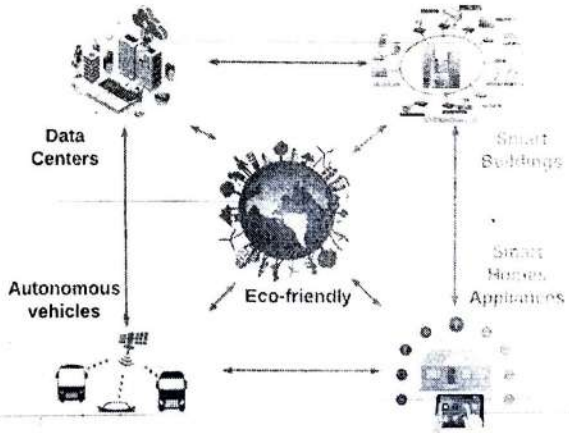

Figure 2: IoT green computing

Internet of things

IoT is the connection of devices to form an intelligent world. This is a paradigm that affects both society and technology. IoT technology involves building an infrastructure for connecting smart objects based on evolving information and network services. The data collected from the device must be processed for analysis and data protection regulations must be guaranteed. IoT is energy efficient when building smart cities. This is because the number of sensor devices and cooperating add-ons makes it easy for them to communicate with each other. Green computing must focus on reducing

Dairy Farm.

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***Abstract*— This document gives formatting instructions for authors preparing papers for publication in the Proceedings of an IEEE conference. The authors must follow the instructions given in the document for the papers to be published. You can use this document as both an instruction set and as a template into which you can type your own text.**

***Keywords*— Include at least 5 keywords or phrases**

Abstract

A dairy farmhouse business is not like any other business. It takes a lot of hard work to run this business properly. So if you are thinking of starting this business then you must read our article. In today'sarticle, we are going to tell you the things that you need to take care of while starting this business. How can you run this business properly? But first of all, you need to know what is the status of this business in your country and how much profit you can earn from it. If you want to start a small business, you can starts a dairy business with government assistance.

Today we are going to give you information about dairy which is also known as a home business but is leading in making a profit Dairy farm business is a simple business but if you want to start a dairy business it requires more effort. In this article, you will get complete information about it, what is neededto start this business and how to make a profit.

Introduction :

Dairy farming can be started by rearing cattle. Milk and milk products are used in almost all households. In the dairy farming business, you can earn money by rearing animals like buffalo, and cows and getting milk from them. Similarly, products such as cheese, curd, ghee, butter, sweets, etc. can be made and sold from milk. All these dishes take more time to prepare. And these can be sold at a higher rate than the cost of milk. Apart from this animal dung can be used in cow dung, and dung can be used asfertilizer on agricultural land. Beneficiaries can also earn

In this milk production business, we keep animals and get milk from these animals and distribute itin the market or nearby villages. If you have a large-scale dairy business, you can approach milk companies and supply milk to them, which earns a good income.

While opening a good dairy farm, we must have the following information-

Cattle Information Cattle Information:

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www.ajrconline.org

REVIEW ARTICLE

Review on Microwave, The General purpose in Microwave Assisted Synthesis for Green Chemistry

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ABSTRACT:
In this review we explain all the detailed information about Microwave assisted synthesis. Now a days the Microwave very much beneficial in to Microwave assisted synthesis reaction for green chemistry work by the various reactions. this is initially used by the save energy and rate of reaction is fast. Microwave synthesis capable of predicting many properties and rate of synthesis reaction is fast in small period of time to get from product. all type chemical reaction synthesis is also done by this microwave. various authors words on their subject by using this Microwave assisted synthesis. I show interest into microwave because of this is very beneficial for performing synthesis of reaction. In microwave various principals are added and this will be beneficial or helpful to guide scientist.

KEYWORDS: Microwave assisted synthesis, green chemistry, Microwave.

1. INTRODUCTION:
Green chemistry is defined as environmentally benign chemical synthesis.in that microwave is a general-purpose green chemistry for performing reaction in small period of time. Microwave initially started used in or released in 1986 by the groups of Gedye and Giguere/ Majetich although the use is microwave heating in chemical purpose can be back to 1950. And originates from scientist Gedye and Giguere started his research microwave synthesis in the using green chemistry. if focusses on a process whether carried out in industry or chemical laboratory. the reduced the use and generation of harmful substance or byproduct. Green chemical deals with environmentally for chemical synthesis to devise pathway for the prevention of pollution according to Paul T. Anastas[1]

Table no: 1

Original author	**Gedye and Giguere**
Developers	America
Initial release	1946
Stable release back to chemical purpose	1950
Website	www.microwaveassistedsynthesis.com

We will use the microwave assisted synthesis in windows environment. microwave is capable for predicting many properties of metal catalysis and heating principle. all types chemical synthesis reaction including the following

- Basic principle of microwave assisted synthesis.
- Theoretical aspects of microwave dielectric heating.
- Microwave accelerated metal catalysis.
- Heterocyclic chemistry using microwave assisted approaches.
- Microwave assisted reduction.
- Microwave assisted multi component reactions.
- Integrating microwave assisted synthesis and solid supported reagents.
- Microwave assisted solid phase synthesis.
- Scale up of microwave assisted organic synthesis[1,2]

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2. PRINCIPLE OF MICROWAVE HEATING:

There are three bases of microwave heating mechanical of microwave heating are following points.

- Dipolar polarization
- Ion conduction
- Dielectric loss[3]

2.1 THEORETICAL ASPECTS OF MICROWAVE DIELECTRIC HEATING:

Theoretical aspects in dielectric heating the degree of Interaction between the microwave electric and magnetic field components with the dielectric or magnetic material determines the rate of energy is dissipated into the material by the various methods including the theoretical aspects of microwave dielectric heating.[4]

2.2 THEORETICAL BASIS OF DIELECTRIC HEATING:

Dielectric heating is depending on use of microwave or also use radio frequency (RF) this wave is heating of insulating other material. There are two types of microwave chambers or cavities can be distinguished in the dielectric heating and the single mode pattern is generated. And multi-mode chambers are large number of resonant patterns is supported.[4,5]

2.3 COMPARISON OF MICROWAVE AND CONVENTIONAL HEATING:

Comparison of microwave and conventional heating in that conventional heating is the process are long duration and more energy is used as compared to in that microwave rate of reaction is fast in small duration of time for green chemistry. The mechanism of drying with microwave energy is quite different as compared to conventional drying. There are some properties of microwave heating[4,6]

- Rate of reaction is fast.
- Small duration period of time on reaction synthesis.
- Product is more precise.[6]

2.4 MICROWAVE ACCELERATED METAL CATALYSIS:

The review highlights microwave accelerated metal catalyzed transformation of aryl and vinyl halides, the less metal catalysis is use in rate of reaction is fast and most importance in save energy to help environment into green chemistry.[7,8]

2.5 HETEROCYCLIC CHEMISTRY USING MICROWAVE ASSISTED APPROACHES:

In that heterocyclic chemistry using microwave assisted is include for organic synthesis. Organic synthesis in that improved rate of reaction and reaction conditions, formation of pure product and more precise[9]

2.6 MICROWAVE ASSISTED REDUCTION:

The microwave assisted reduction is various carbonyl compounds using the solid state supported to the sodium borohydride. Microwave assisted synthesis has been shown to accelerated organic reactions and also used to alternative to conventional heating methods but conventional heating is very slow and large amount of energy are used to the conventional heating[9,10]. In that the microwave assisted reduction in that method solvent free techniques in those reactants and reagent that are supported on solid matrix in solid support following techniques are use.[10]

- Solvent free reaction shown decrease reaction time.
- Increase product yields.
- Solvent free in that more easily scale up for production solvent-based microwave reaction[9,10]

2.7 MICROWAVE ASSISTED MULTI COMPONENT REACTION:

Microwave are used to multi component reaction synthesis in that biologically relevant organic components are used in reaction process. Some points of microwave assisted multi component's reaction are;[11]

- High yields
- Less reaction times
- Selectivity and atom economy simpler, purification techniques[11]

2.8 INTEGRATING MICROWAVE ASSISTED SYNTHESIS AND SOLID SUPPORTED REAGENTS:

Microwave are used to integrating assisted synthesis, and solid supported reagent in that polymer supported reagents are applied to synthesis for the natural product and resulting in purify and efficient synthesis without conventional purification methods but performing microwave heating of reaction are usually are used according to following points[12]

- Heating heterogeneous sample: polymer consideration
- Heating polymer solvent: binary phase method
- Migration of reacting species
- Reaction heating: solvent consideration[12,13]

In that solid supported reagent also called as polymer bound or resin bound reagents, the perform synthetic transformation into manner as non-bound counterparts in that microwave assisted synthesis.[12]

2.9 MICROWAVE ASSISTED SOLID PHASE SYNTHESIS:

Microwave are using to solid phase synthesis in that microwave assisted solid phase are starting from various parts are developed

- Resins for solid phase
- Combinatorial chemistry[13]

Are resins for solid phase and combinatorial chemistry are using microwave synthesis are performed rate of reaction is fast in this synthesis and to get product are more precise and purer.[14-17]

2.10: SCALE UP OF MICROWAVE ASSISTED ORGANIC SYNTHESIS:

Microwave are the single mode reactors have been very successful in part in the field of method development and optimization. In that including the microwave technology for development of completely new reaction. Routes for organic synthesis. So, increasing demand for large scale microwave production and also chemical substance in this microwave industrial accepted technology that can fast reaction and routinely provide products are optioned.[17-20]

Microwave scale up of microwave assisted organic synthesis reactions some properties are following:

- The development of large reactors
- At least in the pilot plant scale to enable multikilogram production of compounds.
- Rate of reaction is fast and increasing demand for large scale[21-24]

3. REVIEW OF LITRETURE:

Microwave radiation, an electromagnetic radiation, is widely used as a source of heating in organic synthesis. The basic mechanisms observed in microwave assisted synthesis are dipolar polarization and conduction. Microwave assisted organic synthesis (MAOS) has emerged as a new “lead” in organic synthesis. Microwave Synthesis - A Potential Tool for Green Chemistry S. Ravichandran and E. Karthikeyan Department of Chemistry, Savitha School of Engineering, Savitha University, Thandalam, Chennai - 602 105, India. Jan-Mar 2011[25].

The term green chemistry is defined as “the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances”. The major applications of green chemistry principles and practice renders control, regulation, and remediation, Microwave assisted synthesis is an important tool for green chemistry. Microwave radiation, an electromagnetic radiation, which is widely used as a source of heating in organic synthesis microwave-assisted synthesis: review of recent developments Neha Gupta department of Chemistry, Dev Samaj College for Women, Ferozepur City.18th-19th march 20017.[26]

Solvent-free methods are especially adapted to organic synthesis under Green Chemistry conditions. When coupled to microwave (MW) irradiation, it results in very efficient and clean procedures with noticeable improvements over classical methods. The most suitable cases involve reactions with polar mechanisms with increase of the polarity during the progress of the reaction and late transition states along the reaction coordinates. To cite this article: A. Loopy, C. R. Chime 7 (2004). © 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved in 2004.[27]

Microwave enhanced one pot three component synthesis of 2-amino-4H-chromenes in aqueous hydrotropic medium is reported. The aqueous hydrotropic medium represents a green solvent under microwave irradiation. The synthetic methodology under microwave irradiation is renewable and sustainable tool of energy saving. Current Research in Green and Sustainable Chemistry June 2020, 100014[28]

In recent era, synthesis of nanoparticles through green method has gained more attention as it is an environmentally friendly approach. Bio-synthesis of palladium nanoparticles through single step and environmental benign microwave irradiation technique, using hemicellulose as reducing agent is reported and biological activity. Biological activity of derived from hemicellulose via microwave assisted green synthesis in 2021[29]

4. CONCLUSION:

the review totally focused on microwave assisted synthesis and reaction using under the green chemistry microwave assisted synthesis is help to green chemistry. So, in this review we will use the microwave assisted synthesis in green chemistry is capable for prediction many properties of microwave assisted synthesis. Basic principle of microwave assisted synthesis. Theoretical aspects of microwave dielectric heating. Microwave accelerated metal catalysis. Heterocyclic chemistry using microwave assisted approaches. Microwave assisted reductions. Microwave assisted multi component reactions. Integrating microwave assisted synthesis. And solid supported reagents. Microwave assisted solid phase synthesis. Scale up of microwave assisted organic synthesis. This are points are covered in this review of microwave assisted synthesis.

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RESEARCH ON ESTERIFICATION REACTION UNDER, MICROWAVE ASSISTED SYNTHESIS OF BUTYL BENZOATE FOR GREEN CHEMISTRY

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***Abstract*: In this research we explain all the detailed information about, Microwave assisted synthesis of Butyl Benzoate for esterification reaction in working green chemistry esterification reaction under Microwave assisted synthesis very much beneficial into assisted synthesis chemical reaction for green chemistry. Work by synthesis of butyl benzoate. This is initially used by the save energy and rate of reaction is fast. On this research work esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is ester obtained by esterification reaction, so alcoholic group are using n-Butanol and carboxylic group benzoic acid in the presence of conc. Sulfuric acid so, finally product butyl benzoate is forming an ester this synthesis microwave capable of predicting many properties and role of synthesis reaction is fast in small period of time to get form product. All type esterification chemical reaction synthesis is also done by microwave. Analyzed product by studying Thin Layer Chromatography. Various authors work on their subject by using this microwave assisted synthesis I show interest into microwave because of this is very beneficial for performing synthesis of butyl benzoate.**

***Keywords*: esterification reaction Microwave assisted synthesis, green chemistry, Microwave, synthesis of butyl benzoate, Thin Layer Chromatography.**

INTRODUCTION 1

Green chemistry is defined as environmentally benign chemical synthesis of esterification reaction Microwave assisted synthesis in that microwave is a general-purpose green chemistry for performing organic synthesis reaction in small period of time and no purification necessary as compared to conventional heating method.[1] Microwave initially started used in or released in 1986 by the groups of Gedye and Giguere/ Majetich although the use is microwave heating in chemical purpose can be back to 1950. Esterification reaction Microwave assisted synthesis if focusses on a process whether carried out in industry or chemical laboratory. The reduced the use and generation of harmful substance or byproduct. On this research work esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is ester obtained by esterification reaction, so alcoholic group are using n-Butanol and carboxylic group benzoic acid in the presence of conc. Sulfuric acid so, finally product butyl benzoate is forming an ester this synthesis microwave capable of predicting many properties and role of synthesis reaction is fast in small period of time to get form product. [1][2] Analyzed product (Butyl benzoate) by studying method: Thin Layer Chromatography. Microwave assisted synthesis is the benefits of microwave esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is some reagent or reaction are benefits of microwave assisted synthesis [3]

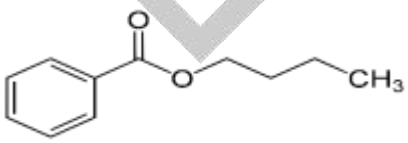


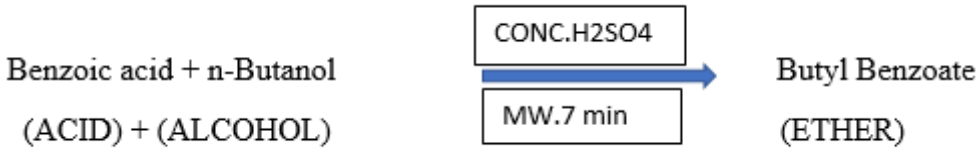
Fig. structure of Butyl benzoate

Butyl benzoate is a benzoate ester obtained by condensation of benzoic acid and butanol. It is used as a perfume ingredient and as a solvent for cellulose ether, a dye carrier for textiles. It has a role as an antimicrobial food preservative, a fragrance and a plant metabolite.[3]

1) Faster reaction synthesis of esterification reaction esterification reaction in microwave 7 min.
2) Better yield and higher purity (microwave synthesis of Butyl benzoate).
3) Energy saving for esterification reaction microwave synthesis of Butyl benzoate.
4) Uniform and selective heating.
5) Esterification reaction microwave synthesis of Butyl benzoate is green synthesis. [4–7]

PRINCIPLE OF ESTERIFICATION REACTION MICROWAVE SYNTHESIS OF BUTYL BENZOATE AS COMPARED TO CONVENTIONAL HEATING METHOD: 2

In microwave assisted synthesis and conventional heating method synthesis are same reaction but only heating method are deferent in microwave heating method are very beneficial for performing synthesis of butyl benzoate. Or other esterification reaction and all chemical reactions. And also rate of reaction is fast. By using dehydrating reagent Sulphuric acid (H2SO4). Also, Sulphuric acid acts as a catalyst in this reaction and removing water molecule. [9-12]



Some esterification reaction synthesis on microwave in general working on following way in short period of time and rate of reaction is fast.[13]

As compared to conventional heating method by synthesis of butyl benzoate in general working on round bottom flask add 3gm benzoic acid and add Butanol 5ml and CONC.H2SO4 are 2 to 3 drops. Then conventional heating 45min product are obtained. Same process working on microwave heating apply (electrical heating is converted to heat energy) product are obtained only 6min.very fast reaction in microwave as compared to conventional heating method by synthesis of butyl benzoate. [14-15]

Butyl Benzoate are compared by two heating method, microwave synthesis heating method and conventional synthesis heating method: 3

Sr.NO.	Heating Method	Green chemistry applies	Temperature 0C	Time	Purity after recrystallization	Stability 1gm in 1ml H2O
1.	Microwave	yes	60 W	6min	96%	Practically insoluble
2.	Conventional	No	60 0C	45min	84%	Practically insoluble

Table No.1

ANALYZED PRODUCT (MICROWAVE SYNTHESIS OF BUTYL BENZOATE) BY STUDYING THIN LAYER CHROMATOGRAPHY:4

Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography in that thin layer of silica gel spread on a glass surface than Butyl Benzoate spot the plate using a capillary tube.[16] Press the tube firmly to the plate in order to deposit the solution. We will use solvent to develop the plate. put solvent in a beaker and insert the plate. Mark sure the line sits above the solvent (Butyl Benzoate) and cover the beaker. than now we watch the plate develop.[16]

Mobile phase are water and methanol (1:2) ratio. And stationary phase by using silica applying spot Butyl Benzoate than calculate difference between Microwave Synthesis product and Conventional synthesis product are following:

Formula: RF Value = $\frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$

i.e., d0 = travel distance of the solvent.

dA = travel distance of the compound A (microwave synthesis product).

dB = travel distance of the compound B (Conventional synthesis product).

R1 = (Ratio of front) value for A = dA /d0.

R2 = (Ratio of front) value for B = dB /d0.

Sr.NO.	Heating Method	travel distance of the solvent. (d0)	travel distance of the compound (d)	Ratio of front value (R)
1.	Microwave	6cm	(Spot A) 5.2cm	0.79
2.	Conventional	6cm	(Spot B) 4.7cm	0.71

Table No.2

Result: The Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography are less absorption in that less affinity, So Butyl Benzoate are movement fast. And as compared to conventional synthesis of Butyl Benzoate are more absorption in that more affinity, So Butyl Benzoate are movement are slow.[17]

REVIEW OF LITRETURE

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Microwave radiation, an electromagnetic radiation, which is widely used as a source of heating in organic synthesis Microwave-Assisted Synthesis: Review of Recent Developments Neha Gupta Department of Chemistry, Dev Samaj College for Women, Ferozepur City.18th-19th march 20017. [15]
Esters are among the highest volume of industrial organic compounds produced. faces serious limitations of low conversion and high reaction time attributed largely to establishment of equilibrium. And then Fischer esterification regarded as the most common and widely practiced process of ester synthesis Journal of Industrial and Engineering Chemistry Volume 103, 25 November 2021, Pages 80-101.[16]
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CONCLUSION: Esterification Reaction Microwave Synthesis of Butyl Benzoate as Compared to Conventional Heating Method to change the alcoholic group (butanol) product Microwave Synthesis of Butyl Benzoate are within 6min are Obtained synthesized product. And Conventional Heating Method synthesis product are within 45min are Butyl Benzoate are Obtained synthesized product.
To Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography and to check purity of Butyl Benzoate was found to be 0.79 As Compared to Conventional Heating Method by Studying Thin Layer Chromatography and to check purity of Butyl Benzoate was found to be 0.71.

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Review On General Purpose Of Catalysis In Green Chemistry

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Abstract

Green chemistry, also known as sustainable chemistry, refers to the development of chemical products and processes that minimize or eliminate the usage and production of harmful compounds. They only utilize environmentally friendly chemicals and chemical procedures. It is built on twelve principles that can be used to develop or reproduce molecules, materials, reactions, and processes that are safer for human health and the environment from the ground up. Green Chemistry decreases the environmental impact of chemical processes and technologies, as demonstrated in this article.

The goal of this research is to learn more about the role of catalysts in green chemical synthesis for a more sustainable future. In the ecologically friendly synthesis of novel and existing compounds, catalysis plays a critical role. Catalyzed processes require less energy to produce and produce fewer by-products, co-products, and other waste items, indicating increased efficiency. Catalysts can be created in such a way that they are not harmful to the environment. Catalysts come in a variety of shapes and sizes, and some of them have positive effects in the chemical industry.

Key words- Biocatalysis, Biomass, Ionic Liquids, Critical Fluids, Microwave Irradiation, Photocatalysis, Green Chemistry

Definition of green chemistry-

Green chemistry, also known as sustainable chemistry, is the development of chemical products and processes that reduce or eliminate the usage and manufacture of harmful compounds.[1] Chemical goods should be designed so that they do not persist in the environment after they have served their purpose and are broken down into environmentally friendly components.[2]

INTRODUCTION TO GREEN CHEMISTRY-

In the early 1990s, the concept of green chemistry was originally proposed. The first volume of the well-established green chemistry journal of the Royal Society of Chemistry was published in 1999, and the green chemistry institute was founded in 1997.[3] Green chemistry processes encompass practically all aspects of chemistry, including inorganic, organic, biochemistry, polymer, environmental, and toxicity. The goals of environmental protection and economic benefit can be achieved through several prevailing trends of the green programme, such as catalysis, bio-catalysis, and the use of safety alternatives: renewable feedstock (biomass), reaction solution (such as water, ionic Liquids, and supercritical liquids), reaction conditions (microwave irradiation), and new synthetic pathways (photo catalytic reaction).[4]

Concept of Pharmaceutical Green Chemistry

Pharmaceuticals are the most dynamic segment of the chemical business. It is at the vanguard of major shifts toward "greener" feedstock, cleaner solvents, alternative methods, and new concepts. All of these measures will improve the pharmaceutical industry's environmental credentials while also lowering costs and materials for manufacturing processes, paving the way for long-term sustainability.Green chemistry is a Hippocratic oath for chemists, and a new generation of scientists and technologists is being formed to analyse the processes and materials used in production and development efficiently in order to protect natural resources and the environment. If no hazardous substances are used or produced, the risk is zero, and there is no need to be concerned about removing hazardous substances from the environment or limiting exposure to them. "Green chemistry is about reducing waste, raw materials, risks, energy, environmental impact, and cost," as the phrase goes.[5]

Scientific Areas for Practical Applications of Green chemistry

The areas proposed for special focus under the green chemistry Principles were the following.

1.Use of alternative feedstock

2.Use of less hazardous reagent

3.Use natural processes like biocatalytic techniques

4.Use of alternative solvents

5.Designe of safer chemicals and products.

Green Chemistry's Latest Trends-

The green program's core goals are achieved through many prominent trends in the design, development, and use of chemical products and processes that decrease or eliminate the use or production of substances that are dangerous to human health and the environment."

a. Catalytic and biocatalytic reaction research in order to obtain highly selective, pure compounds without the formation of toxic byproducts;

b. Searching for new raw materials that are both harmless and renewable, such as biomass;

c. Developing environmentally friendly chemicals that are less toxic;

d. Developing and evaluating new non-toxic, renewable reaction media, such as water, ionic liquids, and supercritical fluids.

e. Developing and evaluating new reaction conditions, such as microwave, ultrasound, and light reactions.[6]

PRINCIPAL IN GREEN CHEMISTRY

There are twelve green chemistry principles that have been created By EPA's Paul Anastas and John Warner, who described their significance in practise in their Green Chemistry Theory and Practice book, published in 1998. Green chemistry principles call for the elimination or reduction of dangerous or harmful compounds from the synthesis, manufacture, and application of chemical products, reducing or eliminating the use of substances harmful to human health and the environment.

"Reducing Risk" and "Minimizing the Environmental Footprint" are two of the principles. In the past, various chemical industries have been associated with risk. Hazardous chemicals to humans and the potential of environmental pollution were linked to new chemical products, giving synthetic chemical materials a "bad name." Energy use, climate change, crisis, and depletion of natural resources are all factors in the environmental footprint.[7]

1.Prevention

2.Atom Economy

3.Less Hazardous Chemical Syntheses

4.Designing Safer Chemicals

5.Safer Solvents and Auxiliaries

6.Design for Energy Efficient

7.Use of Renewable Feedstock

8.Reduce Derivatives

9.Catalysis

10.Design for Degradation

11.Real-time analysis for Pollution Prevention

12 Inherently Safer Chemistry for Accident Prevention

What is Catalysis-

Catalysis is a term used in chemistry to describe the process of modifying the rate of a reaction by using a substance that isn't consumed by the reaction.

How it related to green chemistry

Chemical operations produce large amounts of trash every day. Stoichiometric equivalents, in particular, produce undesirable byproducts such as inorganic salts. More efficient catalytic alternatives are progressively replacing stoichiometric chemical methods, allowing scientists to save energy and resources. Moving away from stoichiometric processes and toward homogeneous and heterogeneous catalytic reactions using organic, organometallic, inorganic, and biological catalysts is referred to as greener catalysis.[8]

Role of Catalyst in green chemistry

Green chemistry is an area of chemistry that focuses on the discovery and use of environmentally friendly chemicals and processes . Catalysis is a key component of green chemistry. Green chemistry, often known as environmentally benign chemistry or sustainable chemistry, minimises toxicity. Its objective is to design and execute pollution avoidance solutions other than waste management that reduce waste, save energy, and reduce natural resource depletion. Green chemistry is considered environmentally friendly because it is thought to reduce carbon emissions and pollution. Catalysis has aided in the reduction of pollution in our environment. Catalysts have been used to improve air quality by removing and controlling NOx emissions, reducing the use of Volatile Organic Compounds (VOCsi), developing alternative catalytic technology to replace the use of chlorine or chlorine-based intermediate in chemical synthesis and waste minimization, and developing alternative catalytic technology to replace the use of chlorine or chlorine-based intermediate in chemical synthesis and waste minimization. Catalysis allows for more efficient and selective reactions, resulting in the elimination of vast volumes of by-products: and other waste chemicals.[9]

Types of Catalysis –

Depending on the number of phases in which the catalytic reaction is carried out, homogeneous or heterogeneous catalysis can be used for synthetic processes.Homogeneous catalysis is a single-phase reaction that is usually liquid/liquid, whereas heterogeneous catalysis is a multi-phase reaction. The use of homogeneous catalysts provides a number of advantages, including decreasing reaction temperatures and thereby saving energy.[10]

The following are some of the catalysts:

1.METAL CATALYST – Using well selected metal catalysis can make a reaction more ecologically friendly. Transition metals are frequently utilized as catalysts in reducing reactions like hydrogenation.Metal catalysts can be pure metals or bimetallic or multimetallic mixtures of metals, or they can be spread on solid supports like silica, alumina, or carbon.[11]

2)METAL OXIDE CATALYST- For catalytic oxidation, transition metal oxides have been utilized. In the production of bulk chemicals, molecular oxygen is preferred, whereas in the production of fine chemicals, hydrogen peroxide is preferred. Although more expensive than molecular oxygen, hydrogen peroxide is environmentally friendly because it is converted to water during the oxidation reaction.Because it is transformed to molecular oxygen, ozone is also environmentally friendly, but its generation necessitates particular handling and equipment.[12]

3)METAL COMPLEXES- In homogeneous catalysis, metal complexes are commonly utilised. A transition metal complex was used to synthesise naproxen with a 97% yield under high pressure. Chiral metal complexes catalyse inhomogeneous phase reactions while also controlling the reaction's stereo specificity.[13]

4)BIOCATALYSTS- Enzyme and antibody catalysts are used in both homogeneous and heterogeneous systems.

•ANTIBODY CATALYSTS- Another form of biocatalyst that is frequently employed is antibody catalysts. Antibody specificity and selectivity are related to the antigen structure required to elicit an immune response.

•ENZYME CATALYSTS- Selectivity is one of the most notable characteristics of enzyme catalysts. They are regioselective, which means they can distinguish between several identical groups within the same molecule. Enzyme catalysis can take place in both aqueous and non-aqueous solvents, including supercritical fluids.[14]

Solid acid and bases As catalyst-

Acid and base catalysed reactions are important in the oil refining and petrochemical sectors, as well as in the production of a wide range of speciality chemicals like medicines, agrochemicals, and flavors and perfumes. In liquid-phase homogeneous systems or on inorganic supports in vapour phase systems, many of these processes require the use of conventional Brnsted acids (H2SO4, HF, HCl, p-toluene-Sulfonic acid) or Lewis acids (AlCl3, ZnCl2, BF3). Similarly,NaOH, KOH, NaOMe, and KOBut are examples of common bases. As a result of their subsequent neutralisation,The formation of inorganic salts that eventually find their way into aqueous streams.[15]

Additional advantages of using solid acids and bases as catalysts include:

-Separation and recycling are made easier, resulting in a faster process and lower production costs.

-Solid acids, such as H2SO4, HF, are safer and easier to handle than their liquid equivalents. Very corrosive and necessitates the use of costly construction materials

-Trace levels of (neutralized) catalyst contamination in the product are often avoided.

When the latter is a dependable.

-Granular chemicals are safer and easier to operate than their liquid counterparts.

Solid Acid Catalysis –

One of the most important applications of heterogeneous catalysis is in acid-catalyzed processes. Solid catalysts are utilised in a wide range of applications. Acidic Clays, zeolites, supported heteropoly acids, and mixed oxides like silica–alumina and sulfated zirconia are among them.Hybrid organic–inorganic materials, such as mesoporous oxides, and organic ion exchange resins Organic sulfonic acid moieties are suspended in the air.[16]

WITH A SOLID BASE

There are much fewer examples of reusable solid base catalysts in use than there are for solid acids.This is most likely due to the fact that acid-catalyzed reactions are far more common in the manufacture of Substances that are widely available. The different types of solid bases that have been reported are similar.Anionic clays, basic zeolites, and anionic clays are all alternatives to the solid acids detailed in the preceding sections.Mesoporous silica grafted with organic bases pendent.[17]

Catalytic C–C Bond Formation

Another important transformation in organic synthesis is the production of C–C bonds, and carbonylation is an important catalytic technique for producing C–C bonds. It's utilised in the bulk chemicals industry to make acetic acid by catalysing the carbonylation of methanol with rhodium and since they are 100 percent atom efficient, they are increasingly being used in fine chemistry.Manufacture of chemicals The Hoechst-Celanese method, for example, is a beautiful illustration of this.Manufacturing of the analgesic ibuprofen, with a production capacity of several thousands of tonnes per year.[18]

TECHNOLOGY OF ENZYMES IN BIOCATALYTIC REDUCTION

Reductions are important in organic synthesis because they lead to chiral compounds with new functionalities.Such processes can be catalysed by enzymes with exceptional stereo-, regio-, and chemoselectivity, resulting in The path to not just high-added-value but also shorter classical synthetic pathways Compounds, as well as bulk chemicals, are available.Enzymes, nature's catalysts, offer nearly limitless access to a wide range of chemical reactions.Reactions. Reductions in particular can result in the formation of not just multiple chiral centres, but also multiple chiral centres.But also new functional groups in products that are in high demand in the pharmaceutical and fine chemical industries.[19]

Are Biocatalytic Reactions Green?

Today, the statement "biocatalysis is intrinsically green" has become a mantra for many Researchers.First of all, researchers should be aware that no chemical transformation (including Biocatalytic reactions) is green, as in all cases resources are consumed and waste is generated, Thereby putting a burden on the environment. We believe that a given reaction of methodology can Be greener than another reaction. Such a comparison, however, should be based on quantitative Data rather than on general statements. Comparative full life cycle assessments (LCA) represent The "gold standard" for such comparisons, but are usually time-intensive due to the large data basis Required for a meaningful comparison. Sheldon's E-factor6 and possibly its derivative, the E+-Factor, taking energy-related CO2 emissions into account,7 represent an acceptable alternative for The preparative chemist.[20]

Conclusion

There is a need to update or adapt traditional procedures that are not environmentally friendly, use dangerous solvents, and are not atom specific in the sense that they do not follow green chemistry principles. This could be beneficial to students' safety while also being environmentally sustainable. For the first time, a new approach has been established. IN organic synthesis, non-conventional approaches are used. Catalysis is crucial in the environmentally friendly synthesis of compounds. By substituting an environmentally friendly synthetic approach for a standard synthetic pathway, several by-products, co-products, possible wastes, and pollutants can be avoided. The reduction of a number of steps that normally occur during synthesis shows the possibility for catalyst to be employed for environmentally friendly synthesis. The use of catalysts in chemical synthesis can be quite beneficial. Inventing environmentally friendly technology and producing ecologically friendly chemicals.

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RESEARCH ARTICLE

Influence of Newly Synthesized Superdisintegrant on Dissolution Rate Enhancement of Carbamazepine using Liquisolid Compact Technique

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ABSTRACT:
The purpose of this study was to manufacture liquisolid compact of high dose poorly water-insoluble drug, Carbamazepine (CBZ) by using novel superdisintegrant for the purpose of fast disintegration and improved its dissolution rate. The solubility of CBZ was analyzed in various non-volatile solvents in order to find the vehicle with the maximum solubility. The dissolving profile of liquisolid compacts was compared to a marketed tablet formulation's dissolution profile. CBZ was found to be much more soluble in polyethylene glycol 200 than in the other solvents. Crosspovidone-containing formulations showed no disintegration, but all other formulations disintegrated after 91.2 seconds. A Starch Glutamate-Croscarmellose Sodium combination has a disintegration time of 42.5 seconds. The optimized batch NSC1 including Starch Glutamate-Croscarmellose Sodium had 94.81 % greater drug release compared to the marketed formulation. This investigation found that the novel superdisintegrant had the fastest disintegration and the highest drug release compared to other disintegrants.

KEYWORDS: Liquisolid Compact, Fast Disintegration, Dissolution Enhancement, Starch Glutamate, Carbamazepine, Neusilin.

1. INTRODUCTION:
To be absorbed from the gastrointestinal tract, the oral solid dose form must dissolve. Water-insoluble drugs have poor dissolving rates and absorption characteristics, which are major concern for the pharmaceutical industry [1]. Liquisolid compacts have recently emerged as a potential approach for enhancing the dissolution rate of poorly soluble drugs[2]. The notion of "liquisolid systems" as defined by Spireas et al. i.e Simple physical blending with selected excipients termed the carrier and coating material can be utilised to transform a liquid into a free flowing, readily compressible, and apparently dry powder[3].

The drug's wetting properties and surface area that is available for dissolution are considerably improved by the liquisolid compact. Water-insoluble compounds in liquisolid compacts are likely to have increased drug dissolution, resulting in improved bioavailability[4]. The liquisolid compact approach has been successfully used to improve the in vitro release of poorly soluble drugs such as indomethacin,[5] piroxicam,[6] griseoful¬vin,[7] ezetimibe,[8] repaglinide,[9] prednisolone,[1] etc. The liquisolid approach has been successfully used to improve the release of low dose, poorly soluble drugs. However, one of this technology's limitations is the conceptualization of a high-dose poorly soluble drug[10]. In order to enhance drug loading, the powder must retain high amount of liquid. However, this may result in poor flow and compression characteristics of the powder. A large amount of carrier and coating component should be used to maintain good flow and compression properties.

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As a result, increasing the capacity for liquid adsorption with carrier and coating component such as Neusilin could be a potential approach to loading a high dose of water insoluble drug. Neusilin US2 is an amorphous synthetic form of magnesium aluminometa silicate[11].

Carbamazepine (CBZ) has been used for over 40 years to treat epilepsy and trigeminal neuralgia. Carbamazepine is taken in doses of 100-200mg once or twice daily. It has a 72-96% oral bioavailability. It is practically insoluble in water. CBZ oral absorption in humans is slow, erratic, and unpredictable due to slow dissolution. One of the most major issues with this drug is its very low solubility in biological fluids, which results in poor bioavailability after oral administration. Many trials have been conducted in order to improve CBZ bioavailability. The use of water-soluble salts and polymorphic forms, the formation of water-soluble molecular complexes, Amorphisation of drug[12], Micronization[13], Solid dispersion[14], Co-grinding[15], Self-emulsifying drug delivery system[16], Nanosuspension[17], Hot melt extrusion[18], Adsorption of drugs to hydrophilic silica aerogels[19], Lyophilization, microencapsulation, and Inclusion Complexation[20,21] are some of the most important formulation tools. Apart from that, this method of liquisolid compact formulation is one of the method for increasing the rate of dissolution of poorly soluble drugs[22]. Superdisintegrants are the most common excipients used in tablet formulations to speed up disintegration in the gastrointestinal environment and thus increase active ingredient release. Disintegration is a critical step in drug release and absorption into the systemic circulation, resulting in pharmacological effects. However, the number of available superdisintegrants is still limited, necessitating the development of more efficient ones. The addition of Starch Glutamate, a hydrophilic amino acid, to the starch may improve its ability to disintegrate. The prepared formulation was characterised and compared to marked formulations (Mazetol)[23].

2. MATERIALS AND METHODS:

2.1. MATERIALS:

CBZ was received as gift sample from Abbott Healthcare Pvt. Ltd. Mumbai and chemicals were obtained from Loba Chem in Mumbai.

2.2. METHODS:

2.2.1. Synthesis of novel superdisintegrant:

To make a starch slurry, 10 parts potato starch were accurately weighed and dispersed in 25 parts distilled water. Weighing and dissolving 10 parts glutamic acid in distilled water, it was added to the starch slurry. The dispersion was conditioned for 16 hours after adjusting the pH to 3.5 with 10ml sodium hydroxide to complete the reaction between potato starch and glutamic acid. The dispersion was washed to remove unreacted glutamic acid after conditioning, and the solid mass was dried at 60°C to yield starch glutamate. To get consistent sized particles, the dried starch glutamate was sieved with a #120 sieve and kept in desiccators[24].

2.2.2. Characterization of novel superdisintegrant:

2.2.2.1. Fourier transformed infrared spectrometer (FTIR):

The molecular substitution of starch glutamate was evaluated using a Fourier Transform Infrared Spectrometer (FTIR). The IR spectrum of starch glutamate was measured using an ATR Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan, IRAFFINITY-1Miracal 10). A small amount of sample was taken and directly put on the ATR diamond. The sample was pressurized using a pressure arm. The spectrum was then scanned in the wavelength range of 4000-400cm^{-1} [23].

2.2.3. Solubility studies:

Saturation solubility studies in four different non-volatile solvents, namely PG (propylene glycol), PEG 200, PEG 400, and Tween 20, were needed to identify the appropriate non-volatile solvent for making liquid medication. Excess amount of carbamazepine was mixed separately with four non-volatile solvents. For 48 hours, the mixtures were shaken on an orbital shaker using the shake flask method. The solutions were then filtered through Whatman filter paper to obtain clear solutions. These filtered solutions were often diluted with 1% SLS (sodium lauryl sulphate) and their drug content was determined using UV spectrophotometry at 285 nm. To calculate carbamazepine solubility, three determinations were performed for each sample[22].

2.2.4. Determination Value:

The flow properties of powder excipients (Neusilin US2) in liquid vehicles were assessed using the "angle of slide" measurement. Several homogeneous liquid vehicle/powder admixtures containing 10 g of carrier or coating ingredients and increasing volumes of liquid vehicle were prepared (PEG 200). The created powder admixtures were placed on polished metal plates, which were gradually tilted until the powder admixture was about to slide, to measure the angle of slide. The angle of slide (ø) was used to describe the angle formed between the plate and the horizontal surface. The flow properties of excipients will be altered due to adsorption of the liquid vehicle. The flowable liquid-retention potential (ϕ-value) of each liquid/powder admixture was evaluated using the following equation.

Φ value = liquid weight/solid weight

The graph were plotted against ϕ -values versus the corresponding angle of slide (ø). The flowable liquid retention potential, ϕ -value, of its powder, which is required for the preparation of liquisolid tablets, was

represented by an angle of slide (for optimal flow properties) corresponding to 330 of a liquid/powder admixture. All measurements were taken in triplicate[25].

2.2.5. Liquisolid system preparation:

The amount of excipients is determined by their ϕ-values and liquid load factors. In the current research, neusilin was used as a carrier and coating material. The liquid load factor (Lf) is calculated using the formula below

Lf = ϕ + ø (1/R)…….... (1)

Lf =W/Q …….. (2)

R=Q/q ……. (3)

Where, Φ and ø are the values of the carrier and the coating powders respectively, while R is excipient ratio[1].

In PEG 200, CBZ was suspended, as indicated in the table no. 3, and a total of 12 batches were formulated, as indicated in the Table No.1.

2.2.5.1. Preparation of CBZ liquisolid compact:

The carbamazepine, carrier and coating material, and other excipients in a liquisolid powder mixture were immediately compacted on a single punch tablet machine to yield tablets with the specified diameter, thickness, and hardness[22].

Table No.1 Formulation of Liquisolid Compact of Carbamazepine

Name of superdisintegrant	Batch code	%CD	LF (mg)	R (mg)	W (mg)	Q (mg)	q (mg)	Superdisintegrnat (mg) 3%,5%, 7%	Total Weight (mg)
Crospovidone	NCP1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NCP2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NCP3	50%	1	20	200	200	10	7%-35.7	545.7mg
Crosscarmallose Sodium	NCS1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NCS2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NCS3	50%	1	20	200	200	10	7%-35.7	545.7mg
Starch Glutamate	NSG1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NSG2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NSG3	50%	1	20	200	200	10	7%-35.7	545.7mg
Starch Glutamate+ Crosscarmallose sodium	NSC1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NSC2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NSC3	50%	1	20	200	200	10	7%-35.7	545.7mg

% Cd= drug Concentration in non-volatile solvent, Lf=liquid load factor,
R= Excipient ratio, W= weight of non-volatile solvent, Q= Carrier, q= coating material.

2.2.6. Liquisolid Compact Evaluation:

2.2.6.1. Tablets' physical parameters:

In triplicate, tablets were tested for weight variation, uniformity of tablet thickness and diameter, friability, and hardness[26; 27].

2.2.6.2. Drug content:

The uniformity of drug content was determined as per IP 1996. The tablets were weighed and powered, and 100mg of drug powder was weighed and transferred to a 100ml volumetric flask containing 60ml of ethanol (95%). To dissolve the drug, the flask was shaken, and the volume was adjusted with ethanol. By using ethanol, 10mL of this solution was diluted to 100mL, and the absorbance of resulting solution at λmax of 285nm was measured [28].

2.2.6.3. Disintegration test:

In a suitable vessel, preferably a 1000ml beaker, the assembly was submerged in liquid medium (ED-2L, Electrolab, Mumbai). The liquid volume must be such that the wire mesh is at least 25 mm below the liquid's surface and at least 25mm above the bottom of the beaker at its highest point. A thermostatic arrangement was made for heating the liquid and maintaining the temperature at 37±2℃. The assembly was submerged in a beaker containing 900ml of distilled water, and the apparatus was operate for the duration specified. The tablet's disintegration time was also recorded. Finally, the assembly was taken out from the liquid[28].

2.2.6.4. Dissolution studies:

The dissolution test was used to compare carbamazepine release from liquisolid tablets and mazetol, a marketed tablet. The USP Apparatus 2 (Electrolab, TDT-06L) was used in conjunction with 900ml of 1% sodium lauryl sulphate solution (1 % SLS) at 37±0.5℃, and rotated at 75rpm. After the specified time intervals, a one millilitre sample was withdrawn, and the sink condition was maintained. The samples were filtered, diluted appropriately, and spectrophotometrically analysed at 285 nm wavelength[29].

2.2.6.5. IR- spectroscopy (FTIR):

IR spectrum of Carbamazepine, PEG200, NeusilinUS2 and optimized formulations NSC1 were recorded using an ATR Fourier Transform Infrared Spectrophotometer (MIRacle 10)[22].

2.2.6.6. Statistical analysis:

To determine whether there was a notable difference in the time required for 100% release of carbamazepine from different formulations and the marketed tablet, a one-way ANOVA with Turkey's multi comparison test was used.

3. RESULT AND DISCUSSION:

3.1. FTIR of carbamazepine

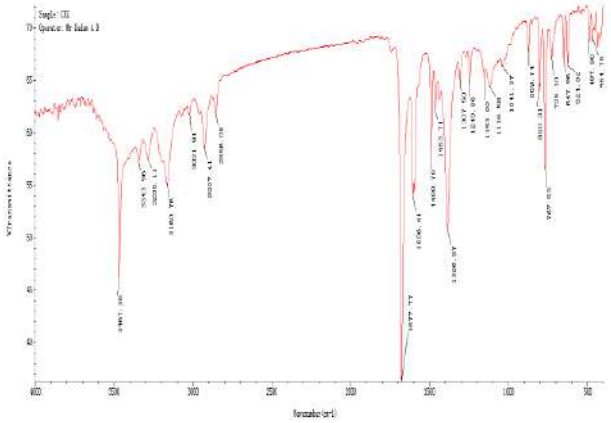

Fig No.1 FTIR Spectrum of Carbamazepine

The FTIR spectra of CBZ showed a characteristic peak at 3467.38 cm^{-1} (-NH_2 vibration), 1677.77cm^{-1} (-C═O vibration), 1606.41cm^{-1} (-C═C vibration).

3.2. Characterization of novel superdisintegrant:

3.2.1. FTIR:

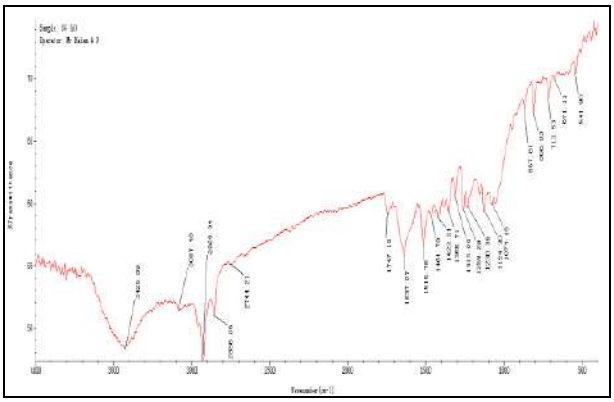

Fig.No.2 FTIR of Starch Glutamate

The FTIR spectrum of starch glutamate revealed a distinct peak at 1637.27cm^{-1} (-R-COO-R′ vibration).

3.3. Solubility studies:

Solubility of carbamazepine in propylene glycol, PEG 200, PEG 400, glycerine and Tween 20 is given in table no.2. Carbamazepine was most soluble in PEG 200 (107.94 mg/ml) and least soluble in Tween 20 (6.84 mg/ml). This is due to the dispersion of a larger fraction of drug in PEG 200, which helps to enhance drug dissolution.

Table No.2 Solubility Data of Carbamazepine

Nonvolatile Solvent	Solubility (mg/ml)
Propylene Glycol	45.10±0.16
PEG 400	68.12±0.29
PEG 200	107.94±0.62
Tween 20	6.84±0.11

3.4. Determination of (ϕ) value:

Relation between angle of slide of carrier and coating material in PEG 200 and corresponding ϕ values is depicted in Fig no.3.

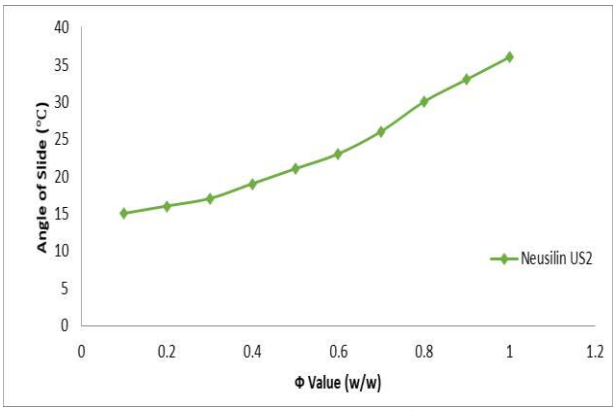

Fig No.3 Liquid Retentions Potential (Φ) of Carrier and Coating Material

3.5. Liquisolid compact evaluation:

3.5.1. Physical parameters of liquisolid compact:

All the physical parameters of liquisolid compact are shown in table no 3. Liquisolid compact containing Neusilin as carrier and coating component showed good compatability, due to its high specific surface area and porosity. Thickness of liquisolid compacts were ranged from 5.33±0.02 to 5.4±0.06557mm and diameter of all the liquisolid compacts was to be in the range of 10±0 to 10.03±0.05744mm as indicated in table no 3. Thickness and diameter of tablet measured by using Vernier caliper.

Tablet hardness test were measured using Monsanto Hardness tester Hardness of tablets was found to be in the range of 3.133±0.05774kg to 3.177±0.04933kg as shown in table no 4.

Due to identical compression force, uniform hardness was achieved.

Tablets were prepared using direct compression method. Because the material was free flowing, uniform weight tablets were obtained as a result of uniform die fill. Tablets were obtained in the 10% acceptable weight variation range as specified by Pharmacopeia. The results are summarizes in table no 3.

Friability of liquisolid compact found to be 0.5129±0.0090 to 0.6003±0.01682% indicated in table no 3. As stated by USP if conventional compressed tablets that loss less than 0.5% to 1% of their weight is generally regarded as acceptable.

Table No. 3 Physical Parameters of Carbamazepine Liquisolid Compact

Formulation Code	Thickness (mm)	Diameter (mm)	Hardness (kg)	Weight Variation (mg)	Friability %
NCP1	5.4±0.06557	10.03±0.05744	3.17±0.0435	524.9±0.5967	0.5335±0.0205
NCP2	5.35±0.03512	10.03±0.05744	3.133±0.05774	533.8±0.4404	0.5129±0.0090
NCP3	5.4±0.01	10.03±0.05744	3.143±0.0589	544.1±0.4318	0.5458±0.0194
NCS1	5.37±0.03	10±0	3.17±0.0435	574.8±0.4894	0.5591±0.02171
NCS2	5.393±0.02082	10.03±0.05744	3.133±0.05774	587.9±0.3426	0.5716±0.0177
NCS3	5.34±0.02646	10±0	3.177±0.04933	600.5±0.4894	0.5514±0.01322
NSG1	5.383±0.01528	10±0	3.173±0.04619	523.9±0.4286	0.5877±0.02307
NSG2	5.383±0.02517	10±0	3.177±0.04933	534.2±0.3712	0.6003±0.01682
NSG3	5.33±0.02	10.03±0.057444	3.173±0.04619	543.2±0.3401	0.5919±0.02657
NSC1	5.427±0.07024	10.03±0.05744	3.2±0.1	577.4±0.9787	0.5312±0.01649
NSC2	5.367±0.04041	10.03±0.05744	3.177±0.06807	587±0.4648	0.5244±0.0203
NSC3	5.37±0.0435	10.03±0.05744	3.15±0.05196	600.6±0.4686	0.553±0.02453

All values are expressed as mean ± SD (n=3).

3.6. Disintegration time:

Neusilin-crosspovidone batches were failed to disintegrate. Batch NSC1 shows fast disintegration i.e. 42.5±0.5774sec. Starch Glutamate batches shows fast disintegration as compare to crosspovidone batches. Disintegration time of liquisolid compact tablets is given in table no 5 and complies as per IP specifications for all formulated batches except formulations containing Neusilin-Neusilin crosspovidone and these batches were failed to disintegrate. Crosspovidone fails to disintegrate Neusilin-Neusilin compact, for disintegration of this compact addition of 10% fujicalin necessary but it shows more disintegration time. Novel superdisintegrant starch glutamate successfully disintegrate Neusilin-Neusilin compact. Hence Liquisolid compact of Neusilin-Neusilin-starch glutamate-coroscarmellose sodium exhibited fast disintegration.

3.7. Drug content:

The requirement for a steady dose of drug between individual tablets is an essential quality attribute for all pharmaceutical formulations. Uniform drug content was observed for all the formulations given in table no 4. Which is as per the IP specification.

Table No 4. Evaluation of Carbamazepine Liquisolid Formulations

Formulation code	Disintegration Time* (sec.)	% Drug Content
NCP1	No Disintegration	80.81±0.4494
NCP2	No Disintegration	79.87±0.2728
NCP3	No Disintegration	81.3±0.7435
NCS1	91.2±0.05774	94.23±0.4494
NCS2	85.3±0.05774	94.46±0.7784
NCS3	85.2±0.05774	95.11±0.6438
NSG1	71.4±0.05774	95.35±1.61
NSG2	74.4±0.05774	95.35±0.7435
NSG3	78.3±0.1528	96.8±1.189
NSC1	42.5±0.5774	98.8±1.189
NSC2	63.7±0.1	97.04±0.3717
NSC3	60.7±1.155	97.23±0.4494

All data is presented as mean ± SD (n=3).

3.8. Dissolution studies:

The results of in vitro percentage amount of drugs are released at varied intervals of time which is plotted against time to obtain the release profiles and are given in fig no 4.

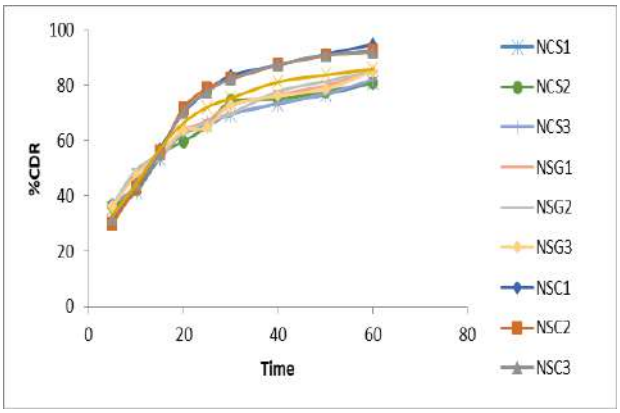

Fig No 4. (A) Dissolution profiles of all batches of liquisolid compact and marketed formulation

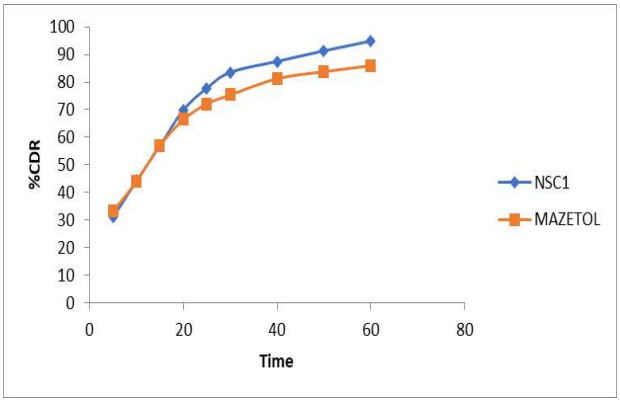

Fig No 4. (B) Dissolution profiles of optimized batches of liquisolid compact and marketed Formulation

Neusilin-crosspovidone showed drug release 80.78±0.5225 to 82.27±1.7121% at the end of 60 minutes. Neusilin-Starch Glutamate, Croscarmellose sodium batch showed drug release 92.27±0.460 to 94.81±0.201% at the end of 60 minutes (shown in table no. 5). Formulations prepared with a novel superdisintegrant demonstrated higher drug release than crosspovidone batches and marketed tablets. As the concentration of crosspovidone increases drug release also increases. Crosspovidone disintegrant failed to

disintegrate liquisolid compact, but a novel superdisintegrant did, and that batch had an 85% drug release rate. Starch Glutamate showed greater drug release than the marketed formulation. At the end of 60 minutes, the marketed formulation had a drug release rate of 86±0.190%. In Neusilin-Starch glutamate, Croscarmellose sodium batches NSC1 batch shows marked increase drug release than other two NSC2, NSC3 batches.

The fact that the novel superdisintegrant and the drug are already in PEG 200 while being carried by the powder particles may account for the increased dissolve rates of liquisolid compacts when compared to marketed tablet. As a result of the quick disintegration and increased wettability and surface availability to the dissolution liquid, its release is expedited. One of the hypothesised methods for explaining the increased dissolving rate from liquisolid compacts is the compacts' wettability by the dissolution media. PEG reduces the interfacial tension between the dissolution media and the tablet surface, allowing drug particles to wet more easily.

Table No 5. Percentage Amount of Drug Release of Liquisolid Compact

Time	%CDR									
(Min.)	NCS1	NCS2	NCS3	NSG1	NSG2	NSG3	NSC1	NSC2	NSC3	Mazetol
5	35.42± 0.4996	36.58± 0.999	37.1± 0.556	35.54± 0.999	36.63± 0.0999	36.17± 0.995	31.15± 0.915	30.06± 0.993	31.85± 0.1731	33.17± 1.130
10	41.44± 1.044	42.32± 0.875	43.66± 1.117	48.93± 1.964	49.51± 0.9591	47.25± 0.996	44.26± 0.807	42.92± 0.870	44.38± 0.9216	44.16± 0.345
15	53.44± 1.096	54.9± 0.979	54.85± 0.891	53.49± 1.088	55.11± 1.271	55.56± 0.991	57.07± 0.466	55.85± 1.723	55.63± 1.947	57.08± 0.523
20	62.39± 0.096	59.87± 0.8834	63.34± 0.2567	63.42± 1.362	62.97± 0.997	63.88± 0.697	69.96± 0.236	71.85± 0.6351	70.71± 2.058	66.45± 0.156
25	65.33± 1.467	65.34± 0.4066	66.81± 0.2581	67.17± 0.8298	66.43± 0.956	64.87± 0.984	77.79± 0.343	79.05± 0.113	77.67± 1.714	72.13± 0.347
30	69.38± 0.2671	74.23± 0.428	69.83± 0.177	72.78± 0.3209	69.74±± 0.956	72.72± 0.986	83.52± 0.550	82.42± 0.965	82.6± 1.144	75.41± 0.583
40	73.28± 0.1788	75.21± 0.2835	73.73± 0.33646	76.53± 0.9379	77.67± 0.247	76.41± 0.219	87.5± 0.454	87.55± 0.368	87.67± 0.5245	81.3± 0.762
50	76.56± 0.09445	77.93± 0.9728	77.07± 0.2701	79.83± 0.4464	81.44± 0.134	78.72± 0.264	91.32± 0.527	90.96± 0.5372	91.14± 1.364	83.87± 0.206
60	80.78± 0.5225	81.12± 0.6438	82.27± 1.7121	85.04± 0.8721	85.08± 0.974	85.32± 0.969	94.81± 0.201	92.67± 0.1041	92.27± 0.460	86± 0.190

All values expressed as mean ± SD (n=3).

3.9. IR- spectroscopy:

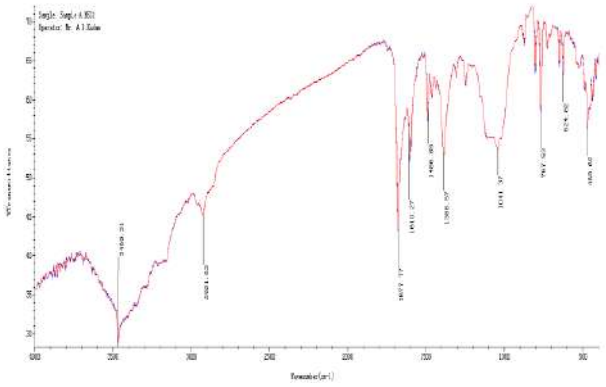

Fig No.5 FTIR Spectrum of Optimized Formulation

The continuous several range of CBZ 3470- 1600cm^{-1} and 1619.29cm^{-1} represent the ammonia and ester group respectively present in the batch NSC1.

However, comparison of the spectra demonstrated no new characteristic peaks in the liquisolid compact formulation which indicated no physical or chemical interaction between CBZ and Starch Glutamate are given in Fig No. 5.

3.10. Statistical analysis:

There was no significant difference (P< 0.05) between the release profiles of the marketed tablet and liquisolid compacts, according to the results of a one-way ANOVA with Turkey's multi comparison test.

4. CONCLUSION:

The results indicated that, liquisolid compacts of CBZ can be prepared using a novel superdisintegrant such as Starch Glutamate. To ensure the safety of newly developed superdisintegrants, the base of synthesis was an endogen amino acid (Glutamic acid), and starch was successfully derivatized with Glutamic acid. The comparison of Starch Glutamate, Croscarmellose Sodium, and Crosspovidone-made tablets revealed that starch Glutamate and Croscarmellose Sodium-made tablets had better disintegration and dissolution behaviours than the others. It was necessary to pick superdisintegrants to maximise drug dissolving in a time when formulators were confronted with a growing number of poorly soluble drugs. The effects of Starch Glutamate, Croscarmellose Sodium, and Crosspovidone on the dissolution rates of poorly soluble drugs were investigated, and it was discovered that Starch Glutamate, Croscarmellose Sodium has the fastest

dissolving rate. The drug release of a Liquisolid compact formulation with Starch Glutamate superdisintegrant was higher than that of other disintegrants and marketed tablets. This study came to the conclusion that Starch Glutamate might be used as a new superdisintegrant in the pharmaceutical industries.

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DEGRADATION STUDY OF DIFFERENT BRANDS OF ANTIPYRETIC TABLETS BY UV SPECTROSCOPY

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ABSTRACT

In the present project forced degradation study of different brands of Paracetamol Tablets were performed. Forced degradation of drug substance was performed by exposing it to Acid, Base, and UV light. The amount of degradation product can be determined with the help of UV spectrophotometer. Forced degradation of drug substance of Pacimol, Pyrigesic and Calpol were observed. In the result negligible difference was observed on exposure to UV. This method can be used successfully for studying the stress degradation factors. Because this method is less time consuming and simple and cost effective. Three different Brands that is Pacimol, Pyrigesic and Calpol of Paracetamol Tablets were used for the study. It was found that very negligible degradation was occurred.

KEYWORDS: UV spectroscopy, Paracetamol, UV Cabinate, Acid Degradation, Base Degradation.

INTRODUCTION

One of the most common symptoms is pain and this is one of the most frequent reasons why people seek medical care. Therefore, it is not surprising that the analgesics are among the most widely used categories of drug. Hence, for the treatment of inflammation and pain Paracetamol is used. Chemically Paracetamol is 4-hydroxyacetanilide. Paracetamol is a weak peripheral cyclooxygenase inhibitor and from the inhibition of prostanoid synthesis in the central nervous system, analgesic effect of Paracetamol may arise. Antipyretic effect of Paracetamol is reported to inhibit prostaglandin synthesis at the level of the hypothalamus causing alteration in body temperature.[1]

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum. When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. In many laboratories, spectrophotometric method was used due to less equipment cost and economical maintenance advantages. By the help of this technique, the UV absorbance spectra are measured at 200–400 nm. In accordance with the International Conference on Harmonization guideline, the force degradation state of active pharmaceutical substance includes acidic, basic and photolytic conditions. We already performed these types of degradation studies which are useful for pharmacy profession. Basic parameters for drug degradation studies are acid/base stress testing, humidity and with temperature, photo degradation. Forced degradation of drug was performed with acidic, basic and Uv light condition. Forced degradation of drug substance in UV light was performed by exposing the drug substance to UV light.[2]

MATERIALS

For the present project work different paracetamol tablets brands were used such as Calpol 500 mg tablets of GlaxoSmithKline Pharmaceutical Limited, Pyrigesic 500 mg tablets of East India Pharmaceutical Works Limited, Pacimol 500 mg tablets of Ipca Laboratories Limited and Febrex Indoco Remedies Limited. 1M NaOH, 1M HCL, reference standard Paracetamol, Pyrex type stirrer, measuring cylinder, pipette, funnel, beaker and volumetric flask, petri dish, cuvettes, butter paper, Whatman filter paper No. 44, spatula, tissue paper were used. Freshly laboratory prepared distilled water was used to wash glasswares. Weighing Balance, Shimadzu UV spectrophotometer.

METHOD

Preparation of 1mole/liter NaOH

In 100 ml volumetric flask, accurately 4 g NaOH was dissolved and to make up the volume up to 100 ml, deionized water was added.[3]

Preparation of 1 mole/liter HCl

A total of 8.36 ml hydrochloric acid (37% 12 mol/L) was took accurately analytical grade in 100 ml volumetric flask to make up the volume up to 100 ml by adding deionized water. [4]

Preparation of paracetamol stock solution (API)

1. Weigh accurately 100 mg of Paracetamol IP pure powder and add 15 ml of 0.1 N NaOH and dilute up to 100 ml with distilled water (1000 ug /ml).
2. Prepare 6 standard dilutions from above solutions by diluting 0.5 ml, 1 ml, 1.5 ml, 2 ml 2.5 ml, 3 ml to 100 ml with distilled water.
3. Run these 6 standard dilutions of 5, 10, 15, 20, 25 and 30 ug/ml concentration for absorbance against blank. Plot calibration curve between absorbance versus concentration at 257 nm.
4. Find out equation of line i.e Y= mx+c. [5]

Preparation of Tablet (Test) solution

Weigh the 10 tablets of different brand of Paracetamol with the help of clean and dry mortar and pestle Calculate average weight of powder. Take Powder was equal to 20 mg of Paracetamol of each brands. Paracetamol powder which is equal to Calpol (25.44mg), Pacimol (22.68mg), Pyrigesic (22.72) were accurately weighed. In the 100 ml volumetric flask, all of 3 brands powders transferred individually. These powder samples were dissolved and shaked with water and finally make up the volume up to 100 ml respectively for each sample. A total of 20 mg/100 ml concentration solution was preferably obtained. By using spectrophotometer at 257 nm wavelength individually all brands absorbance were determined.[6-10]

Procedure for forced degradation studies

1. For acid

Forced degradation of drug substance in acidic media was performed by taking 5 ml of 20 mg/100 ml of Pyrigesic, Pacimol and Calpol in 3 separated testtubes, then 5 ml of 1 mol/L HCl was added in each test tube. The sample was left for 30 min. Solution was transferred to a separated cuvette after the time period completion and UV absorbance of the solution was measured at the 257 nm wavelength.[6-10]

2. For base

Forced degradation of drug substance in basic media was performed by taking 5 ml of 20 mg/100 ml solution of Pyrigesic, Pacimol and Calpol in 3 separated test tubes, then 5 ml of NaoH was added in each test tube and the sample was left for 30 min, and then UV absorbance of solution was measured at 257 nm wavelength.[6-10]

3. For UV light

Forced degradation of drug substance in UV light was performed by taking the 5 ml of 20 mg/100 ml solution of Pyrigesic, Pacimol and Calpol, then 5 ml of water was added in each test tube and these test tubes were exposed to UV light for 30 min, and then UV absorbance of solution was measured at 257 nm wavelength.[6-10]

RESULT

Preparation of callibration curve

Table 1: Absorbance of standard paracetamol solution.

Sr. no.	Standard Concentration	Absorbance
1	0	0
2	5	0.0600
3	10	0.1204
4	15	0.1779
5	20	0.2105
6	25	0.3155
7	30	0.3685

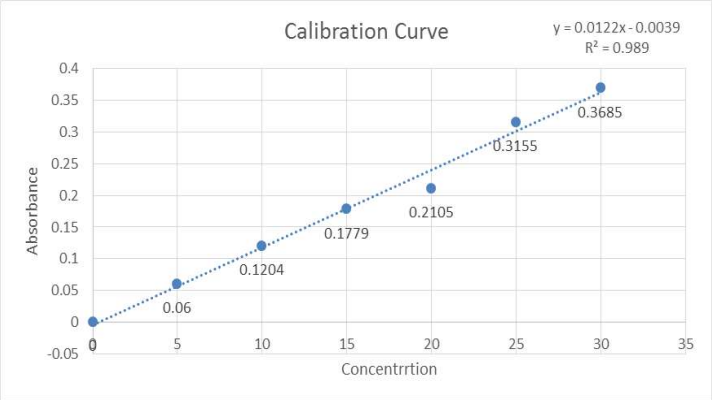

Fig. no. 1: Callibration curve of standard paracetamol solution.

Forced degradation studies

The degradation study was conducted on three brand of Paracetamol which are Pyrigesic 500 mg tablets of East India Pharmaceutical Limited, Pacimol 500 mg tablets of IPCA Laboratories Limited and Calpol 500 mg tablet of Glaxosmithkline Pharmaceutical Limited.

When Paracetamol brands were treated with the 1 mol/L HCL, it showed small degradation. When Paracetamol brands were treated with the 1 mol/L NaOH drugs, it showed degradation. When exposed to UV light showed more degradation. Table 1 represents the UV absorption of different brands of the Paracetamol before and after exposing to the degradation environment. We concluded according to our results that when the Calpol introduced into acidic medium 1 mol/L HCL, it showed degradation that is (78.11%) Pacimol showed degradation in acidic medium that is (71.71%) Pyrigesic also gave greater results on exposure to acidic medium (70.63%) respectively. Similarly on exposure to 1 mol/L NaOH basic medium, the Calpol showed the (82.91%) degradation whereas Pacimol showed degradation to minor extension that is (74.62%) while Pyrigesic gave moderate results on exposure to basic medium (69.36%) respectively. When Calpol (52.83%), Pacimol (59.51%) and Pyrigesic (61.98%) exposed to UV light for 30 min and evaluated for degradation studies, it also showed minor changes in concentration respectively for degradation studies. Results of degradation studies are given in Tables 2.

Table 2: Absorbance of different brand of paracetamol.

Tablet	Treatment	1	2	3	Average	Percentage
Calpol	Before	2.4436	2.4436	2.4559	2.4477	84.93%
	Acid Treatment	2.2518	2.2924	2.3098	2.2846	78.11%
	Base Treatment	2.3872	2.3872	2.3979	2.3907	82.90%
	UV Treatment	1.7423	1.7423	1.7328	1.549	52.83%
Pacimol	Before	2.2291	2.2441	2.2291	2.2341	77.30%
	Acid Treatment	2.0555	2.0861	2.0911	2.0775	71.71%
	Base Treatment	2.1549	2.1611	2.1611	2.1590	74.62%
	UV Treatment	1.7328	1.7328	1.7423	1.7359	59.51%
Pyrigesic	Before	2.0705	2.0915	2.0915	2.0845	71.96%
	Acid Treatment	2.0362	2.0457	2.0604	2.0474	70.63%
	Base Treatment	2.0087	2.0087	2.0177	20117	69.36%
	UV Treatment	1.8124	1.8013	1.8013	1.8005	61.98%

DISCUSSION

In the present project work all the brands of Paracetamol were exposed to different degradation parameters, there was small degradation in the active ingredient of the brands of Paracetamol. The brands i.e. Calpol, Pacimol and Pyrigesic when they come in contact with different degradation parameters (before, acid, base, and UV) showed degradation of drug substance. According to specification of United State Pharmacopoeia, the content official limit of not less than (98%) and not more than (101%) the labeled amount. We have

concluded from our studies that Paracetamol less degrades in acidic and basic medium as compared to UV light treatment.

CONCLUSION

In the present project degradation study was performed as per ICH guideline. Paracetamol tablets were expose at different condition like Acid, Base and UV light and it was found that some amount of degradation was occurred at different conditions. In Calpol tablet degradation study it showed degradation in UV treatment more prominent. Similarly Pacimol and Pyrigesic also showed more degradation in UV treatment as compared to Acid and Base treatment.

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CLEANING VALIDATION OF TABLET COMPRESSION MACHINE BY SWAB SAMPLING

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ABSTRACT

Validation of cleaning technique using swab sampling and application in determining residual L-ascorbic acid in production area equipment, as well as confirmation of cleaning procedure efficiency. After producing L-ascorbic acid 10 mg chewable tablets, the swab sampling and UV method for residual determination of in L-ascorbic acid swab samples from equipment surfaces were established and verified. For L-Ascorbic Acid in tablet form, a unique, safe, and sensitive method of spectrophotometric measurement in the UV region has been devised. Water as diluents were used to develop and validate the technique for L- Ascorbic Acid.

KEYWORDS: Cleaning Validation, Linearity, Swab, Absorbance, UV Spectroscopy, L-Ascorbic Acid.

INTRODUCTION

Validation is documented evidence that a planned procedure will function consistently according to the previously stated results. Process validation is defined by the quality systems regulation as establishing, by objective evidence, that a process consistently generates a result or product that meets its pre-determined specifications. A quality system's purpose is to generate products that are suitable for their intended usage on a consistent basis.

Cleaning validation is proof that an approved cleaning operation will result in equipment that is suitable for the processing of pharmaceutical products. Cleaning pharmaceutical equipment is becoming increasingly important in terms of regulatory compliance. To evaluate a cleaning procedure, a sample of the product contact surfaces of the equipment must be

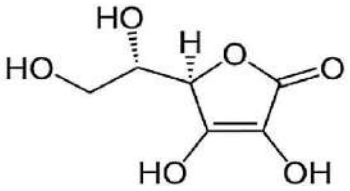
taken to determine the level of residuals present. Swab sampling, rinse sampling, coupon sampling, placebo sampling, solvent sampling, and product sampling are the six sampling procedures. It's critical to use the right procedure for detecting residue in the cleaning sample. The test method used to validate the cleaning procedure should be able to precisely quantify the concentration of any substances of interest that may be present in the sample. UV spectroscopy, HPLC, GC, HPTLC, atomic absorption spectroscopy, fluorimetry, and simple photometry are some of the specialised analytical procedures utilised for cleaning validation. Visual examination, gravimetric analysis, pH, conductivity, microscopy, titration, and the total organic carbon method are examples of non-specific analytical procedures often used for cleaning verification. The accuracy, precision, linearity, specificity, range, and LOQ/LOD would all be included in the method validation SOP. The scientific basis for cleaning validation is usually mentioned in the protocol's limit section. The scientific rationale for the real restriction should be logical, thorough, and simple to comprehend.

Fibrous material is used in the swab sampling method to swab a surface in order to collect samples. Direct surface sampling method is another name for the sample technique. Textiles that are woven or nonwoven and have a plastic handle make up the fibre component of swabs. This approach involves pre-wetting a swab (i.e., a fibrous substance) with a suitable solvent before sampling a residue that is soluble in that solvent. Prior to sampling, the edges of the swab head are typically squeezed against the walls of the vial or test tube to eliminate excess solvent. This is important because too much solvent may leave interesting extractable materials on the surface, acting as a source of residues and producing inconsistent findings. A suitable extraction solvent is used to extract the analyte that needs to be analysed and measured from the swab head after sampling has been completed across a predetermined area. The extracted solvent may be the same as or different from the solvent used to moisten the cotton swab.

Swab sample recovery should be regarded as a serious concern since studies on swab recovery show that if a residue is present on an equipment surface, it may be accurately assessed and quantified by using analytical methods and sampling techniques. Developing a consistent level of recovery from the equipment surface is the goal of the scientific approach known as a recovery study. It should be demonstrated that recovery is feasible from all product contact materials sampled in the apparatus using all the sampling techniques. The size, shape, and characteristics of the swab head, as well as the characteristics of the swab

handle (such as flexibility and length), all affect the recovery of residues from surfaces. Sampling recovery studies are lab experiments using coupons of equipment made of various construction materials (such as stainless steel, glass, PTFE, and EPDM) that have been sampled and have been contaminated with residues to be analysed. The normal range of acceptable variation for recovery outcomes at a single spiking level is between 15% and 30% RSD. One would prefer to recover 100% of the challenge, however depending on the sample conditions and the residue, recoveries may only be restricted to 75% to 80%.[1,2]

L- Ascorbic Acid



Molecular formula	-	$C_6H_8O_6$
Molecular Weight	-	176.12
IUPAC Name	-	(5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one
Appearance	-	White or light-yellow crystalline powder
Melting Point	-	190^0 C
Boiling Point	-	553^0 C
Soluble in	-	Water, Glycerol, Ethanol, Polypropylene glycol

Vitamin C is another name for it. L-ascorbic acid is a water-soluble vitamin that can be found in citrus and other fruits and vegetables, as well as being purchased as a dietary supplement. Scurvy is a disease that can be prevented and treated with this supplement. L-ascorbic acid is a vitamin that helps with tissue healing, collagen creation, and the enzymatic manufacture of some neurotransmitters.

Ascorbic acid is a water-soluble vitamin that is found in nature (Vitamin C). Ascorbic acid is a powerful reducing and antioxidant that aids in the battle against bacterial infections, detoxification, and collagen production in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries. Vitamin C is found in citrus fruits and vegetables and cannot be synthesised or stored by humans, thus it must be taken through the diet.

L-ascorbic acid is a crystalline powder that is white to pale yellow in colour and has a nice sharp acidic taste. It is almost odourless.[3,4]

❖ MATERIALS AND METHODS

L- Ascorbic acid (Reference std.) by S. D. Lab, Distilled water by Distillation Unit of Equitron, Texwipe swab sticks by Somya Digital Technologies New Delhi, Tablet Compression Machine supplied by Nikhil's Scientifics, UV Spectrophotometer (Systronics).[1,4]

❖ Selection of Analytical Performance Characteristics

The analytical performance criteria listed below were chosen for use in analytical technique validation for cleaning swab samples: Maximum detection, Blank swab interference analysis, Linearity, and Range, Precision, and LOD and LOQ, as well as drug recovery from spiked SS plates (accuracy).[4,5]

❖ ANALYTICAL METHOD VALIDATION

1. Detection of λ Maxima for L- Ascorbic acid

Weighed accurately 100mg of working standard and transferred into 100 ml volumetric flask and made up the volume with water to 100 ml. 1 ml of above solution was taken in another 100 ml volumetric flask and made up the volume to 100 ml with water (10ppm).

2. Blank Swab Interference Analysis

Blank swab solution

6 Texwipe swabs were placed in various stopper test tubes with 100 ml water. After 2 minutes of sonication, the swab was withdrawn from the sample solution. A homogeneous solution was prepared. By scanning a 10 ppm standard solution, the absorbance of the standard solution, blank solution, and blank swab solution was determined.

The purpose of this investigation was to see if the swab and cleaning agent interfered with the absorbance of L- Ascorbic acid at the maxima.[1,4]

3. Linearity

Prepared the test solution using L- Ascorbic acid working standard at concentration level 2, 4, 6, 8 and 10 ppm. Measured the absorbance of all solutions at determined maximum

wavelength in duplicate. Plot the graph between absorbance (y-ordinate) and concentration (x-abscissa) and determined the regression and correlation coefficient (r^2).[1,5,6]

4. Precision

Repeatability of Measure of Absorbance

To check repeatability, measurement of absorbance 10 ppm solution of drug was measured 6 times at λ max and % RSD was calculated.[4,5]

- **Reproducibility of Measurement of Absorbance**

The absorbance of different concentrations was measured in three replicates to ensure repeatability.

- **Intraday analysis**

Intraday analysis was determined by analyzing the 5 drug concentration in triplicate at same day and % RSD was calculated. In this study 2,4,6,8 and10 ppm solution was prepared in triplicate form and the absorbance was determined at λ max of drug.[1,5,6]

❖ **Interday Analysis**

Inter day precision is determined by analyzing drug daily for three days.[7,8]

5. Recovery of drug from Spiked SS Plates

Determined the recovery of the method by applying the method to SS plates to which known amount of analyte (L- Ascorbic acid) has been added.

Spiking with solution of 50, 100 and 200 ppm

Spiked uniformly three 5 X 5 cm^2 three separate SS plate (316 SS grade) with 400 µl solution of 50, 100 and 200 ppm standard solutions respectively with the help of a micro pipette and allowed the surfaces to dry. This procedure was performed in triplicate.

Test solutions

Took out the swabs from stoppered test tubes and squeezed the excess swabbing solvent by pressing it with the wall of the test tube. Swabbed the dried spiked plate as per procedure of sampling of swab on equipment (vertically and horizontally). Using single swab from one spiked location (plate). Placed this spiked swab in stopper test tube containing 10 ml of water and sonicated the test tube for about 10 min. to extract the drug in solution. Swabbed at 9 plates (3 concentrations in triplicate). Measured the absorbance of each of the swab test

solutions at determined λmax. Compared all these swabbed solutions of 50, 100 and 200 ppm with standard solution of 2, 5, 10 ppm and % recovery should not be less than 80 %. A recovery of >80% is considered good, >50% reasonable and, <50% questionable. The amount of drug found in test solution was calculated by determining concentration of drug in test solution.[8,9]

6. Limit of Detection

Preparation of standard solution (10 ppm)

Took 100mg of drug (working standard) to 100 ml volumetric flask and made up to 100 ml with water. 1 ml of this solution was taken in 100 ml volumetric flask and made up 100 ml with water. From stock solution prepared 100 ml each of 0.1, 0.2, 0.3, 0.4, 0.5 ppm by suitable dilutions.[10]

Using the following formula, calculate the LOD using the absorbance of all of the above concentrations:

$$LOD = 3.3\ \sigma / S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

7. Limit of Quantitation

Determined the concentration at which the analytical method can quantify the analyte using the absorbance obtained from the Limit of Detection. The following formula can be used to determine LOQ.[10,11]

$$LOD = 10\sigma/S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

➢ **TABLET COMPRESSION MACHINE**

❖ **Selection of swab sampling points of equipments**

Following are the swab sampling point for the cleaning validation

A. Granulation Area: Vibro Sifter, Multimill, Rapid Mixer Granulator, Fluidised Bed Drier, Bin Blender, IPC, Tipper

B. Compression Area: Compression Machine, Deduster (LHS), Metal detector (LHS), Deduster (RHS), Metal detector (RHS)

C. Inpection Area: Tablet capsule sorter,

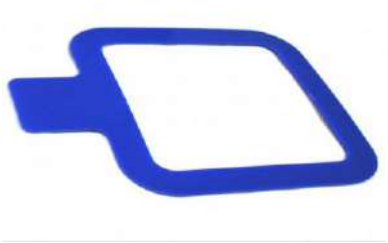
D. Coating Area: Auto coater, Roll Compactor

F. Packaging Area: Blister Packing Machine[1,4,12]

❖ Procedure of swab sampling from the equipment

Swab samples were collected from the different locations of the equipments

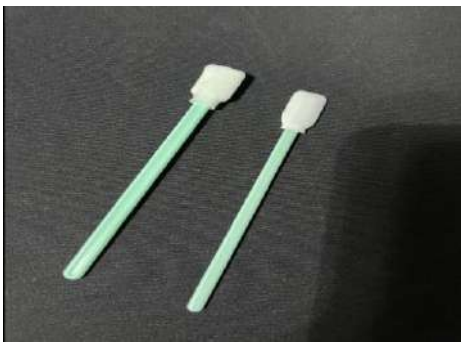
- Swab samples was done in the following manner Sampling area = 5 X 5 cm = 25 cm2.



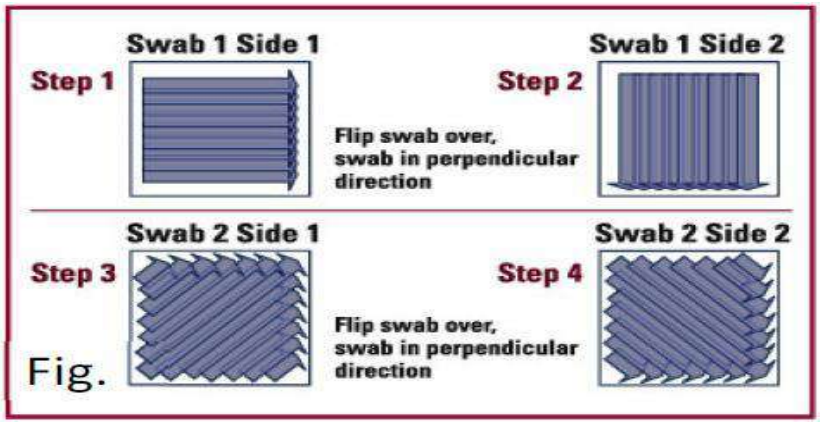
5×5 cm^2 Sterile Plastic Swabbing Template

❖ Sampling patterns

As illustrated in the diagram, wiped the defined area in both directions. Applied only one time. The surface was not rubbed in to and forward movement. Swabbed the specified area and stored in a test tube containing 10 ml of water then stoppered the test tube. This sample was then analyzed by the UV spectrometer.[4,13]



Swab Sticks



Sampling Pattern

❖ **Procedure for Analysis of the Samples**

Preparation of standard solution (10 ppm)

About 100mg of L- ascorbic acid was taken in 100 ml volumetric flask. Then made up with water and placed in sonicator to prepare uniform solution. Took 10 ml of this solution in 100 ml volumetric flask and made up with water. Took the absorbance in UV spectrophotometer. Preparation of sample solution swab is placed in a test tube filled with 10 ml of water and sonicated for 10 min. Took the absorbance of solution in UV spectrophotometer. The amount of drug present in each swab was calculated.[14]

❖ **Optimization and validation of cleaning procedure for L- ascorbic acid tablets on compression machine**

Cleaning validation was performed to demonstrate that the cleaning technique was effective for residues according to the predetermined L-ascorbic acid acceptable limit in the equipment train.[6,9,15]

❖ **Sampling procedure**

After cleaning, a swab sampling process was utilised to determine the amount of drug residue left in the equipment. Sterile cotton swabs with a polypropylene swab stick were utilised in an HDPF container for this procedure. Swabs were immersed in distilled water and saturated for 15 minutes before being used to collect samples. A 5 cm × 5 cm swab area was chosen for each sampling location and swabbed according to the swabbing pattern. The pattern of swabbing and pressure applied was such that it collected the maximum residue present in the selected area. All this operation was done with care and wearing powder free sterile gloves in hands. The swab wiped from selected area was placed in the sterile HDPF containing 10 ml

of distilled water and capped securely. Each tube was sonicated for 5 min; the extract was collected and analyzed.[4,11,16]

❖ Sampling locations

It is important to include swab samples from the equipment's most difficult to clean and worst-case locations, but the sampling locations were chosen to be representative of all sections of the equipment, including easy-to-clean surfaces.

The given piece of equipment had a 99 percent surface that was easily accessible, cleanable, and eye catching. Only 1% or less of the equipment surface was difficult to clean, hence it was presumed that the equipment was as filthy as the hard-to-clean surface samples.[17]

Tablet Compression Machine

Dies and Punches

Feeding hopper

Outlet Hopper

Tablet Scraper

❖ **Cleaning procedure**

After compressing the L-ascorbic acid 10 mg tablets, the tablet machine was cleaned according to industry standard procedures for cleaning pharmaceutical equipment. Cotton swabs were used to collect the samples, which were wiped over a 5 cm5 cm region of predetermined sampling locations. The samples were evaluated and compared to the set of acceptance criteria.

Following steps were followed in the cleaning procedure of the tablet machine after compression of the L- Ascorbic Acid 10 mg tablet.

- The machine's main power supply was turned off.
- The pressure that was exerted to the machine's roller during compression was released.
- Powders, containers, and tablets from previous batches were removed from the producing area.
- A dry towel was used to remove the loosely stuck powder from the machine.
- Compression machine was dissembled as follows:
- Unscrew the top screws to remove the hopper from the spindle.
- The door of machine were opened.
- By unscrewing the upper screws, the feed frame was removed from the die table.
- The tablet discharge chute was removed from the machine after the screws were loosened.
- Washing was performed on all of the above-disassembled pieces.
- The machine's bottom side cover was removed.
- The higher punches were removed by removing the upper punch guide and using the flying wheel to rotate the turret.
- Using an allen key, the turret's die locking screw was released, and dies were removed by pressing through the bottom guide hole with the use of a die driving road.

- Cleaning of die and punches
- With the help of the dry towel, the adhering powder was removed from the punches.
- Clean wet towels dipped in purified water were used to clean punch bowls.
- Wet cloths dipped in purified water were inserted into the hole to clean the dies. A moist cloth dipped in purified water was used to wipe the outside of the dies.
- After a visual check, cleaned punches and dies were stored in the proper cabinet.
- A clean, wet towel dipped in purified water was used to clean the die lock.
- After cleaning, the die lock was let to air dry.
- Cleaning of machine
- A wet cloth dipped in purified water was used to clean the machine's top base, upper roller, upper cam track, and bottom track, followed by a dry cloth clean.
- The vacuum cleaner was used to remove all adherent material from the turret, which was then wiped clean with a clean cloth soaked in water before being cleaned with a clean cloth soaked in IPA.
- A dry clean towel was used to clean the upper punch shank, lower punch shank, and die pocket.
- A clean dry cloth was used to wipe the machine's non-contact parts.
- Doors were cleaned with a purified water-soaked cloth followed by a dry clean cloth.
- Washing
- To remove adherent materials on SS surfaces, the hopper, feed frame, liner removal plate, and discharge chute were flushed with purified water for at least 4 minutes.
- Final rinse and drying
- Finally, the feed frames, hopper from the inside, liner, and discharge chute were rinsed with purified water for no more than 4 minutes. The excess water was wiped away with a dry, clean cloth.
- Items were carried to the machine area once they had been cleaned.
- The machine was marked as "ready to use."[1,5,7,18]

RESULT AND DISCUSSION

1. Detection of λmax of L- Ascorbic acid

The 10 ppm solution was scanned and λmax was found to be 295.4 nm.

2. Blank swab interference

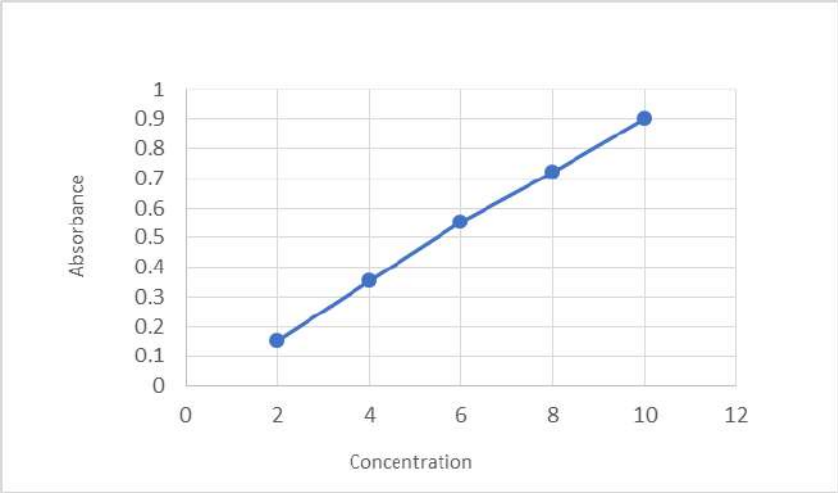
This study was performed to check the interference on the absorbance of L- Ascorbic acid at the maxima due to swab and cleaning agent.

Solutions	Standard solution	Swab Solution 1	Swab Solution 2	Swab Solution 3	Swab Solution 4	Swab Solution 5	Swab Solution 6
Absorbance at 295.4 nm	0.8996	0.0008	0.0012	0.0005	0.0011	0.0010	0.0017

3. Linearity

Linearity of the analytical method was its ability to elict test result that are directly proportional to concentration of the drug substance taken for test, within a given range of 2-10 ppm.

Conc. (ppm)	2	4	6	8	10	Correlation coefficient	Intercept	Slope
Absorbance	0.1522	0.3542	0.5526	0.7158	0.8999	0.9992	-0.0221	0.0928



4. Precision

Precision is the measure of either degree of reproducibility or repeatability of analytical method. It is expressed as standard deviation or coefficient of variance.

Sr. No	1	2	3	4	5	6	Mean+ Std. Deviation	% RSD
Absorbance	0.8952	0.8999	0.9002	0.8970	0.8986	0.8996	0.8988+ 0.8993	100.05

Reproducibility of absorbance

- **Intraday analysis**

Intraday analysis was determine by analyzing drug as per procedure for three times in the same day.

Sr. No	Conc. (ppm)	Absorbance			Mean	Standard Deviation	% RSD
1	2	0.1519	0.1523	0.1522	0.1521	0.00020	0.1368
2	4	0.3541	0.3536	0.3545	0.3540	0.00045	0.1273
3	6	0.5529	0.5531	0.5529	0.5529	0.00011	0.0208
4	8	0.7162	0.7158	0.7165	0.7161	0.00035	0.0490
5	10	0.8991	0.8999	0.9001	0.8997	0.00052	0.0588

- **Interday analysis**

Interday precision was determined by analyzing drug as per procedure daily for three days. Reproducibility was evaluated by coefficient of variation.

Sr. No	Conc.(ppm)	Absorbance			Mean	Standard Deviation	% RSD
		Day 1	Day 2	Day 3			
1.	2	0.1569	0.1601	0.1612	0.1593	0.0022	1.4014
2.	4	0.3521	0.3451	0.3496	0.3489	0.0035	1.0166
3.	6	0.5495	0.5461	0.5529	0.5494	0.0034	0.6187
4.	8	0.7222	0.7124	0.7162	0.7169	0.0049	0.6891
5.	10	0.8999	0.9011	0.9021	0.9010	0.0011	0.1222

5. Recovery of drug from Spiked SS Plates

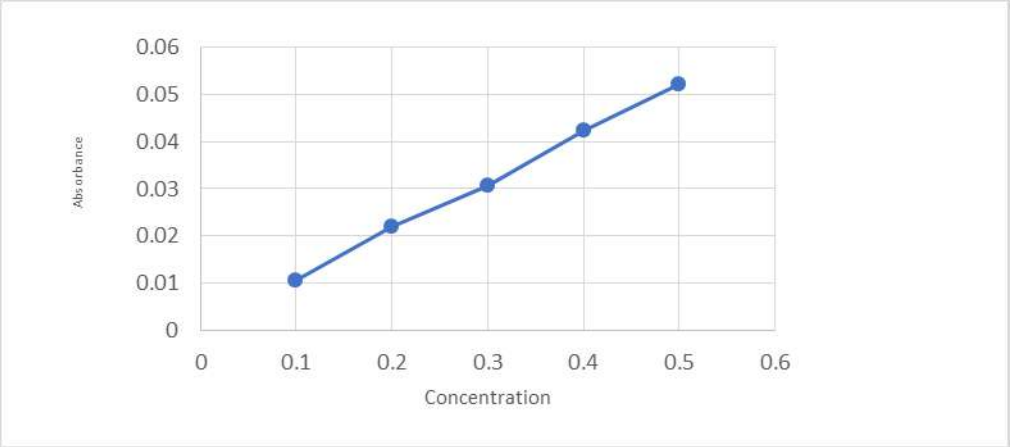
Recovery study was performed by spiking the different concentration solution on SS plate and then swabbed by swab sticks. Then analysis in UV and compared the absorbance of test solution with standard solution results.

Conc.	Abs. 1	Abs. 2	Abs. 3	Mean	Std. abs	Conc.(ppm)	% Recovery
50	0.1502	0.1255	0.1519	0.1425	0.1522	18.5	92.5
100	0.4132	0.4267	0.4293	0.4230	0.4409	35.9	89.7
200	0.8581	0.8473	0.8575	0.8543	0.8999	71.3	88.7

6. Limit of Detection

It was calculated based on standard deviation of response and slope of calibration curve

Conc. (ppm)	Absorbance		Mean absorbance
	Absorbance 1	Absorbance 2	
0.1	0.0108	0.0109	0.0108
0.2	0.0221	0.0219	0.0220
0.3	0.0301	0.0305	0.0303
0.4	0.0472	0.0469	0.0470
0.5	0.0525	0.0524	0.0524



Took the absorbance of all the above concentration and calculated the LOD by using following formula:

$$LOD = 3.3\ \sigma / S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

LOD = 3.3× 0.0172 / 0.1082

= 0.0570 / 0.1082

= 0.5143 µg/ml

7. Limit of Quantitation

LOD = 10× 0.0172 /0.1082

= 0.1727 / 0.1082

= 1.5957 µg/ml

➢ **Tablet Compression Machine**

L- Ascorbic acid tablets 10 mg were manufactured in order to test the cleaning procedure on the tablet machine. The purpose of this validation was to demonstrate the cleaning procedure's effectiveness and uniformity. Validation was also carried out in order to meet the development method's regulatory requirements. After production, tablet compression machine was subjected to above cleaning procedure. The results of the visual and chemical inspections were documented.[4,8,18]

Sr. No	Sampling Point	Absorbance	Concentration
1.	Turret	0.0109	0.35
2.	feeding Hopper	0.0016	0.24
3.	Outlet Hopper	0.0080	0.25
4.	Dies	0.0054	0.29
5.	Punches	0.0102	0.34

CONCLUSION

Cleaning procedure was optimized for determination of drug residues on different parts of tablet machine in different steps of cleaning. On the tablet compression machine, cleaning validation was performed. L- Ascorbic acid tablets were formulated for cleaning validation study. The drug's chemical residue was detected within the predetermined acceptance parameters at various areas of the equipment surfaces.

To show cleaning validation, swab sampling and the UV method were devised and validated for quantifying L- Ascorbic acid residues on stainless steel surfaces of plant equipment following manufacturing of L- Ascorbic acid 10 mg chewable tablets. Selective, accurate, precise, and linear methods with the suitable swab wipe approach were discovered.

The swab sampling and UV approach can be successfully applied in cleaning validation for quantitative measurement of L- Ascorbic acid residues following the manufacture of L- Ascorbic acid chewable tablets in different pharmaceutical quality control laboratories.[18,19]

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Review Article

MICROWAVE ASSISTED SYNTHESIS: A GREEN CHEMISTRY APPROACH

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Abstract: Green Chemistry with its twelve principles would like to see changes in the conventional chemical synthesis and the use of less toxic starting materials. Green Chemistry would like to increases the efficiency of synthetic methods, to use less toxic solvents, reduce the stages of the synthetic routes and minimize waste as far as practically possible. In this way, chemical synthesis will be part of the effort for sustainable development. Microwave assisted synthesis has revolutionized chemical synthesis. Small molecules can be built in a fraction of the time required by conventionalmethods. In conventional heating methods oil bath or hot plate are used as a source of heat to a chemical reactionMicrowave irradiation is widely used as a source of heating in chemical synthesis.The basic mechanisms observed in microwave assisted synthesis are dipolar polarization and conduction. Microwave-assisted synthesis provides clean synthesis with the advantage of enhanced reaction rates, higher yields, greater selectivity, and economic for the synthesis of a large number of organic molecules, have provided the momentum for many chemists to switch from conventional heating method to microwave assisted chemistry.Microwave-assisted synthesis is rapidly becoming the method of choice in modern chemical synthesis and drug discovery. The present article will highlight the applications of microwave-assisted synthesis in organic synthesis, inorganic synthesis, polymer synthesis, nanotechnology, peptide synthesis and discuss the basic mechanism involved in microwave heating.
Key words: Microwave heating, Green chemistry, Microwave synthesis, Microwaves.

INTRODUCTION

The term Green Chemistry is becoming the worldwide term used to describe the design of chemical products and processes that reduce or eliminate the use or generation of substances hazardous to human health.[1] The term was coined by the US Environmental Protection Agency and has been defined as: the utilization of a set of principles that reduce or eliminate the use or generation of hazardous substances in the design, manufacture and application of chemical products.[2] This goal can be achieved by use of twelve principles of Green Chemistry which are as follows.
(1) It is better to prevent waste than to treat or clean up waste after it has been created. (2) Synthetic methods should be designed to maximize the incorporation of all materials used in the process, into the final product. (3) Synthetic methods should be designed to use and generate less hazardous/toxic chemicals. (4) Chemical products should be designed to affect their desired function while minimizing their toxicity. (5) The use of solvents and auxiliary substances should be made unnecessary wherever possible and innocuous when used. (6) Energy requirements of chemical processes should be minimized, and synthetic methods should be conducted at ambient temperature and pressure if possible. (7) A raw material should be renewable rather than depleting whenever practicable. (8) Unnecessary derivatization should be minimized or avoided if possible. (9) Catalytic reagents are superior to stoichiometric reagents. (10) Chemical products should be designed so that at the end of their function they break down into innocuous degradation products that do not persist in the environment. (11) Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances. (12) Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents.[2-4]

Organic synthesis on a large scale involves the use of basic chemical ingredients from the petrochemical sector and catalysts; and after the end of the reaction, separation, purification, storage, packaging, distribution etc. Conventional methods of organic synthesis usually need longer heating time, tedious apparatus setup, which result in higher cost of process and the excessive use of solvents/reagents. During these processes there are many problems of health and safety for workers in addition to the environmental problems caused by their use and disposition as waste. Green Chemistry would like to increases the efficiency of synthetic methods, to use less toxic solvents, reduce the stages of the synthetic routes and minimize waste as far as practically possible.[5] Microwave synthesis is considered as an important approach toward green chemistry, because this technique is more eco-friendly. Due to its ability to couple directly with the reaction molecule and by passing thermal conductivity leading to a rapid rise in the temperature, microwave irradiation has been used to improve many organic syntheses.[6,7]

Microwave chemistry is the science of applying microwave radiation to chemical reactions. Microwave synthesis represents a major breakthrough in synthetic chemistry methodology; a dramatic change in the way chemical

synthesis is performed. Conventional heating, long known to be inefficient and time consuming, has been recognized to be creatively limiting too. Microwave synthesis gives the chemists more time to expand their creativity, test new theories and develop new processes. Instead of spending hours or even days synthesizing a single compound, chemists can now perform the same reaction in minutes. The problem associated with waste disposal of solvents has been overcome by performing reactions without a solvent under microwave irradiation. Coupling of microwave irradiation with the use of mineral-supported catalysed reactions, under solvent-free conditions, provides clean chemical processes with the advantage of enhanced reaction rates, higher yields, greater selectivity, and greater ease of manipulation. Thus microwave synthesis acts as a potential tool for green chemistry.[6, 8]

Microwave irradiation provides an alternative to the conventional methods, for heating or introducing energy into the system. It utilizes the ability of mobile electric charges present in liquid or conducting ions in solid to transform electromagnetic energy into heat. Microwave radiations are electromagnetic waves. In the electromagnetic spectrum, the microwave radiation region is located between infrared radiation and radio waves. Microwaves have wavelength of 1 mm to 1 m corresponding to frequencies between 0.3 and 300 GHz. Telecommunication and microwave radar equipment occupy many of the band frequencies in this region. Microwave dielectric heating; uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. This technology opens up new opportunities to the synthetic chemist in the form of new reactions that are not possible using conventional heating.[7,9]

MECHANISM OF MICROWAVE HEATING

All the materials are not susceptible to microwave heating as response of various materials to microwave radiation is diverse. Based on their response to microwaves, materials can be broadly classified as follows:

(1) Materials that are transparent to microwaves, e.g. sulphur
(2) Materials that reflect microwaves, e.g. copper
(3) Materials that absorb microwaves, e.g. water

Microwave absorbing materials are of utmost important for microwave chemistry and three main different mechanisms are involved for their heating namely: Dipolar polarization, Conduction mechanism and Interfacial polarization.[10]

Dipolar polarization:

For a substance to generate heat when irradiated with microwaves it must possess a dipole-moment. It is the electric field component of the microwave radiation, rather than magnetic field component that is responsible for heating, when a dipole tries to reorient itself with respect to an alternating electric field; it loses energy in the form of heat, by molecular friction. Dipolar polarization can generate heat by either interaction between polar solvent molecules such as water, methanol and ethanol; or interaction between polar solute molecules such as ammonia and formic acid. The key requirement for dipolar polarization is that the frequency range of the oscillating field should be appropriate to enable adequate inter-particle interaction. If the frequency range is very high, inter-molecular forces will stop the motion of a polar molecule before it tries to follow the field, resulting in inadequate inter-particle interaction. On the other hand, if the frequency range is low, the polar molecule gets sufficient time to align itself in phase with the field. Microwave radiation has the appropriate frequency (0.3-30 GHz) to oscillate polar particles and enable enough inter-particle interaction. This makes it an ideal choice for heating polar solutions.[11, 12]

Conduction mechanism:

The conduction mechanism generates heat through resistance to an electric current. The oscillating electromagnetic field generates an oscillation of electrons or ions in a conductor, resulting in an electric current. This current faces internal resistance, which heats the conductor. A solution containing ions, or even a single isolated ion with a hydrogen bonded cluster, in the sample the ions will move through the solution under the influence of an electric field, resulting in expenditure of energy due to able to believe that the more polar the solvent, the more readily the microwave irradiation is absorbed and the higher the temperature obtained. Where the irradiated sample is an electrical conductor, the charge carriers (electrons, ions, etc.) are moved through the material under the influence of the electric field, resulting in a polarization. These induced currents will cause heating in the sample due to any electrical resistance. Major limitation of the method is that it is not applicable for materials with high conductivity, since such materials reflect most of the energy that falls on them.[11]

Interfacial polarization:

The interfacial polarization method can be considered as a combination of both the conduction and dipolar polarization mechanisms. It is important for heating systems that comprise a conducting material dispersed in a non-conducting material. For example, consider the dispersion of metal particles in sulphur. Sulphur does not respond to microwaves and metals reflect most of the microwave energy they are exposed to, but combining the two makes them a good microwave-absorbing material. However, for this to take place, metals have to be used in powder form. This is because, unlike a metal surface, metal powder is a good absorber of microwave radiation. It absorbs radiation and is heated by a mechanism that is similar to dipolar polarization. The environment of the metal powder acts as a solvent for polar molecules and restricts the motion of ions by forces that are equivalent to inter-particle interactions in polar solvents. These restricting forces under the effect of an oscillating field induce a phase lag in the motion of ions, resulting in random motion of ions, and ultimately heating of the system.[13-15]

MICROWAVE VERSUS CONVENTIONAL SYNTHESIS

Conventional synthesis usually involves the use of a furnace or oil bath which heats the walls of the reactors by convection or conduction (Figure 1). The core of the sample takes much longer to achieve the target temperature. This is a slow and inefficient method for transferring energy into

the reacting system. On the other hand in microwave assisted synthesis microwave penetrates inside the material and heat is generated through direct microwave-material interaction (**Figure 1**). Microwave-assisted synthesis has several advantages over conventional reactions in that the microwave allows for an increase in reaction rate, rapid reaction optimization, and rapid analogue synthesis. It also uses both less energy and solvent, and it enables difficult compound synthesis. Specifically, microwave synthesis has the potential to impact upon medicinal chemistry efforts in at least three major phases of the drug discovery process: lead generation, hit-to-lead efforts, and lead optimization. Microwave chemistry can be carried out very efficiently in a parallel format using dedicated rotors or microtiter plate systems. Several hundred reactions can be performed in a single microwave experiment using multimode microwave devices. Researchers have shown the benefits gained by employing microwave heating in tandem with combinatorial chemistry.[16, 17]

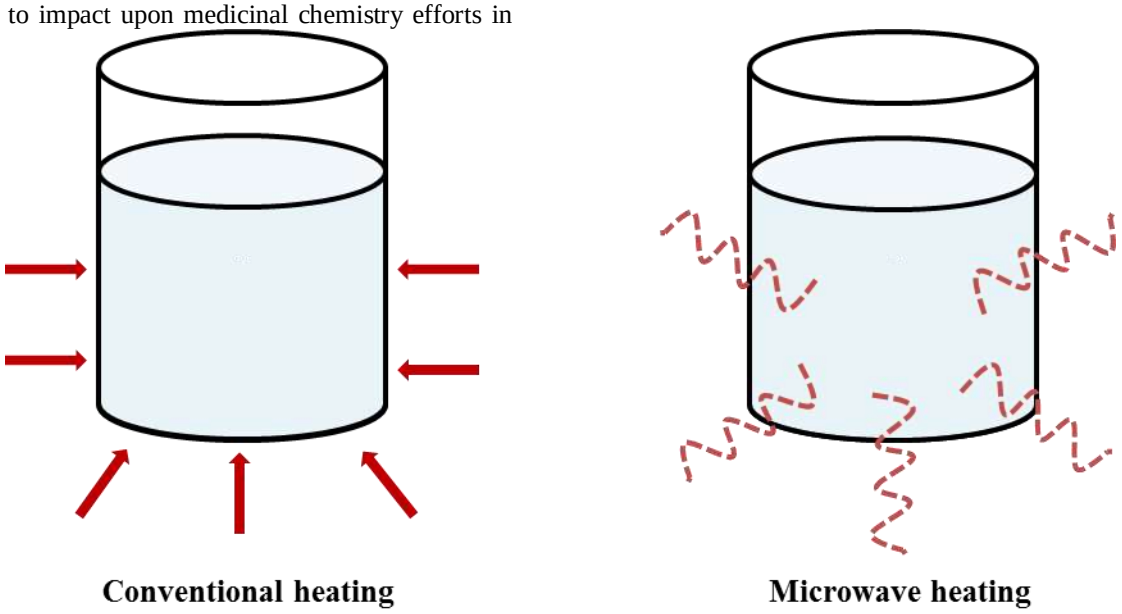


Figure 1: Comparison of microwave heating versus conventional heating[18]

A few reactions which were carried out using microwave heating and compared with conventional heating indicating time and energy efficiency of the technique are compiled in **Table 1**.

Table 1: Comparison of reaction times using microwave versus conventional heating[19]

Compound synthesized	Reaction time: Microwave	Reaction time: Conventional
Methyl benzoate	5 minutes	8 hours
4-nitrobenzyl ester	2 minutes	1.5 hours
Zeolite synthesis	30 seconds	60 minutes
Cubanite	3 minutes	3 days
$NaAlH_4$	2 hours	8 hours
$CuBi_2O_4$	5 minutes	18 hours
Ag_3In	2 minutes	48 hours

MICROWAVE SYNTHESIS APPARATUS

The apparatus for microwave assisted synthesis include; single-mode microwave ovens, and multi-mode microwave ovens.[9]

Single-mode microwave apparatus:

The differentiating feature of a single-mode apparatus is its ability to create a standing wave pattern. This interface generates an array of nodes where microwave energy intensity is zero, and an array of antinodes where the magnitude of microwave energy is at its highest. One of the limitations of single-mode apparatus is that only one vessel can be irradiated at a time. However, the apparatus is user-friendly. An advantage of single-mode apparatus is their high rate of heating. This is because the sample is always placed at the antinodes of the field, where the intensity of microwave radiation is the highest. These apparatus can process volumes ranging from 0.2 to about 50 ml under sealed-vessel conditions, and volumes around 150 ml under open-vessel conditions.Single-mode microwave ovens are currently used for small-scale drug discovery, automation and combinatorial chemical applications.

Multi-mode microwave apparatus:

An essential feature of a multi-mode apparatus is the deliberate avoidance of generating a standing wave pattern inside it. The goal is to generate as much chaos as possible inside the apparatus. The greater the chaos, the higher is the dispersion of radiation, which increases the area that can cause effective heating inside the apparatus. As a result, a multi-mode microwave heating apparatus can accommodate

a number of samples simultaneously for heating, unlike single-mode apparatus where only one sample can be irradiated at a time. Owing to this characteristic, a multi-mode heating apparatus is used for bulk heating and carrying out chemical analysis processes such as ashing, extraction, etc.In large multi-mode apparatus, several litres of reaction mixture can be processed in both open and closed-vessel conditions. A major limitation of multi-mode apparatus is that, heating samples cannot be controlled efficiently because of lack of temperature uniformity.[20-24]

BENEFITS OF MICROWAVE ASSISTED SYNTHESIS

Microwaves can accelerate the rate of reaction, provide better yields and higher purity, uniform and selective heating with lower energy usage, achieve greater reproducibility of reactions and help in developing convenient and cleaner synthetic routes. The main advantages of microwave assisted organic synthesis are:

Faster reaction: Based on experimental data it has been found that microwave-enhanced chemical reaction rates can be faster than those of conventional heating methods by as much as 1,000-fold. The microwave can use higher temperatures than conventional heating system, and consequently the reactions are completed in few minutes instead of hours, for instance, synthesis of fluorescein, which usually takes about 10 hours by conventional heating methods, can be conducted in only 35 minutes by means of microwave heating.

Better yield and higher purity: Less formation of side products are observed using microwave irradiation, and the product is recovered in higher yield. Consequently, also the purification step is faster and easier. For example, microwave synthesis of aspirin results in an increase in the yield of the reaction, from 85 % to 97 %.

Energy saving: Heating by means of microwave radiation is a highly efficient process and results in significant energy saving. This is primarily because microwaves heat up just the sample and not the apparatus, and therefore energy consumption is less.

Uniform and selective heating: In conventional heating, the walls of the oil bath get heated first, and then the solvent. As a result of this distributed heating in an oil bath, there is always a temperature difference between the walls and the solvent. In the case of microwave heating, only the solvent and the solute particles are excited, which results in uniform heating of the solvent. Selective heating is based on the principle that different materials respond differently to microwaves. Some materials are transparent whereas others absorb microwaves.

Green synthesis: Reactions conducted using microwaves are cleaner and more eco-friendly than conventional heating methods. Microwaves heat the compounds directly; therefore, usage of solvents in the chemical reaction can be reduced or eliminated. Synthesis without solvent, in which reagents are absorbed on mineral support, has a great potential as it offers an eco-friendly green protocol in synthesis. The use of microwaves has also reduced the amount of purification required for the end products of chemical reactions involving toxic-reagents.

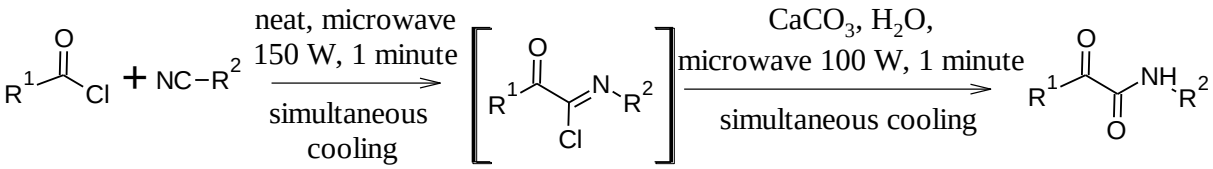
Reproducibility: Reactions with microwave heating are more reproducible compared to the conventional heating because of uniform heating and better control of process parameters. The temperature of chemical reactions can also be easily monitored.[25-31]

LIMITATIONS OF MICROWAVE ASSISTED SYNTHESIS

The yield obtained by using microwave apparatus available in the market is limited to a few grams. Although there have been developments in the recent past, relating to the scalability15 of microwave equipment, there is still a gap that needs to be spanned to make the technology scalable. The use of microwaves as a source of heating has limited applicability for materials that absorb them. Microwaves cannot heat materials such as sulphur, which are transparent to their radiation. Improper use of microwave heating for rate enhancement of chemical reactions involving radioisotopes may result in uncontrolled radioactive decay. Certain problems, with dangerous end results, have also been observed while conducting polar acid-based reactions, for example, microwave irradiation of are action involving concentrated sulphuric acid may damage the polymer vessel used for heating. Conducting microwave reactions at high-pressure conditions may also result in uncontrolled reactions and cause explosions. Health hazards related to microwaves are caused by the penetration of microwaves. While microwaves operating at a low frequency range are only able to penetrate the human skin, higher frequency-range microwaves can reach body organs. Research has proven that on prolonged exposure microwaves may result in the complete degeneration of body tissues and cells. It has also been established that constant exposure of DNA to high-frequency microwaves during a biochemical reaction may result in complete degeneration of the DNA strand.[19, 32, 33]

ENHANCED MICROWAVE SYNTHESIS

Recently, an alternative method for performing microwave-assisted organic reactions, termed Enhanced Microwave Synthesis (EMS), has been examined. By externally cooling the reaction vessel with compressed air, while simultaneously administering microwave irradiation, more energy can be directly applied to the reaction mixture. EMS ensures that a high, constant level of microwave energy is applied. Simultaneous cooling enables a greater amount of microwave energy to be introduced into a reaction, while keeping the reaction temperature low. This results in significantly greater yields and cleaner chemistries. EMS was employed in the synthesis of a variety of α-keto amides (Scheme 1) to support a protease inhibitor discovery project. This may eventually lead to improved treatments for stroke, Alzheimer's disease, and muscular dystrophy. Under conventional heating conditions, this took between 2 to 6 hours for completion; whereas under optimized EMS conditions, the two steps were completed in 2 min and in 21-74% yields.[34, 35]



Scheme 1: Improved Synthesis of α-keto Amides by Enhanced Microwave Synthesis

APPLICATIONS OF MICROWAVE ASSISTED SYNTHESIS

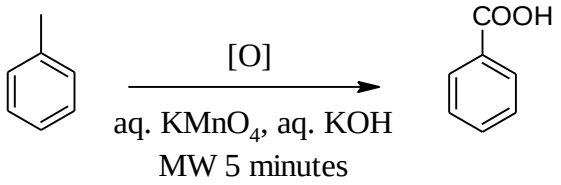
Application of microwave irradiation in chemical synthesis involves its use in the acceleration of chemical synthesis. Microwave-enhanced synthesis results in faster reactions, higher yields, and increased product purity. In addition, due to the availability of high-capacity microwave apparatus, the yields of the experiments have now easily scaled up from milligrams to kilograms, without the need to alter reaction parameters. Microwave-assisted synthesis can be suitably applied to the drug discovery process.[33]

Organic synthesis:

Microwave-assisted organic synthesis has been the foremost and one of the most researched applications of microwaves in chemical reactions. Literature survey reveals that scientists have successfully conducted a large range of organic reactions. These include Diels-Alder reaction, Ene reaction, Heck reaction, Suzuki reaction, Mannich reaction, Hydrogenation of [beta]-lactams, Hydrolysis, Dehydration, Esterification, Cycloaddition reaction, Epoxidation, Reductions, Condensations, Cyclisation reactions, Protection and deprotection, etc.[34]

Microwave-assisted organic synthesis is being widely applied in the pharmaceuticals industry, particularly for developing compounds in the lead optimization phase of drug discovery and development. In this phase, chemists use diverse synthetic techniques to develop candidate drugs from lead compounds. Based on reaction conditions, organic synthesis reactions can be conducted in the following techniques.

(1) **Microwave-assisted organic synthesis at atmospheric pressure:** Microwave-assisted organic synthesis can be most conveniently conducted at atmospheric pressure in reflux conditions, for example, oxidation of toluene to benzoic acid (**Scheme 2**) with $KMnO_4$ under normal conditions of refluxing takes 10-12 hours compared to reaction in microwave conditions, which takes only 5 minutes. **Table 2** shows an increased yield of 200 % for the oxidation of hexanenitrile and 150 % for the hydrolysis of cyclohexene when the reaction is conducted in the microwave batch reactor.[36, 37]



Scheme 2: Oxidation of toluene to benzoic acid with $KMnO_4$

Table 2: Heterogeneous reactions under microwave and classical heating[36]

Chemical reaction	Time (minutes)	MW Yield (%)	Classical Yield (%)
Hydrolysis of hexanenitrile	60	40	26
Oxidation of cyclohexene	60	26	12

(2) **Microwave-assisted organic synthesis at elevated pressure:** Microwaves can be used to directly heat the solvents in sealed microwave-transparent containers. The sealed container helps in increasing the pressure in the reactor, which facilitates the reaction that will take place at much higher temperatures. This results in a substantial increase in the reaction rate of microwave-assisted organic synthesis.[12]

(3) **Microwave-assisted organic synthesis under solvent-free conditions:**Microwave-assisted solvent-free organic synthesis has been developed as an environmentally friendly process as it combines the selectivity associated with most reactions carried out under microwaves with solvent and waste-free procedures in which organic solvents are avoided throughout all stages. The solvent-free organic syntheses are of three types: (i) reactions using neat reactants; (ii) reactions using solid-liquid phase transfer catalysis (PTC); and (iii) reactions using solid mineral supports. The microwave-assisted reaction could be completed within two to three minutes, compared to conventional oil-bath heating at 75 °C for 40 hours.[12, 38]

Inorganic synthesis:

A variety of materials such as carbides, nitrides, complex oxides, silicides, zeolites, apatite, etc. have been synthesized using microwaves. A series of A_3B and A_4 type mesoporphyrinic complexes were synthesized with superior yields using microwave irradiation under solvent-free conditions. Solvent-free synthesis by microwave irradiation has been successfully applied to obtaining mesoporphyrinic compounds because the absence of solvent from the reaction environment has the effect of decreased interaction time between reactant molecules and improves the reaction yield. Two new iso-structural coordination polymers with novel anionic metal-organic frameworks were synthesized using

microwave-assisted technique. Microwave-assisted synthesis of pinacol boronates from aryl chlorides catalysed by a palladium/imidazolium salt system was reported.[39-43]

Synthesis of nanotechnology products:

Amongst the several methods that exist for synthesizing of nanoparticles, the use of microwave assisted synthesis has shown promise. Synthesis of silver nanoparticles from silver nitrate employing starch as the reductant as well as stabilizing agent has been carried out under direct heating, controlled heating and microwave irradiation. The microwave irradiation was considered as better for reduction of silver ions to silver nanoparticles. It also afforded smaller particle sizes and particle size distribution. Compared to conventional methods, microwave assisted synthesis was faster and provided particles with an average particle size of 12 nm. Nanostructures with smaller sizes, narrower size distributions, and a higher degree of crystallization were obtained under microwave heating than those in conventional oil-bath heating. The gold nanoparticles have been prepared by microwave high-pressure procedure with alcohol as the reducing agent. A method has been reported for microwave-assisted non-aqueous synthesis of zinc oxide nanoparticles. Particularly the fast reaction rates, better product yields and the possibility to automatically combine different experimental parameters makes microwave-assisted synthesis suitable for the studies of the influences of the reaction conditions on the morphology and sizes of zinc oxide nanoparticles particles, which determine its properties and applications. Pt/C and $PtCo_3O_4$/C nano catalysts were prepared using microwave assisted methods. The results of XRD and TEM revealed that the prepared catalysts have small and uniform shapes with high dispersion ability. The developed approach is a useful method for preparing platinum and platinum supported electrocatalysts, which can be used in the field of fuel cells and other related fields. Strontium stannate ($SrSnO_3$) nanostructures were obtained by microwave-assisted calcination of a $SrSn(OH)_6$ precursor powder. Compared to other conventional calcination methods mentioned in the literature, this procedure led to a remarkable decrease of the reaction time and the synthesis temperature owing to direct interaction of radiation with the material.[9, 44-50]

Polymer synthesis:

Polymer chemistry, including ceramic processing, forms the single-largest application area of microwave chemistry. The use of polar reactants in polymerization reaction results in controlled synthesis and a combination of this with direct heating of reactants makes microwave heating an economically viable option. Using microwave radiation in curing has greatly increased the rate of the reactions. It has been found that the rate of a curing reaction, using microwaves, is not dependent on the power applied but on the way the pulse is applied. Controlled solvent-free synthesis and modification in polymer materials can be rapidly and effectively done with the help of microwave heating using large scale reactors The first microwave assisted organic synthesis of Poly Lactic Acid was carried out with SnOct as catalyst by using toluene as a solvent.[51, 52]

Peptide synthesis:

A microwave-assisted, rapid solid phase peptide synthesis procedure has been reported. The synthesis protocol is based on the use of cycles of pulsed microwave irradiation with intermittent cooling of the reaction during the removal of the Fmoc protecting group and during the coupling. The desired nonapeptide was obtained in highest yield and purity by employing MicroKan technology. The protocols for the synthesis of cystine-rich peptides in the presence of microwave radiation with solid phase peptide synthesis have been reported. The method is broadly applicable for a wide range of peptides using Boc-SPPS, especially for SPPS of large peptides via native chemical ligation. Microwave radiation produces peptides in high yield and with high purity, and the time for the assembly of approximately 30 amino acids peptide chains was reduced to an overnight reaction in the automated microwave-assisted synthesis. The applications of microwaves in the field of peptides and glycopeptides have been reported.[53-56]

Synthesis of radiopharmaceuticals:

Microwave-assisted organic synthesis at an elevated pressure has been used in pharmaceutical industry for the synthesis of radiopharmaceuticals. During pre-clinical trials, these radiopharmaceuticals are used as tracers to generate a nuclear medical image. A multi-mode microwave oven was used in the first trial of this kind and it was observed that the rate of reaction increased substantially. This has resulted in the enhanced use of microwaves to produce radiopharmaceuticals. Advantages of microwaves include the fast reaction rates and high yield of the reaction. This can be attributed to the short half-life of reactants, for example, saving five minutes in a synthesis with carbon-11 resulted in an enhanced production rate of 15%. It has also been observed that several reactions could only be achieved by using microwaves.[57-60]

CONCLUSION

Microwave-assisted synthesis is a convenient way toward the goal of green chemistry.Microwaves irradiation can be used to in chemical synthesis as a heat source; it is veryefficient and can be used to significantly reduce reaction times of numerous synthetically useful chemical transformations.Thus, microwave-assisted synthesis has advantages over conventional technology: it is more energy efficient and it can lead to improved isolated yields of products with green synthesis.The advantages of this enabling technology have, more recently, been exploited in the context of multistep total synthesis and medicinal chemistry/drug discovery, and have additionally penetrated related fields such as polymer synthesis, material sciences, nanotechnology and biochemical processes. In order to achieve further development inthis field, novel instruments, which give rise to reproducible performances and that constitute aminimal hazard should be used instead of the domestic microwave ovens.

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REVIEW ARTICLE

Quality by Design (QbD) concept Review in Pharmaceuticals

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ABSTRACT:

Quality by Design (QbD) refers to a holistic approach towards drug development. Quality by design is a vital part of the modern approach to pharmaceutical quality. The purpose of this practice school topic is to discuss the pharmaceutical Quality by Design (QbD) and illustrate how it can be used to ensure pharmaceutical quality. The QbD is a systemic approach to pharmaceutical development. It means designing and developing formulations and manufacturing processes to ensure predefined product quality. Some of the QbD elements include: Defining Quality target product profile, identifying critical quality attributes, link the drug excipients attributes, establishing design space, control strategy, critical process parameters and product life cycle management. Using QbD, pharmaceutical quality is assured by understanding and controlling formulation and manufacturing variables. A new approach to drug development could increase efficiencies, provide regulatory support and flexibility, and offer important business benefits throughout the product's life cycle. This PS topic explores the processes used in developing a market formulation and required supportive data, particularly in light of the industry's current movement toward submissions based on QbD. The work also facilitates the adoption and implementation of QbD. Principles in the development of pharmaceutical industries. Successful implementation of QbD concepts requires cooperation across a multitude of company teams, from R&D to manufacturing to quality control and regulatory affairs. This is necessary to ensure that QbD concepts are incorporated not only when the first activities are initiated around a product's design but also during the design of the process used to make the product and other activities associated with a product's life cycle. The application of the concept of quality by design (QbD) presented in this paper aligns with the principles of ICH Q8, Q9 and Q10 guidelines.

KEYWORDS: Control strategy, Critical material attributes, Critical process parameters, Design space, Quality by design.

INTRODUCTION:

Quality by Design (QbD) was first described by Joseph M. Juran. and applied heavily, particularly in the automotive industry. The fundamental premise behind QbD is that quality can be "designed in" to processes through systematic implementation of an optimization strategy to establish a thorough understanding of the response of the system quality to given variables, and the use of control strategies to continuously ensure quality.

The FDA has recently begun to advocate the QbD methodology for the pharmaceutical sector. In order to describe quality by design, we must first define what we mean by quality. In a 2004 paper, Janet Woodcock (Director for the Centre for Drug Evaluation and Research) defined pharmaceutical quality as a 'product that is free of contamination and reproducibly delivers the therapeutic benefit promised in the label to the consumer'. This explanation focuses on the QbD for generic drugs. The concept of QbD was mentioned in the ICH Q8 guidance, which states that "quality cannot be tested into products, i.e., quality should be built in by design". This paper discusses the pharmaceutical quality by design and describes how it can be used to ensure pharmaceutical quality with emphasis on solid oral dosage forms of small molecules. The pharmaceutical industry works hard to develop, manufacture, and bring

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to market new drugs and to comply with regulatory requirements to demonstrate that the drugs are safe and effective. A new approach to drug development could increase efficiencies, provide regulatory relief and flexibility, and offer important business benefits throughout the product's life cycle. This topic explores the processes used in developing a market formulation and requisite supportive data, particularly in light of the industry's current QbD concept as part of its two-year initiative, movement toward submissions based on quality by design (QbD). DA introduced the Pharmaceutical cGMPs for the 21st Century: A Risk-Based Approach Pharmaceutical cGMP initiative (also referred to as the pharmaceutical cGMP Initiative or 21st Century Initiative) in 2002. QbD is not a new concept from a pharmaceutical technology perspective. It is, however, a new concept relative to pharmaceutical regulatory review and submission. As a systematic and prospective approach to product design, process design and control, process performance and continuous improvement, QbD designs quality into the manufacturing process. By doing so, QbD encourages innovation, continuous quality improvement, and science-and risk based regulatory processes and ensures the availability of high-quality medicines to the consumer.[1,2,3,4]

Design:

Product is designed to meet patient needs and performance requirements. Process is designed to consistently meet product quality attributes. Impact of starting raw materials and process parameters on product quality is understood. Critical sources of process variability are identified and controlled. The process is continually monitored and updated to allow for consistent quality over time.[5]

Quality:

"The degree to which a set of inherent properties of a product, system or process fulfils requirements" (ICH Q9).

"Good pharmaceutical quality represents an acceptably low risk of failing to achieve the desired clinical attributes."[1]

Definition of QbD [ICH Q8 (R1)]

A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.[5]

Definition of PAT [FDA PAT Guidelines, Sept. 2004]

A system for designing, analysing and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of new and in-process materials and processes, with the goal of ensuring final product safety.[5]

Process Analytical Technology

The concept actually aims at understanding the processes by defining their Critical process parameters, and accordingly monitoring them in a timely manner (preferably in-line or on-line) and thus being more efficient in testing while at the same time reducing over-processing, enhancing consistency and minimizing rejects.

The FDA has outlined a regulatory framework for PAT implementation. With this framework–according to Hinze "the FDA tries to motivate the pharmaceutical industry to improve the production process". Because of the tight regulatory requirements and the long development time for a new drug, the production technology is "frozen" at the time of conducting phase-2 clinical trials.

PAT allows for and encourages continuous process manufacturing improvement. It uses real-time information to reduce process variation and manufacturing capability and demands a solid understanding of the various processes involved in the operation. Simply put PAT is a real-time testing and adjustment based on the complete understanding of how the components and related processes affect the final product. This is in accordance with the fundamental principle that quality cannot be tested but is instead built into the medicinal product by design.[6]

PAT is a system for

- Designing, analysing and controlling manufacturing.
- Timely measurements.
- Critical quality and performance attribute.
- Raw and in-process materials.
- And processes.[6]

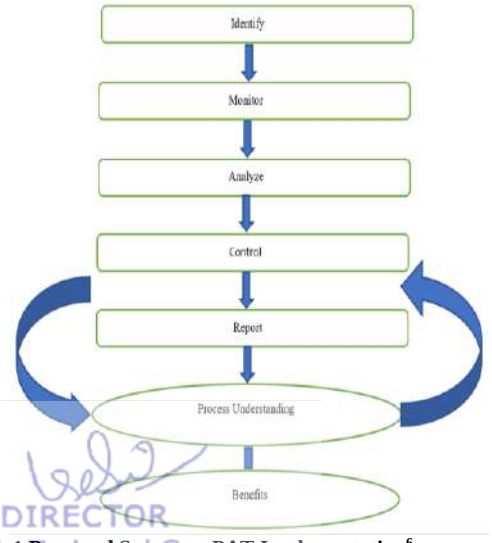

Fig.No:1 Proposed Steps to a PAT Implementation[6]

Benefits of QbD:

- QbD is good Business.
- Eliminate batch failures.
- Minimize deviations and costly investigations.
- Avoid regulatory compliance problems.
- Organizational learning is an investment in the future.
- QbD is good Science.
- Better development decisions.
- Empowerment technical of staff.[7,8,9]

Opportunities of QbD:

- Efficient, agile, flexible system.
- Increase manufacturing efficiency, reduce costs and project rejections and waste.
- Build scientific knowledge base for all products.
- Better interact with industry on science issues.
- Ensure consistent information.
- Incorporate risk management.[6,8]

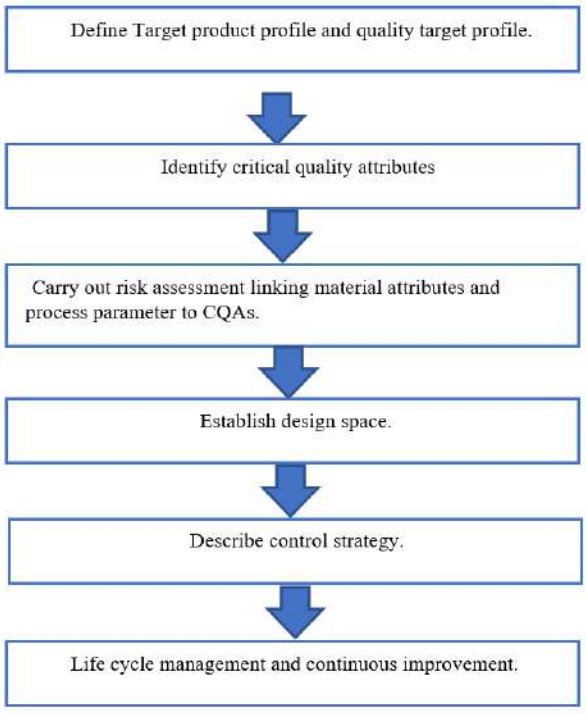

Fig.No:2 Flow of Quality by Design[1]

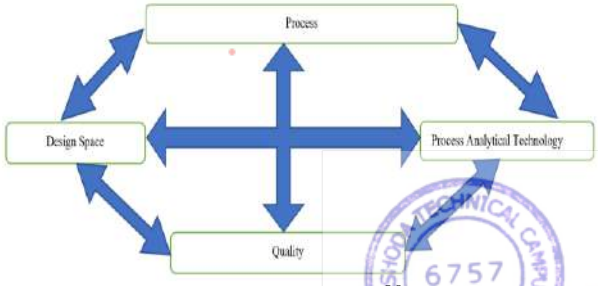

Fig.No:3 Process, Quality, Design and PAT[5,6]

Steps of Quality by Design:

1. Target Product Profile (TPP):

FDA published a recent guidance defining a Target Product Profile (TPP): "The TPP provides a statement of the overall intent of the drug development program, and gives information about the drug at a particular time in development. Usually, the TPP is organized according to the key sections in the drug labelling and links drug development activities to specific concepts intended for inclusion in the drug labelling." When ICH Q8 says that pharmaceutical development should include "...identification of those attributes that are critical to the quality of the drug product, taking into consideration intended usage and route of administration", the consideration of the intended usage and route of administration would be through the TPP.

Identifying Quality Target Product Profile (Qtpp):

"Begin with the end in mind" By Beginning with the end in the mind, the result of development is robust formulation and manufacturing process with an acceptable control strategy that ensures the performance of the drug product. The quality target product profile (QTPP) is "a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product." The QTPP is an essential element of a QbD approach and forms the basis of design of the generic product. The quality target product profile (QTPP) is a quantitative substitute for aspects of clinical safety and efficacy.[1,2,5,13] Quality target product profile (QTPP) Includes, but not limited to:

- Dosage form.
- Route of administration.
- Strength.
- Release or Delivery of the drug.
- Pharmacokinetic characteristics e.g., dissolution, aerodynamic performance.
- Drug product quality characteristics for intended use e.g., sterility, purity.[1,2,5]

2. Identifying Critical Quality Attributes (CQA):

Definition: ICH Q8 (R1) defines CQAs as physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The International Society of Pharmaceutical Engineers (ISPE) and Product Quality Lifecycle Implementation (PQLI) defines critical quality attributes (CQAs) as physical, chemical, biological or microbiological properties or characteristics that need to be controlled (directly or indirectly) to ensure product quality. CQA has been used by some to describe elements of the QTPP (such as dissolution) while others have used CQA to describe mechanistic factors (such as particle size and hardness) that determine product

performance. Thus, CQA is used to describe both aspects of product performance and determinants of product performance. It was stated that the ICH working definition of CQA was: "A CQA is a quality attribute (a physical, chemical, biological or microbiological property or characteristic) that must be controlled (directly or indirectly) to ensure the product meets its intended safety, efficacy, stability and performance". This CQA definition implies that the intended safety, efficacy, stability and performance are not CQAs. Safety and efficacy clearly fall under the domain of the TPP But if stability and performance are not CQA and not part of the TPP, then what are they? We are thus compelled to acknowledge that there is an intermediate category of product performance (or surrogates for quality) that we have defined as the QTPP.[1,2]

3. Critical Process Parameter:
Critical process parameter (CPP) is defined as any measurable input (input material attribute or operating parameter) or output (process state variable or output material attribute) of a process step that must be controlled to achieve the desired product quality and process uniformity. In this view, every item would be a process parameter. Surrogates for quality) that we have defined as the QTPP.
For a given unit operation, there are four categories of parameters and attributes:

- Input material attributes
- Output material attributes
- Input operating parameters
- Output process state conditions.

Critical Process Parameter:
A parameter is Critical when a realistic change in that parameter can cause the product to fail to meet the QTPP. Uniqueness of Critical Process Parameters: Because of the broadness of the CPP definition it is possible for two investigators to examine the same process and come to a different set of CPP. The set of CPP is not unique, but the chosen set must be sufficient to ensure product quality. Different sets of CPP can have several origins. One is that the definition of operating parameters depends on the engineering systems installed on a piece of process equipment.[1,2,10,12]

4. Risk Assessment and Design Space:
Quality Risk Management (ICH Q9) indicates that, the manufacturing and use of a drug product necessarily entail some degree of risk. Risk assessment is a valuable science-based process used in science-quality risk management that can aid in identifying which material attributes and process parameters potentially have an effect on product CQAs. Risk assessment is typically performed early in the pharmaceutical development process and is repeated as more information becomes available and greater knowledge is obtained. Risk assessment tools can be used to identify and rank parameters (e.g., process, equipment, input materials) with potential to have an impact on product quality, based on prior knowledge and initial experimental data.

Design space ICH Q8 (R1) defines Design space as, the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post-approval change process. Many believe design space and QbD are interchangeable terms. This is incorrect. For generic-drug applications, design space is optional. QbD can be implemented without a design space because product and process understanding can be established without a formal design space. It should be pointed out that implementation of QbD is strongly encouraged by FDA. For some complex drug substances or drug products, implementation of QbD is considered a required component of the application. Submission of a design space to FDA is a pathway obtaining the ability to operate within that design space without further regulatory approval.[1,2,14]

5. Defining Control Strategy ICH Q8 (R1):
It defines control strategy as A planned set of controls, derived from current product and process understanding that ensures process performance and product quality.

Minimal and enhanced approaches As in ICH Q8(R), a distinction may be drawn between a minimal and an enhanced control strategy approach. In a Minimal Control Strategy, drug product quality is controlled primarily by intermediate (in process material) and end product testing. In an Enhanced Control Strategy drug product quality ensured by risk-based control strategy for well understood product and process, and quality controls are shifted upstream, with the possibility of real-time release or reduced end-product testing.

Developing the Control Strategy:
Development of a Control Strategy requires a structured process, involving a multi-disciplinary team of experts, linking pharmaceutical development to the manufacturing process, and engineering controls of process equipment.

The PQLI Control Strategy Team has proposed a Control Strategy Model that facilitates understanding and that may be used a cross-functional communication tool.
Personnel at all levels should be able to understand the way control strategy links from CQAs to operational aspects to ensure, for example that:

- Chemists understand in-process controls are established to keep the process inside the design

space and seek opportunities for simplification of controls, as knowledge is gained.
- Engineers know how equipment operating conditions impact product quality.
- Quality Assurance professionals know where the highest risks are in the process.[1,5]

6. Control Strategy and the Product Lifecycle:

The Control Strategy is related to the level of process understanding at a given time, and evolves as manufacturing experience increases. The originally specified measures, controls or models may be modified or even removed, or the need for additional controls may be identified. Other revisions to the Control Strategy may relate to continual improvement, for example the introduction of improved analyser or control technology. Periodic reviews of risk assessments and mitigation should be conducted to determine the appropriateness of the Control Strategy based on product manufacturing history. Failure or deviations should be investigated and the effectiveness of the control system considered in relation to the identified root cause. Corrective and preventive actions should be applied and the Control Strategy updated as necessary (including any regulatory actions required) in the light of new product and process knowledge. Implementing PAT in the Control Strategy will require the application of process models (multivariate prediction models) that either predicts CQAs or CPPs or a combination of both. These models may require frequent updates, depending on the maturity of the model (e.g., the amount of data and their variability within the model), as well as the kind of data that has been included to reflect variability in scale, equipment, analytical set-up, sampling, and site. A monitoring program for verifying the validity of process models should be established and be based on a risk analysis of the model itself and include possible ways to verify the model by other means. One example would be to compare the predicted CQA value to a conventional analytical method. The monitoring program should include requirements for when a model has to be updated (e.g., change of raw material supplier or deviations resulting in increased knowledge).[1,2,11]

CONTINUOUS IMPROVEMENT:

"Continuous improvement is an essential element in a modern quality system that aims at improving efficiency by optimizing a process and eliminating wasted efforts in production. These efforts are primarily directed towards reducing variability in process and product quality characteristics."

The backbone for Continuous Improvement is the Pharmaceutical Quality System. PQS should facilitate continual improvement and help to: "Identify and implement appropriate product quality improvements, process improvements, variability reduction, innovations and pharmaceutical quality system enhancements, thereby increasing the ability to fulfil quality needs consistently. Quality risk management can be useful for identifying and prioritizing areas for continual improvement. "Continuous improvement is not the same as corrective actions preventative actions (CAPA).[1,11,15]

CONCLUSION:

The goal of a well-characterized method development effort is to develop a reliable method that can be implemented with a high degree of assurance to consistently produce data meeting predefined criteria when operated within defined boundaries. QbD can be applied to the development and evaluation of analytical methods. QbD gives an idea about the process development with very detailed analysis of every single part involved in it that can maintain products quality at extreme level. Quality by Design's steps have accurate understanding of product and process development that can avoid unnecessary variables and problems in manufacturing of product that can evaluate and keep consistency in quality of product.

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FORMULATION AND EVALUATION OF ASCORBIC ACID EFFERVESCENT GRANULES

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ABSTRACT

Effervescent granules have occupied a unique place in the field of pharmaceutics. Widely use in clinical diagnosis of heart burn, urinary tract infection, acidity. The aim of these study to design and optimize effervescent granules of ascorbic acid. Eight different formulation of ascorbic acid effervescent granules was prepared and formulations are made up of chemical ingredients such as citric acid, tartaric acid, sodium bicarbonate and calcium carbonate. Effervescent granules were prepared by heat or fusion method. The study also focused on the water of crystallization concept which is related to the reaction between citric acid, tartaric acid and sodium bicarbonate. Evaluation test were performed such as disintegration test, amount of carbon dioxide, pH of formulation. There were eight different formulation prepared and denoted as F1, F2, F3, F4, F5, F6, F7 and F8. Fifth formulation that is F5 gives precise result. They give significantly low disintegration time, pH within range and also amount of carbon dioxide in accepted range. F5 give significant result as compared to other formulations.

KEYWORDS:- Effervescent granules, Disintegration time, Damped mass, Heat method, Water of crystallization, Ascorbic acid.

INTRODUCTION

Effervescence is Latin word it means escape of gas from an aqueous solution. Effervescent granules have short half-life as react rapidly with polar solvent or water. There is a liberation of carbon dioxide gas due to chemical reaction between acid and base.[1]

Effervescent granules are one of the solid dosages forms that is taken orally. In daily life, use of effervescent preparation is increasingly due to many advantages such as good stability, quickly dissolve, masking of unpleasant taste and ease administration with highly compliance in patients with difficulty in swallowing of pills and tablet. The bioavailability of low absorbed drugs can be increased by effervescent granules preparation.[2]

Effervescent granules are spherical in shape and very coarse in nature. They were prepared by hot method. Effervescent granules are not administered directly. They are intended to be dispersed in water before use. Effervescent granules are a type of compound powder. For dissolution of effervescent granules only water is used due to acid dissociate in the water and produce hydrogen ions which is needed for evolution carbon dioxide gas.[3]

Due to the high content of carbonate salt, upon the ingestion of drug solution, the gastric pH is temporarily elevated, resulting in first gastric emptying. This in turn promotes drug adsorption from the upper small intestine, which is primary site of drug absorption. Effervescent granules are responsible for higher bioavailability and fast disintegration rates. Within a couple of minutes, the granules are completely dissolved and the drug become available in solution.[4]

The ideal disintegration time for effervescent granules is 6 to 9 sec. While disintegration time for uncoated tablet, coated tablet, film coated tablet and enteric coated tablet is 15min, 60min, 30min and 60min respectively.[5]

Ascorbic acid or vitamin C is water soluble vitamin. They widely used in prevention of scurvy disease. They also prevent oxidation of molecules inside a body. So, they act as potent antioxidant agent. Vitamin C naturally found in citrus fruit, lemon, oranges. Ascorbic acid involved in production of collagen fibres. Also play a vital role in diagnosis of cancer. Vitamin C is potent anti-inflammatory, antibacterial, immunostimulant agent. It is potent antioxidant and cofactor of gene regulating enzymes. Ascorbic acid enhanced the action of B cells and T cells.[6]

The scientist research on the deficiency of vitamin C cause major disease known as scurvy. Ascorbic acid is essential part of diet. Bruising, bleeding gums, weakness, fatigue and rash are among scurvy symptoms. Minimum intake of ascorbic acid causes haemodynamic instability.[7]

Concept of water crystallization

Crystals of some compound seem to be dry or anhydrous but actually contain some amount of water molecule attached to them. This water molecule is called water of crystallization. Fixed number of water molecules present in formula unit of salt. Hydrated salt contain water in its structure that cause crystallization.

When all excipients and active drug mixed together in a clean porcelain dish. As the heat were provided to the porcelain dish and powder mixture get converted into damp mass or lumps due to water of crystallization. When heat is provided water molecules is released out and water of crystallization is taken place.

Citric acid is reacted with sodium bicarbonate is gives sodium citrate, carbon dioxide, water molecules are release

$$C6H8O7 + 3NaHCO3 \longrightarrow C6H5Na3O + 3CO + 3H2O$$

Tartaric acid is reacted with sodium bicarbonate it gives sodium tartrate, carbon dioxide, water molecule is release.

$$C4H6O6 + 2NaHCO3 \longrightarrow C4H5Na3O + 3CO + 3H2$$ [8]

MATERIALS AND METHODS

Chemical used for this formulation are purchased from SD Lab of Mumbai. The excipients were used for preparation such as- Sodium bicarbonate (S D LAB CHEM MUMBAI), Ascorbic acid (S D LAB CHEM MUMBAI), Calcium bicarbonate (S D LAB MUMBAI), Citric acid (S D LAB MUMABAI), Tartaric acid (S D LALB MUMBAI)

Instruments – Hot Plate, Weighing Balance

Glassware – Volumetric flask, stirrer, beaker

Preparation of effervescent granules

There were six formulations of effervescent granules are prepared. Formulation batches are denoted by symbol F.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Ascorbic acid	1gm	1gm	1gm	1gm	1gm	1gm	1gm	1gm
Citric acid	1gm	0.1gm	1gm	0.1gm	0.1gm	1gm	1gm	0.1gm
Tartaric acid	0.1gm	1gm	1gm	0.1gm	0.1gm	1gm	0.1gm	1gm
Sodium bicarbonate	1.5gm	1.5gm	1gm	1.5gm	1gm	1.5gm	1gm	1gm
Calcium Carbonate	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm

Heat method or fusion method

All excipients were accurately weighted and added into clean porcelain dish. These porcelain dishes were placed on the hot plate at 50 degrees Celsius. There is a formation of lumps due to loss of water. After formation of damped mass, they were passed through sieve number 12 as obtained granules. These granules were dried at 60-degree Celsius.[9]

Evaluation test

1) Disintegration time

About 1gm of effervescent granules was added in 50ml water at 25 degrees Celsius. The stop watch were started. The granules when enter into water they instantly dispersed, formation of bubbles and carbon dioxide is released. When liberation of gas is stopped than, that time is consider as disintegration time.[10]

2) Amount of carbon dioxide

This method is used to determine the amount of carbon dioxide liberate from effervescent granules. weight of empty 50ml volumetric flask was taken (W1). About 50ml of prepared sulfuric acid solution was added in flask. One gram of effervescent granules was added into solution. After addition of granules there was formation of bubbles and CO2 was liberated from solution. A weight of flask after liberation of gas was taken (W2). A difference between W1 and W2 was calculate. This weight or value consider as total amount of liberation of carbon dioxide (T).[11]

By formula,

W1 + 500mg - W2 = T

Method of preparation of 50 ml sulfuric acid –

A clean and dry 50 ml volumetric flask was used. In flask 15-20 drops of water were added and then 10 ml of concentrated sulfuric acid was added. Final volume was made up to 50 ml with water.

3) pH of solution

About 1gm of effervescent granules was added in 50ml of water at 25 degrees Celsius the effervescent granules were kept in the beaker which allowed it completely to dissolve. The pH was measured using digital pH apparatus.[12]

RESULT

Evaluation test were performed on each formulation, the pH and disintegration time was shown in table

Table 2: pH and Disintegration time of effervescent granules.

Formulation Batches	pH	Disintegration time (sec)
F1	4.8	9.5
F2	4.4	9.1
F3	2.75	8.8
F4	6.1	8.5
F5	4.1	6.3
F6	4.0	6.7
F7	3.9	6.9
F8	3.5	6.8

Amount of carbon dioxide test was performed on the formulation the total liberation of carbo dioxide was shown in table 3

Table 3: Total amount of carbon dioxide gas evolved from effervescent granules.

Formulation	Weight of empty flask (W1)	Weight of flask after addition of 500mg effervescent granules	Weight of flask after liberation of gas (W2)	Total CO2 evolved
F1	85.672	86.112	86.0569	0.056 gm
F2	86.210	86.710	86.596	0.141 gm
F3	86.150	86.650	86.122	0.528 gm
F4	93.662	94.162	93.621	0.541 gm
F5	89.991	90.491	90.368	0.123 gm
F6	98.771	99.271	98.677	0.594 gm
F7	91.263	91.763	91.261	0.492 gm
F8	93.220	93.720	93.505	0.215 gm

DISCUSSION

The pH values of effervescent granules were in range between 2 to 6. This pH values of granules were considered as ideal values according to IP. The amount of carbon dioxide ranged between 0.528gm to 0.141gm for all formulations F1to F8 table 3. The formulation batches contain high amount of sodium bicarbonate and tartaric acid than produced more carbon dioxide. But the magnitude of effect for sodium bicarbonate was more than tartaric acid. At low concentration citric acid produce more carbon dioxide.

CONCLUSION

According to study it is confirm that F5 give significant result. they were prepared by using 1gm ascorbic acid, 0.1 citric acid ,0.1gm tartaric acid 1gm sodium bicarbonate ,0.203 calcium carbonate, 0.015gm sodium saccharin, 0.03gm aspartame. F5 gives pH value 4.1, disintegration time 6.3sec and amount of carbon dioxide is 0.123gm all outcomes of evaluation test within a standard range. Therefore, F5 formulation consider as an ideal formation of ascorbic acid effervescent granules.

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STABILITY STUDY OF DIFFERENT MARKETED BRANDS OF DICLOFENAC SODIUM AND PARACETAMOL TABLETS BY USING SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The forced degradation study of different marketed formulation of combined tablets of Paracetamol and Diclofenac was performed. Paracetamol and Diclofenac was exposed to different conditions according to International Conference on Harmonization guideline. The amount of degradation product can be calculated with the help of UV spectrophotometer. The official test limits according to Indian Pharmacopoeia/United States Pharmacopoeia were considered. The marketed brands of Diclofenac Sodium and Paracetamol are Deemol-500,DIK-MR,Oxan Plus used in study as this method is less time consuming and simple and cost effective. The brands Deemol-500, DIK-MR, Oxan Plus when come in contact with different degradation parameters acid, base, and UV treatments they show negligible effects.

KEYWORDS: UV Spectroscopy, Paracetamol and Diclofenac Sodium, UV Cabinet, Degradation.

INTRODUCTION

Diclofenac Sodium and Paracetamol belongs to a class of medications known as a non-steroidal anti-inflammatory drug (NSAID) or pain killer. Combined Diclofenac Sodium and Paracetamol are widely useful for the treatment of painful musculoskeletal joint conditions like osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.[1]

Diclofenac and Paracetamol contain Diclofenac (analgesic) and Paracetamol (fever reducer/mild analgesic) effective against painful musculoskeletal pain, joint pain, and skeletal muscle spasms.[2] Diclofenac works by blocking the action of a chemical messenger known as cyclooxygenase (COX) which causes pain and swelling at the injured or damaged tissue site. On the other hand, Paracetamol acts as a mild analgesic (mild pain reducer) and antipyretic (fever reducer), which enhances the pain relief action Diclofenac.[2] Additionally, it also helps to relieve toothache, ear pain, backache and other musculoskeletal related pain.

Spectrophotometric technique is based on measuring the absorption of a monochromatic light in the near ultraviolet region (200-380 nm) by colourless complex. UV spectrophotometer can also be use for stress degradation.[3] According to International Conference of Harmonization (ICH) guideline the active pharmaceutical ingredient is focused to various forced degradation conditions involves photo acid/base stress testing, temperature, photo degradation and or with humidity, time, pH variation (low and high)[4] Thermal and/or humidity stress testing is performed by exposing the drug substance to thermal/humidity conditions in due course which causes the substance to degrade forcefully to its main components.[5] UV degradation is a main trouble in frequent UV unstable products which are made up of natural and synthetic polymers as they break or disintegrate when exposed to constant sunlight. As the attack is depend on the extent and degree of exposure, nonstop exposure is a more serious problem than intermittent exposure. Acid or base stress testing is used for the evaluation of forced degradation of a drug substance.[6] This test involves degradation of a drug substance by exposure to basic or acidic medium over time to its primary degradation products. Acid or base hydrolysis occur in labile carbonyl functional groups which are esters (lactones), amides (lactams), aryl amines, carbamates, imides, imines and alcohols. Forced degradation is capable of demonstrating that the chosen technique is stability indicating that is the technique use to identify the increase in the degradation product and the subsequent loss of active components.

Simultaneous Equation Method

Simultaneous equation (SE) is typically applied to estimate drug combinations that contain two drugs or more than two drugs in combined dosage form. If the sample contains two absorbing components x & y and each absorbs at the λ_{max} of the other (λ_1& λ_2). It may be

possible to determine both drugs by the technique of simultaneous.[7]

MATERIALS

Paracetamol and Diclofenac brands used were Deemol-500 500 mg tablets of Saint Michael Biotech, DIK-MR 500 mg tablets of Remex Healthcare Limited and Oxan Plus 500 mg tablets of Creative Remedies (AHD) Limited,1 mol/L HCl, 0.1N NaOH, reference standard Diclofenac Sodium, Paracetamol and distilled water were used, Pyrex type stirrer, measuring cylinder, pipette, beaker and volumetric flask were used, The glassware washed with distilled water, UV Cabinet, Weighing Balance, and Spectrophotometer were used in this study.

Methods

1. Preparation of standard solution of Paracetamol

30 mg pure sample of Paracetamol weighed and dissolved in 15 ml 0.1N NaOH and distilled water and final volume is adjusted with 100 ml l to obtain stock solution of 300 ug/ml, from this stock solution take 1 ml volume and diluted to 10 ml to get 30 µg/ml solution.[8]

2. Preparation of standard solution of Diclofenac Sodium

30 mg pure sample of Diclofenac Sodium weighed and dissolved in 15 ml 0.1N NaOH and distilled water and final volume is adjusted with 100 ml to obtain stock solution of 300 µg/ml, from this stock solution 1 ml volume was taken and diluted to 10 ml to get 30 µg/ml solution.[9]

3. Preparation of 0.1 N NaOH

2 gm of NaOH pellets were weighed and its solution is prepared in 500 ml volumetric flask by distilled water.[10]

4. Preparation of test/sample solution of combined tablet of Paracetamol and Diclofenac Sodium

10 tablets of combined tablet of Paracetamol and Diclofenac Sodium of Deemol-500, DIK-MR, and Oxan Plus brand were taken, weighed and average weight was calculated then triturated to form homogeneous mixture. Quantity of triturated powder equivalent to 30 mg of Paracetamol and 30 mg of Diclofenac Sodium transferred to separate 100 ml of volumetric flasks and 15ml 0.1N NaOH and distilled water was added and final volume adjusted to 100 ml then diluted to get 30 µg/ml of Paracetamol and Diclofenac Sodium respectively considered as test sample solution.[11]

5. Absorbance of standard solution of Paracetamol was taken λ_1 and λ_2.
6. Absorbance of standard solution of Diclofenac Sodium was taken at λ_1 and λ_2.
7. $^{1\%}$1cm was calculated for each at λ_1 and λ_2 and noted mean $A^{1\%}$1cm taken for calculation.
8. λ_1 and λ_2 were obtained after scanning standard solution range 400-200 nm at UV spectrophotometer.
9. Absorbance was recorded for test sample solution of combined tablet at λ_1 and λ_2.
10. Calculations were done for concentration (µg/ml) using simultaneous equation method and reported.
11. Percent purity and standard deviation were calculated and reported.
12. Final result was reported as percent purity +5.0.

Procedure for forced degradation studies

1. For acid

Forced degradation of drug substance in acidic media was performed by taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, and Oxan Plus in 6 separate test tubes, and then 5 ml of 1 mol/L HCl was added in each test tube. The sample was left for 30 min. Solution was transferred to a separated cuvette after the time period completion and UV absorbance of the solution was measured at the 257 nm and 275 nm wavelength.

2. For base

Forced degradation of drug substance in basic media was performed taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, Oxan Plus in 6 separate test tubes, then 5 ml of NaoH was added in each test tube and the sample was left for 30 min, and then UV absorbance of solution was measured at the 257 nm and 275 nm wavelength.

3. For UV light

Forced degradation of drug substance in UV light was performed by taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, Oxan Plus in 6 separate test tubes then 5 ml of water was added in each test tube and these test tubes were exposed to UV light for 30 min, and then UV absorbance of solution was measured at the 257 nm and 275 nm wavelength.[10]

RESULT AND DISCUSSION

We have conducted the degradation study on three brands of Diclofenac Sodium using were Deemol-500 500 mg tablets of Saint Michael Biotech, DIK-MR 500 mg tablets of Remex Healthcare Limited and Oxan Plus 500 mg tablets of Creative Remedies (AHD) Limited. When Diclofenac Sodium brands were treated with the 1 mol/L HCL, it showed availability of different brands. When Diclofenac Sodium brands were treated with the 0.1N NaOH drugs, it showed the increased availability and absorbance respectively. When exposed to UV light, changes had been observed respectively. Table 1 represents the UV absorption of different brands of the Diclofenac Sodium before and after exposing to the degradation environment. We concluded according to our results that when the Deemol-500 introduced into acidic medium 1 mol/L HCL, it showed degradation of Paracetamol that is (18.93%) and Diclofenac Sodium that is (72.73) DIK-MR showed degradation of Paracetamol (63.13%) and Diclofenac Sodium (68.50%) in acidic medium. Oxan Plus also gave greater results of Paracetamol that is (76.20%) and Diclofenac Sodium (63.62%) on exposure to acidic medium respectively. Similarly on exposure to 0.1N NaOH basic medium, the Deemol-500 showed the degradation of Paracetamol (17.43%) and Diclofenac Sodium (74.29%) respectively.

whereas DIK-MR showed degradation of Paracetamol (60.20%) and Diclofenac Sodium (61%) while Oxan Plus gave moderate results which are Paracetamol (73%) and Diclofenac Sodium (61.33%) on exposure to basic medium respectively. When Deemol-500 exposed to UV light it gives results Paracetamol (42.48%) and Diclofenac Sodium (71.20%) respectively, whereas DIK-MR shows (64.66%) and (63%) degradation of Paracetamol and Diclofenac Sodium respectively, Oxan Plus shows (70.73%) of Paracetamol and (64%) of Diclofenac Sodium degradation when exposed to UV light for 30 min, it also showed minor changes in concentration respectively for degradation studies. Results of degradation studies are given in Tables 1 and 2.

Table 1: Absorbance of different brands of Paracetamol and Diclofenac Sodium.

Tablet	Treatment	Absorbance	
		Paracetamol	Diclofenac Sodium
Deemol-500	Before	λ_1=257=0.8298 λ_2=275=0.7972	λ_1=257=0.6317 λ_2=275=0.6541
	Acid Treatment	λ_1=257=0.5025 λ_2=275=0.4932	λ_1=257=0.4220 λ_2=275=0.3556
	Base Treatment	λ_1=257=0.3818 λ_2=275=0.3219	λ_1=257=0.2825 λ_2=275=0.2522
	UV Treatment	λ_1=257=0.5620 λ_2=275=0.4820	λ_1=257=0.5180 λ_2=275=0.4280
DIK-MR	Before	λ_1=257=0.6888 λ_2=275=0.6532	λ_1=257=0.7312 λ_2=275=0.6920
	Acid Treatment	λ_1=257=0.6620 λ_2=275=0.6432	λ_1=257=0.4212 λ_2=275=0.3813
	Base Treatment	λ_1=257=0.4020 λ_2=275=0.3813	λ_1=257=0.3930 λ_2=275=0.3720
	UV Treatment	λ_1=257=0.6020 λ_2=275=0.5813	λ_1=257=0.5520 λ_2=275=0.5218
Oxan Plus	Before	λ_1=257=0.7810 λ_2=275=0.7220	λ_1=257=0.7312 λ_2=275=0.6810
	Acid Treatment	λ_1=257=0.7030 λ_2=275=0.3820	λ_1=257=0.6030 λ_2=275=0.6820
	Base Treatment	λ_1=257=0.4513 λ_2=275=0.4130	λ_1=257=0.5820 λ_2=275=0.5412
	UV Treatment	λ_1=257=0.6614 λ_2=275=0.6413	λ_1=257=0.6830 λ_2=275=0.6520

Table 2: Absorbance of different brand of Paracetamol and Diclofenac Sodium in percentage.

Tablet	Treatment	Absorbance	
		Paracetamol	Diclofenac Sodium
Deemol-500	Before	57%	75.11%
	Acid Treatment	18.93%	72.73%
	Base Treatment	17.43%	74.29%
	UV Treatment	42.48%	71.20%
DIK-MR	Before	75.11%	70.03%
	Acid Treatment	72.73%	68.50%
	Base Treatment	74.29%	61.05%
	UV Treatment	71.20%	63.20%
Oxan Plus	Before	78%	66.15%
	Acid Treatment	76.20%	63.62%
	Base Treatment	73%	61.33%
	UV Treatment	70.23%	64.10%

In present study the stability testing was performed as per ICH guidelines. The degradation like Acid degradation, Base degradation and UV degradation were performed and trace degradation was found.

CONCLUSION

It was used to study the degradation as per ICH guideline. Paracetamol and Diclofenac Sodium was found to be degraded in almost all type of condition (acidic, basic and uv light) Degradation of different brands of Paracetamol and Diclofenac Sodium were

carried out in different condition showed more changes in different medium.

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POST MARKET IN-VITRO QUALITY CONTROL EVALUATION FOR DIFFERENT BRANDS OF PARACETAMOL TABLETS AVAILABLE IN INDIAN MARKET

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ABSTRACT

Paracetamol scientifically known as acetaminophen is quite popular over-the-counter (OTC) form of analgesic and antipyretic. It is widely used in most of the countries. Acetaminophen is active metabolite of phenacetin. Chemically, it is 4-hydroxy acetanilide (acetaminophen). Paracetamol has medical uses such as it is commonly used for the relief from headache, minor pains, aches and is a major ingredient in numerous cold and flu remedies. It can be used in all ages of people for reducing fever. The main objective of this case study is to check and compare the quality of marketed finished product of paracetamol tablet formulation which are locally and commonly available in Indian pharmaceutical market manufactured by various pharmaceutical companies as India is one of the biggest pharmaceutical product producers in the world. This study includes the randomly selected four different brands (A, B, C, D) as Calpol 500, Pyrigesic, Pacimol 500 and Febrex of paracetamol conventional tablets of 500 mg strength of active pharmaceutical ingredient of paracetamol from local medical pharmacy stores. These different brand tablets were compared by the in-vitro test accordingly the test procedure given in IP and USP standards and unofficial test standards which are also integral part of this quality control tests. The test parameters for quality assessment and evaluation of tablets are weight variation i.e., weight uniformity, friability, hardness, disintegration time and drug assay content by UV spectrophotometer were performed as per standard of pharmacopoeias. The results are then formed according to the limit ranges of pharmacopeial standards.

KEYWORDS: Paracetamol, Quality Control, Weight Variation, Disintegration Time, Drug Assay.

1. INTRODUCTION

Paracetamol or acetaminophen is active metabolites of phenacetin. It is a widely used over-the-counter analgesic and antipyretic. Chemically, it is 4-hydroxy acetanilide (acetaminophen). Paracetamol is approved for reducing fever in people of all ages. It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. It is classified as a non-steroidal anti-inflammatory drug (NSAID) by some sources and not as an NSAID by others, while most sources implicitly distinguish them, for example by mentioning both NSAIDs and paracetamol in the same sentence. Paracetamol has few anti-inflammatory effects in comparison to NSAIDs. Paracetamol is available in different dosage form: tablet, capsules, drops, elixirs, suspension and suppositories.[1,2]

The concept of total energy quality control refers to the produce a perfect product by a series of measures requiring an organized effort by the entire company to prevent or eliminate errors at every stage in production. Although the responsibility for assuring product quality belongs principally to quality assurance personnel, it involves many departments and disciplined lines within a company. To be effective, it must be supported by a team effort. Quality must be built into a drug product during product and process design, and it is influenced by the physical plant design, space, ventilation, cleanliness, and sanitation during routine production. The product and process design begins in research and development, and includes preformulation and physical, chemical, therapeutic, and toxicologic considerations.[3,4]

The assurance of product quality depends on more than just proper sampling and adequate testing of various components and the finished dosage form. Prime responsibility of maintaining product quality during production rests with the manufacturing department.[3,4]

For the conventional tablets weight variation, friability, disintegration, dissolution, drug assay, uniformity of contents is the evaluation test those are required to perform to confirm about the quality of tablet. Friability is the tested for a tablet to see whether the tablet is stable to abrasion or not, it is tested by using Roche friabilator and 1% maximum loss in the weight after friability test is allowed. Weight variation test is performed to check that the manufactured tablets have a uniform weight. Disintegration test is performed to see how much time a tablet takes to break down in to the small particles. The drug assay study

provides the information how much practically available in the given dosage form and after comparing with the theoretical value, a result about the efficacy can be given.[3,4,5]

2. MATERIALS

For the present case study different paracetamol tablets brands were used such as Calpol 500 mg tablets of GlaxoSmithKline Pharmaceutical Limited, Pyrigesic 500 mg tablets of East India Pharmaceutical Works Limited, Pacimol 500 mg tablets of Ipca Laboratories Limited and Febrex Indoco Remedies Limited. 0.1M NaOH, reference standard Paracetamol, double distilled deionized filtered water is used. Pyrex type stirrer, measuring cylinder, pipette, funnel, beaker and volumetric flask, petri dish, cuvettes, butter paper, Whatman filter paper No. 44, spatula, tissue paper were used. Freshly laboratory prepared distilled water was used to wash glasswares. Wensar high precision balance, Friabilator, Disintegrator, Monsento hardness tester, Shimadzu UV spectrophotometer, Systronics UV vis double beam spectrophotometer.

3. METHODS

3.1. Weight variation test

Weigh individually randomly selected 20 tablets and calculate the weight of each individual tablet. Not more than 2 tablets of individual weight deviates than the average weight by the percentage given as per Indian Pharmacopoeia limits. The process repeated same for each brand of conventional paracetamol tablets.[6,7]

3.2. Friability test

For each of the brands, 10 tablets were selected and carefully dusted before testing and weighed. Then the tablets were placed in the drum of friability tester and rotated at the speed of 25rpm for 4 minutes. After 100 revolutions and de-dusting, tablets were re-weighed and the friability percentage was calculated by the following equation:[6,7,8]

Formula: $\% \text{ Friability} = \frac{\text{Initial weight } (W_1) - \text{Final weight } (W_2)}{\text{Initial Weight } (W_1)} \times 100$

3.3. Disintegration test

Six tablets were randomly selected from each brand and placed in the disintegration apparatus, which is filled by 900 mL of distilled water (disintegration medium) maintained at 37±1°C. The time taken to disintegrate the tablet and pass through the mesh was recorded and

the mean of time taken was calculated. The process repeated same for each brand of conventional paracetamol tablet.[6,7,8]

3.4. Hardness test

To conduct the hardness test, 10 tablets of each brand were randomly selected and the crushing strength of the tablets was measured by using instrument Monsento hardness tester. The average hardness of the tablet was calculated. The process repeated same for each brand of conventional paracetamol tablet.[8,9]

3.5. Assay of paracetamol

Weigh and powder 20 tablets. A quantity of powder containing about 0.15 mg of paracetamol, add 50 ml of 0.1M NaOH, dilute with 100 ml of water, shake for 15 minutes add sufficient water to produce 200 ml. Mix, filter and dilute 10.0 ml of filtrate to 100 ml with water. To 10.0 ml of resulting solution add 10 ml of 0.1M NaOH, dilute to 100.0 ml with water and mix. Measure the absorbance of the resulting solution at maximum at about 257 nm. Calculate the content of $C_8H_9NO_2$ taking 715 as specific absorbance at 257 nm.[10]

4. RESULT AND DISCUSSION

After random selection of tablets, the weight variation, friability, disintegration time, hardness and drug assay were performed as per the Indian Pharmacopoeia and United States Pharmacopoeia.

4.1. Weight variation test

The result of weight variation of twenty randomly selected tablets of each brand are as follows:

Table 1: Weight variation test for four different brands of paracetamol tablets.

No. of tablets	Calpol 500 (A) Avg. Weight = 629 mg			Pyrigesic (B) Avg. Weight = 568 mg		
	Weight (mg)	Weight Variation	% Weight Variation	Weight (mg)	Weight Variation	% Weight Variation
1	662 mg	7	1	569 mg	1	0.17
2	628 mg	1	0.15	567 mg	1	0.17
3	626 mg	3	0.47	566 mg	2	0.35
4	629 mg	0	0	580 mg	12	2.11
5	629 mg	0	0	563 mg	5	0.88
6	633 mg	4	0.63	566 mg	2	0.35
7	629 mg	0	0	565 mg	3	0.52
8	624 mg	5	0.79	573 mg	5	0.88

9	630 mg	1	0.15	566 mg	2	0.35
10	630 mg	1	0.15	570 mg	2	0.35
11	631 mg	2	0.31	576 mg	8	1.40
12	622 mg	7	1	571 mg	3	0.52
13	625 mg	4	0.63	563 mg	5	0.88
14	627 mg	2	0.31	556 mg	12	2.11
15	635 mg	6	0.95	576 mg	8	1.40
16	638 mg	9	1.43	569 mg	1	0.17
17	634 mg	8	1.27	576 mg	8	1.40
18	645 mg	18	2.54	571 mg	3	0.52
19	626 mg	3	0.47	564 mg	4	0.70
20	631 mg	2	0.31	570 mg	2	0.35

No. of Tablets	**Pacimol 500 (C) Avg. Weight = 560 mg**			**Febrex (D) Avg. Weight = 581 mg**		
	Weight (mg)	Weight Variation	% Weight Variation	Weight (mg)	Weight Variation	% Weight Variation
1	569 mg	9	1.60	581 mg	0	0
2	565 mg	5	0.89	581 mg	0	0
3	567 mg	7	1.25	577 mg	4	0.68
4	557 mg	3	0.53	579 mg	2	0.34
5	569 mg	9	1.60	579 mg	2	0.34
6	542 mg	18	3.21	583 mg	2	0.34
7	563 mg	3	0.53	593 mg	12	2.06
8	558 mg	2	0.35	581 mg	0	0
9	562 mg	2	0.35	586 mg	5	0.86
10	569 mg	9	1.60	579 mg	2	0.34
11	557 mg	3	0.53	586 mg	5	0.86
12	564 mg	4	0.71	580 mg	1	0.17
13	559 mg	1	0.71	575 mg	6	1.03
14	558 mg	2	0.35	583 mg	2	0.34
15	560 mg	0	0	576 mg	5	0.86
16	559 mg	1	0.17	577 mg	4	0.68
17	555 mg	5	0.89	589 mg	8	1.37
18	553 mg	7	1.25	585 mg	4	0.68
19	560 mg	0	0	583 mg	2	0.34
20	557 mg	3	0.53	567 mg	14	2.40

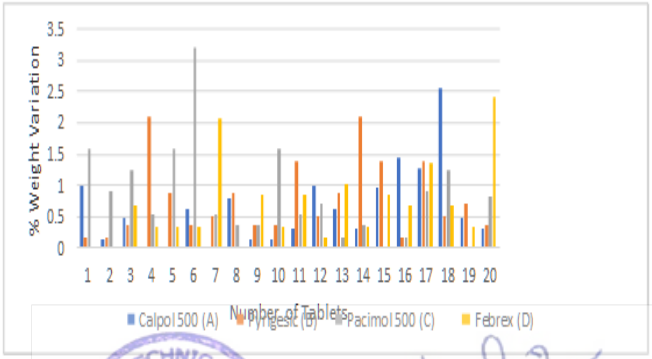

Figure 1: Weight variation test for four different brands of paracetamol tablets.

As per the IP the weight variation limit for the tablet which is having the weight equal or more than 250 mg is ±5 % and the given results shown that all the twenty randomly selected tablets of all four brands are having weight variation less than ±5 % which proves that the four brands (A, B, C, D) of paracetamol tablets those are available in the Indian pharmaceutical market passed the official weight variation test.

4.2. Friability test: The friability test was conducted in Roche friabilator by using 10 tablets, the results of all different brands are as follows:

Table 2: Friability test for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
% Friability	0.61%	0.77%	0.87%	0.27%

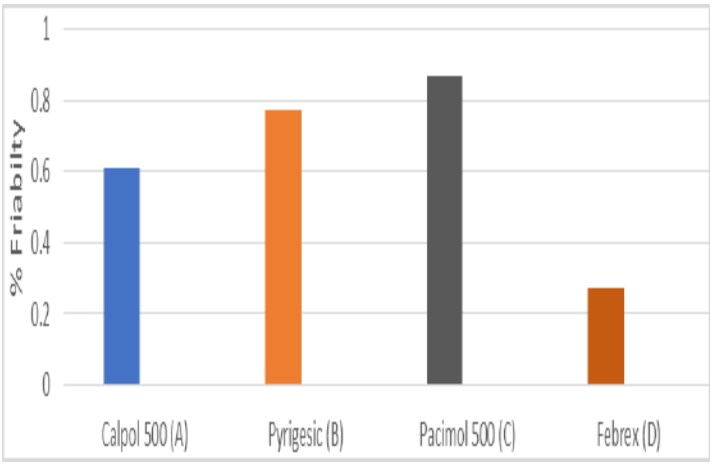

Figure 2: Friability test for four different brand of paracetamol tablets.

The results of friability test shows that all the four brands of paracetamol tablets for Calpol 500 (A) is 0.61%, Pyrigesic (B) is 0.77%, Pacimol 500 (C) is 0.87% and Febrex (D) is 0.27% which are under the pharmacopoeia limits 1% means as per IP standard. All these brands of paracetamol tablets those are available in Indian pharmaceutical market are having good strength and can tolerate the shocks during transportation handling of these tablets.

4.3. Disintegration test: The disintegration test was performed in the distilled water at 37 ±2°C in the Almicro Disintegration instrument. The results of all four brands are as follows:

Table 3: Disintegration time for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
Disintegration time	1 min.	2:49 min.	2 min.	9 min.

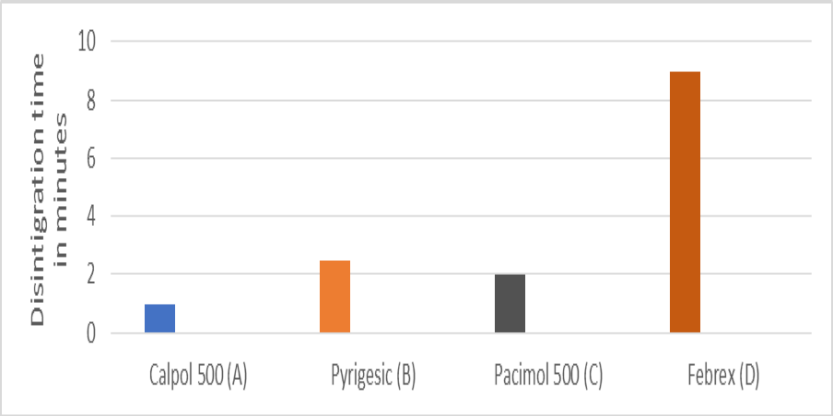

Figure 3: Disintegration time for four different brands of paracetamol tablets.

The results of disintegration test shows that all four different brands of paracetamol are as Calpol 500 (A) is 1 min., Pyrigesic (B) is 2:49 min., Pacimol 500 (C) is 2 min. and Febrex (D) is 9 min. tablet disintegration time is less than the standard disintegration time (15 minute) for uncoated tablet as per IP standards which proves that all these brands of paracetamol tablet pass the quality control limits as per the pharmacopoeia. The brand A disintegration time is about 1 min. means it disintegrates very fast so it might be possible that the drug will be available very fast for absorption as well as the onset of time will be very less.

4.4. Hardness test: The hardness test is conducted with Monsanto hardness tester by using 10 tablets, the results of all different brands as follows:

Table 4: Hardness test for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
Hardness Kg/cm^2	6.2 kg/cm^2	7.6 kg/cm^2	10.2 kg/cm^2	11.4 kg/cm^2

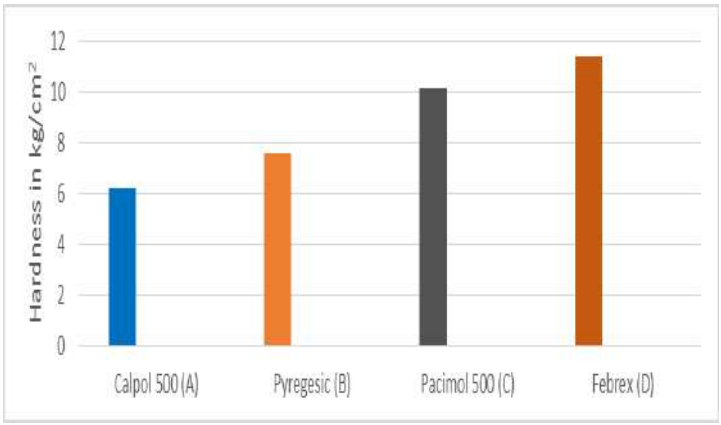

Figure 4: Hardness test for four different brand of paracetamol tablets.

The result of hardness test for all four brands of paracetamol tablets are Calpol 500 (A) is 6.2 kg/cm^2, Pyrigesic (B) is 7.6 kg/cm^2, Pacimol 500 (C) is 10.2 kg/cm^2, Febrex (D) is 11.4 kg/cm^2. The limit for hardness as per USP is 4 to 10 kg/cm^2 as per the pharmacopoeia the range of hardness closely near to 10 kg/cm^2 can be approved to pass the test. High crushing strength is attributed to a high compression force, high binder concentration or excess volume of granulating fluid. Although all uncoated brands of Paracetamol tablets have very high hardness, they still exhibited very good quality control parameters such as dissolution profile, disintegration time and chemical content determination. This indicates that hardness test is not a critical quality control parameter.

4.5. Drug assay content: To confirm the amount of paracetamol drug in the tablet drug assay was performed for all four different brands, the results are as follows:

Table 5: Drug assay content for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
%Drug content	95.13%	97.20%	102%	98%

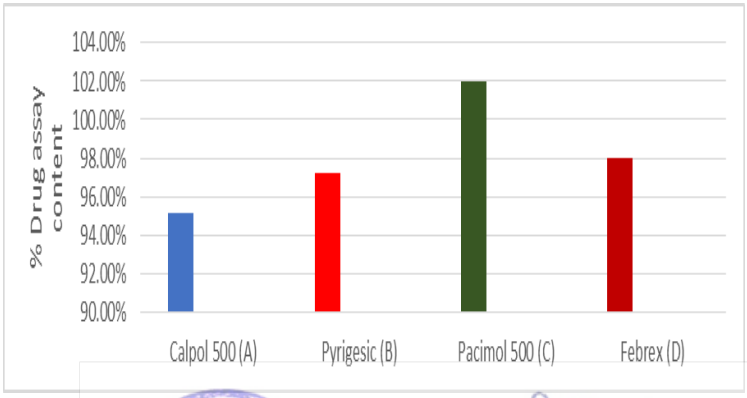

Figure 5: Drug assay content of four different brands of paracetamol tablets.

The results of drug assay of four different brands of paracetamol tablets are Calpol 500 (A) is 95.13%, Pyrigesic (B) is 97.20%, Pacimol 500 (C) is 102% and Febrex (D) is 98%. It shows that amount of paracetamol drug available in all these formulations is between 95% to 105% as per IP standard range it means drug are available as per pharmacological action. There was no statistically significant difference between the different brands of the paracetamol tablets. Furthermore, all the brands of the tablets passed the test for the content of paracetamol.

CONCLUSION

As post market evaluation of approved medicines is essential to monitor, it will meet the desired standards of quality, safety and therapeutic efficacy of medicine for the consumers. For present case study all quality control test for four different brands of conventional paracetamol tablets available in Indian market were assessed. In present case study different quality control parameters were studied such as weight variation, friability, disintegration time, hardness and drug assay content. All values will be compared with the standards of Indian Pharmacopoeia and United States Pharmacopoeia. This study revealed that all brands of paracetamol tablets met the IP and USP specifications.

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TRADITIONAL HERBAL SYRUP: A REVIEW

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ABSTRACT
Syrups, solutions, emulsions, or suspensions containing one or more active ingredients in an appropriate vehicle are examples of liquid oral formulations. Some oral liquid formulations are made by diluting concentrated liquid preparations for drops or syrups in a suitable vehicle. Syrups are aqueous formulations with a sweet flavor and the appropriate viscosity. A suitable combination of polyols, sweetening agents, aromatic, and flavoring agents can be used to achieve an acceptable sweet taste. The stability of the active and inactive ingredients is a significant concern for the formulator in liquid oral formulation. Active ingredients are typically lower stability in aqueous formulations than in solid dosage forms. As a result, it is critical to stabilize and preserve the water-containing liquid oral formulation. Herbal medicine refers to the use of extract for therapeutic purposes, and the majority of herbal syrup was initially obtained from plants. In addition to alternative dosages from natural medications, herbal syrups were also developed. Herbal syrup is now utilized to treat a variety of conditions and to alleviate disease symptoms. Herbal syrup is characterized as a prepared, combined, and concentrated decoction with honey sugar or, on occasion, alcohol. The base of this syrup is a strong herbal decoction, which is thickened and preserved by mixing it with sugar honey. Herbal plants and formulations are used to treat a number of illnesses, like as cough syrup and other illnesses. This review discusses the extraction processes, standardization, phytochemical analysis and evaluation parameters of herbal syrup.

INTRODUCTION
Herbal Syrup: Herbal syrup it is a defined as a prepared and combination and concentration decoction with Honey sugar or either some time use alcohol. The base of such a syrup is a powerful herbal decoction, and thickening it with sugar honey helps to preserve it. Herbal plants and formulations are used to treat a variety of ailments, including cough syrup and other illnesses. Many varieties of herbal plants are utilized for cough syrup, including pudina, Tulsi, Cinnamon, and honey, and the entire plant has been used for manufacturing herbal medicine for many years.

Herbal formulation is the most often used method of health treatment in both developed and developing countries. The cough syrup medication is a liquid dose form, and the use of oral liquid pharmaceuticals has been confirmed on the basis of basic simplicity of administration to those who have difficulty swallowing solid prescription dosages. Syrup is a concentrated solution made of sugar and pure water. In syrup, as opposed to other types of syrup solutions.

The syrup may or may not contain medication or a flavoring agent mixture. Flavored or non-medicated syrup is syrup that does not contain medication but does contain a flavoring agent. Flavored syrup is frequently used as a vehicle for the unpleasant test results of medications (found as) in medicated syrups. The presence of syrup in high concentrations predisposes them to bacterial infection, so they frequently as a preservative.[1]

Syrups are a popular delivery vehicle for anti-tumor medications because they are easier to swallow (ingest) than tablets and capsules. This medication is quickly noticed. There are synthetic cough preparations available, but they have a number of negative side effects. As a result, the current study demonstrated that violet herbal cough syrup contains natural elements with no side effects. In general, health professionals are having difficulty accessing effective and safe natural treatments (therapy). A number of allopathic medication products have not been studied on a large scale, and they are generally solid without knowledge of their mechanism of action or side effects.

Even though the use of complementary medication is sometimes helpful and the confirmation of the effectiveness of some of this all-medication literature is limited, they are frequently sold with the drug store.[2] A successful formulation of liquid as well as other dosage forms necessitates a combination of scientific acumen and pharmaceutical "art".[3] Because harmful changes

occur more easily in solution, oral liquid medicines are gradually being replaced by tablets and capsules. Nonetheless, a large number of liquid oral preparations are still available in the official books.

In fact, the absorption of medicaments in solution from the GI tract into the systemic circulation is expected to be faster than that of other oral dosage forms of the same medicinal agent. Ayurvedic formulations are typically administered orally, and the majority of orally administered Ayurvedic formulations are in the form of a liquid drug or drug combination. Herbal medicinal combination, on the other hand.

Types of herbal syrup

1. Flavored syrup
2. Medicated syrup
3. Artificial syrup.[4]

Herbal syrup is manufactured by combining a concentrated decoction of herbs with honey or sugar, as well as alcohol. The herbal syrup is created through a decoction process. By combining a herb decoction with sugar, the formulation can be thickened and preserved. This was responsible for extending the shelf life of the formulation. The addition of sweetener can also help to improve the palatability of some herbs. The syrup that was eventually obtained was delicious. As defined, it is a thick, sticky liquid made up of a concentrated solution of sugar and water, with or without the addition of flavorings, agents, or medicinal substances.[5]

Although most traditional healthcare systems are effective, they lack proper standardization. Standardization is an crucial step in establishing consistent biological activity, a specified chemical profile, or just a quality assurance programme for herbal formulation production and manufacturing. As a prerequisite for global harmonization, WHO has issued specific guidelines for assessing the safety, efficacy, and quality of herbal medicines. As a result, a polyherbal syrup was created by combining dried powder decoctions of various herbs. The current study includes the standardization of raw materials for their identity, quality, and development of polyherbal syrup, as well as the standardisation of the produced formulation and accelerated stability studies.[6]

IDENTIFICATION, EVALUATION AND STANDARDIZATION OF CRUDE DRUGS

In recent era, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals, and cosmetics. There are around 6000 herbal manufacturers in India. Ayurvedic medications are manufactured in about 4000 units. World Health Organization (WHO) provide guidelines for the herbal standardization and analysis of herbs. WHO Guidelines for Herbal Drug Standardization and Evaluation The WHO guidelines for herbal drugs can be summarized as follows:

1. Identity of the drug: Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
2. Physicochemical character of the drug: Physical and chemical identity, Chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal tests, quantitative estimation techniques, and so on.
3. Pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index etc.
4. Toxicity details: - pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like E. coli, Salmonalla, P.aeroginosa, S. aureus, Enterobacteria etc.
5. Microbial contamination.
6. Radioactive contamination.[7]

Herbal Drugs: The herbal drugs define as whole or plants parts, algae, fungi in unprocessed state usually in dried form but sometimes fresh. Because of the ever-increasing use of plant-based medicines and the rapid growth of the global market for these products, the safety and quality of medicinal plant materials and final herbal medicines has become a major issue for the public health establishment.[8] There is significant diversity in the quality management of such materials and products, which has an influence on population health as contaminants in herbal medicines may represent preventable dangers It has implications for consumers, as well as international trade.[9]

The International Conference of Drug Regulatory Authorities (ICDRA) and the National Centres participating in the WHO Drug Monitoring Programme asked WHO to develop and continuously revise technical guiding principles on quality, safety, and efficacy of herbal medicines in order to reduce the risk of adverse events caused by precarious and low-quality herbal medicines. The process of standardisation is concerned with the physicochemical analysis of crude medication, completed product safety, effectiveness, and consistency evaluation, safety and risk qualifications based on experience, consumer product information stipulation, and product endorsement.[10]

Because polyherbal formulations combine more than one herb to provide the ideal therapeutic effect, evaluation is critical for maintaining the quality and safety of the product. It decreases batch-to-batch variation and assures the efficacy, safety, quality, and sufficiency of polyherbal formulations. This is accomplished by limiting the intrinsic divergence of natural product composition through the application of quality assurance practises to agricultural manufacturing procedures should take into contemplation each and every one phase that adds to the quality of the herbal drugs, specifically

accurate identity of the sample, organoleptic assessment, pharmacogenetic study, volatile matter, quantitative analysis, phytochemical evaluation test for the presence of xenobiotics, microbial load testing, toxicity study and biological activity.[11]

The various parameters for identification, evaluation and standardization.[7,12]

Sr.No.	Methods	Evaluation Parameters
1.	Authentication	A. Parts of plants collect like leaf, flower, root, stolen B. Regional status C. Family D. Biological source E. Chemical constituents
2.	Marphology or Organoleptic evaluation	A. Odour B. Taste C. Size D. Shape E. Special feature
3.	Microscopy evaluation	A. Leaf content B. Trichomes C. Stomata D. Quantitative microscopy
4.	Chemical evaluation	A. Chemical test B. Chemical assay C. Phytochemical screening
5.	Physical evaluation	A. Moisture content B. Viscosity C. Melting point D. Solubility E. Optical rotation F. Refractive index G. Ash value H. Extractive value I. Volatile oil content J. Foreign matter etc.
6.	Biological evaluation	A. Microbial contamination B. Pesticides contamination C. Pharmacological activity of drugs

EXTRACTION TECHNIQUES

Maceration, digestion, percolation, infusion, decoction, hot continuous extraction (Soxhlet), counter current extraction, aqueous-alcoholic extraction by fermentation, supercritical fluid extraction, microwave-assisted extraction, ultrasound extraction (sonication), and distillation techniques (steam distillation, water distillation, phytonic extraction) are all examples of medicinal plant extraction techniques (with hydro fluorocarbon solvents). Hydro water and steam distillation), hydrolytic maceration followed by distillation, expression, and effleurage (cold fat extraction) are all options for aromatic plants. Headspace trapping, solid phase micro extraction, protoplast extraction, and micro distillation are some of the most recent aromatic plant extraction technologies.[13]

1. **Plant tissue homogenization:** Researchers have employed homogenization of plant tissue in a solvent extensively. Fresh plant components are ground to fine powder in a blender, then mixed with a specific amount of solvent and shaken rapidly for 5 to 10 minutes or after 24 hours, the extract is filtered after that.. To evaluate the concentration, the filtrate can be dried under decreased pressure and redissolved in the solvent. However, other researchers centrifuged the filtrate to clarify the extract.[14]
2. **Serial exhaustive extraction:** Another popular extraction approach comprises sequential extraction with changing polarity solvents to assure that a wide polarity range of components can be extracted, from a non-polar (hexane) to a more polar (methanol). Some researchers use an organic solvent to do soxhlet extraction of dried plant material. This approach is not suitable for thermolabile chemicals because prolonged heating may cause degradation.[14]
3. **Soxhlet extraction:** When the target molecule has a low solubility in a solvent in which the impurity is insoluble, soxhlet extraction is required. If the desired component has a high solubility in a solvent, it can be separated from the insoluble substance using simple filtration. The advantage of this approach is that instead of passing multiple batches of warm solvent through the sample, only one batch

is recycled. This approach is not suitable for thermolabile chemicals since prolonged heating can cause degradation.[15]

4. **Maceration:** In maceration (for fluid extract), whole or grinded plant-drugs are held in contact with the solvent in a tight-fitting container for a set period of time, with regular agitation, until all soluble materials is dissolved. This approach is most effective when dealing with thermolabile pharmaceuticals.[16]
5. **Decoction:** This method is used for the extraction of the water soluble and heat stable constituents. This process involves boiling a crude medication in water for 15 minutes, chilling, filtering, and pouring enough cold water through it to generate the required volume.[17]
6. **Infusion:** It is a dilute solution of the crude medications' readily soluble components. Fresh infusions are made by macerating materials in cold or hot water for a small time period.[17]
7. **Digestion:** This is a type of maceration in which the maceration extraction process is heated gently. When a relatively raised temperature is not undesirable and the menstrual solvent efficiency is increased, it is employed.[17]
8. **Percolation:** In the production of tinctures and fluid extracts, this is the method most commonly used to extract active substances. In most cases, a percolator (a thin, cone-shaped jar with openings on both ends) is utilised. The solid materials are soaked with an adequate amount of the prescribed menstruum and let to stand for around 4 hours in a tightly sealed container, following which the mass is compressed and the percolator's lid is closed. A shallow layer of menstruum is poured above the mass, and the combination is macerated for 24 hours in a closed percolator. The percolator's outlet is then opened, allows the inside liquid to gradually drop out. As required, menstruum is added more and more till it percolate reaches approximately three-quarters of the finished product's volume. After pressing the marc, the liquid is poured into the percolate. The required amount of menstruum is added, and the mixed liquid is purified by filtration or standing followed by decanting.[18]
9. **Sonication:** Frequency of ultrasound waves ranging from 20 to 2000 kHz are used in the technique, which enhances the permeability of cell walls and causes cavitation. Although the method is effective in particular situations, such as rauwolfia root extraction, its use on a broad scale is limited due to the increased costs. One downside of the process is the known but rare adverse effect of ultrasonic energy (more than 20 kHz) on the active ingredients of medicinal plants, resulting in the production of free radicals and, as a result, unwanted alterations in the drug molecules.[18]

PHYTOCHEMICAL ANALYSIS

Phytochemical examination as per the standard methods:[13,19]

Sr.No.	Phytoconstituent	Test
1.	Alkaloids	Mayer's test
		Dragendorff's test
		Wagner's test
		Hager's test
2.	Glycosides	Legal's test
		Keller-killiani's test
		Borntrager's test
3.	Carbohydrates	Molisch's test
		Fehling's test
		Benedict's test
4.	Tannins	Ferric chloride test
		Gelatin test
		Lead acetate test
5.	Phytosterols	Liebermann-burchard's test
		Salkowski's test
6.	Reducing sugars	Fehling's test
		Benedict's test
7.	Flavonoids	Ferric chloride test
		Shinoda test
		Alkaline reagent test
		Lead acetate test
8.	Saponins	Foam test
9.	Proteins and amino acids	Biuret test
		Ninhydrin test

EVALUATION PARAMETRS

1. Colour

The syrup's colour is examined directly with our naked eye.[20,21]

2. Odour

Individually, 5ml of final syrup was smelled, and the odour was identified.[20,21]

3. Taste

To determine the taste, a pinch of the final syrup was placed on the tongue's taste bud.[21]

4. Determination of pH

Take 10ml of final syrup in the volumetric flask and fill up the volume upto 100ml with distilled water. A digital pH meter was used to measure the pH.[21,22]

5. Determination of viscosity

The viscosity of syrup can be measured using an ostwald viscometer. First, carefully clean the ostwald viscometer with warm chromic acid or acetone. Fill the water up to the mark "G" in the dry viscometer and place the viscometer vertically on a suitable platform. Take note of the time it takes for water to flow from mark A to mark B. Repeat the filling operation at least three times and record the time to acquire reliable readings. Now rinse the viscometer and fill it with test liquid (syup) till mark A, then calculate the time it takes for the liquid to flow to

mark B. A specific gravity bottle can be used to determine density.[22,23]

Formula for viscosity

$$\text{Viscosity} = \frac{\text{Density of test liquid} \times \text{Time required to flow test liquid} \times \text{Viscosity of water}}{\text{Density of water} \times \text{time required to flow water}}$$

6. Determination of density

The density of syrup can be calculated using the specific gravity of the bottle. Use chromic acid or nitric acid to thoroughly clean the specific gravity bottle. Rinse the bottle two to three times with distilled water. Take note of the weight of the empty dry bottle with the capillary tube stopper (w1). Fill the bottle with unknown liquid, close it, and wipe the excess liquid out of the bottle with unknown liquid in analytical balance (w2). Finally, compute the weight in grammes of an unknown liquid (w3).[23]

Formula for density:

$$\text{Density of liquid under test (syrup)} = \frac{\text{Weight of liquid under test}}{\text{Volume of liquid under test}}$$

$= w3/v$

7. Determination of specific gravity

After cleaning with chromic acid or nitric acid, rinse the bottle with filtered water two to three times. If necessary, rinse and dry the bottle. Take the weight of an empty dry bottle with a capillary tube stopper (w1). Fill the bottle with distilled water, screw on the stopper, and wipe away any surplus liquid from the outside of the tube. And, using an analytical balance, weigh the bottle with distilled water (w2). After emptying and drying, repeat the procedure by replacing water with the liquid under test (syrup). Weigh the container with the stopper and the liquid under test on an analytical balance (w3).[23]

Formula for specific gravity

$$\text{Specific gravity of liquid under test (syrup)} = \frac{\text{Weight of liquid under test}}{\text{Weight of water.}}$$

$= w3/w2.$

ADVANTAGES OF HERBAL SYRUP

1. Production costs are low.
2. With chronic conditions, it is effective.
3. Various options are available.
4. They could have less negative side effects.
5. It's simple to adapt the dose to the weight of the child.
6. There is no need for nursing.
7. They are usually harmless.
8. Herbs can be found almost anywhere.
9. As a syurp is sweet in flavour, it's good patient complimac, especially for paediatric patients.
10. Because of the high osmotic pressure, it acts as a preservative by inhibiting the growth of bacteria, fungi, and mould.

DISADVANTAGES OF HERBAL SYRUP

1. There are no dosing instructions.
2. Wild herbs provide a risk of poisoning.
3. Solid sedimentation occasionally results in the formation of a foot.
4. It is impossible to attain dose precision unless the syrup is packaged in unit doses.
5. If preservation is not added in the correct proportion, microbiological contamination can occur.
6. An additional drawback of herbal medication is the risk of self-dosing of herbs, which is very rare.

CONCLUSION

In today's world, herbal products are a symbol of safety, as contrast to synthetic pharmaceuticals, which are considered unsafe to both humans and the environment. Herbs have been valued for decades for their medicinal, flavouring, and aromatic properties. When designing a herbal medication formulation, it is essential to have a complete knowledge of the drug's organoleptic properties, phytoconstituents, pharmacological action, and standardisation in relation to numerous parameters using various approaches.

Monographs, which are compiled in standard books such as the Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India, Wealth of India, and Ayurvedic Formulary, provide all the details for the various tests to be performed in order to determine the conformity of the crude or formulated herbal drug with the standards established. The involved governing authorities, such as CDSCO and the US-FDA, have established numerous rules on the standards of herbal pharmaceuticals, as well as standard testing techniques to determine the drug's conformity with prescribed standards.

The herbal syrup is a sweet, viscous, concentrated, or a nearly saturated aqueous solution of sucrose-containing 66.7% w/w of sugar (USP contains 64.74%w/v of sugar) having a specific gravity of 1.31. Syrups should be kept in a cool, dark place, in a well-dried, filled, and well-stoppered bottle. They are kept at a temperature of no more than 25°C. A bottle should be filled, tightly closed, and kept dry. Syrups are self-preservatives. Preservatives such as methylparaben, sodium benzoate, benzoic acid, glycerin, and others are used to prevent bacteria and mould growth.

The world of herbal medications is vast and there is still much to learn about them. It’s time to spread awareness about them all across the world.

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EFFECTS OF VERAPAMIL AND FERULIC ACID AGAINST CHEMICALS INDUCED CONVULSIONS IN ALBINO MICE

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ABSTRACT

Background: The currently available antiseizure drugs have a low therapeutic index and provided emerge satisfactory seizure control in only 60-70% of patients. Calcium channel blocker have shown potentials of a useful add-on drug for the available antiepileptic drugs. Role of oxidative stress in epileptogenic process has been supported in various studies. **Objectives:** To study Potentiation effect of verapamil and Ferulic acid against pentylenetetrazole and picrotoxin induced convulsions in mice. M**ethods:** For this study, swiss albino mice were used. Effects of verapamil and ferulic acid alone and in combination with diazepam (4mg/kg) were studied. Onset of convulsions and duration of convulsions, percentage protection was considered as the index for antiepileptic activity. **Result:** Verapamil (20mg/kg) produced non significant antiseizure effect and ferulic acid at dose (75mg/kg) reduced the convulsions and myoclonic jerk but verapamil and ferulic acid in combination with diazepam potentiate the antiepileptic effect. **Conclusion:** Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid

KEYWORD: Verapamil, Diazepam, Ferulic acid, Picrotoxin induced seizures, Pentylenetetrazole induced seizures.

1. INTRODUCTION

Epilepsy a chronic disorder of heterogeneous symptoms characterized by recurrent seizures,

of cerebral brain.[1-3] It is the second most common chronic neurological condition observed in worldwide.[4] Seizures that can happens spontaneously and repeatedly are knows as outward signs of epilepsy.[5] Epileptic seizures are characterized by increasing excitability in brain structures (such as within the cortex and subcortical area).[6]

Approximately 1 % of to the world population has epilepsy[7,8] The therapeutic objectives of the treatment of epilepsy is complete seizures control without excessive side effect. Uncontrolled epilepsy can result neuropsychiatric and social impairment, lower quality of life and higher risk of death.[9] Many of the existent ACD produce a host of undesirable side effects including teratogenesis, drowsiness, mental dullness, nausea, ataxia, hematologic changes, hirsutism, weight gain. For these reasons, new ACD are needed to improve seizure control and reduce the side-effect profile (Gasior et al., 1997).[10,11]

The history of hypertension appears to be an independent risk factor for new onset unprovoked seizures.[12] Overwhelming evidence indicate that calcium ions plays an essential role in the pathophysiology of epilepsy. During seizures one can observe a decrease in extracellular calcium concentration prior to onset of seizures activity followed by increase in the intracellular calcium concentration. An important characteristics all CCBs is their ability to inhibit the inward flow of calcium[13] CCBs have several advantages over the existing antiepileptic drugs,such as no effect on hepatic microsomal enzymes ,devoid sedation and wide therapeutic range.[14]

Some conventional antiepileptic drugs induced oxidative stress which limit their clinical condition. Ferulic acid is phenolic phytochemical with antioxidant and neuroprotective properties that prompted to evaluate its therapeutic potential in epilepsy. Which is usually associated with oxidative stress.[15]

2. MATERIALS AND METHODS

2.1 Animals

Swiss Albino mice of body weight 20-30 g were procured from Animal House of Yashoda Technical Campus, Faculty of Pharmacy, satara (Dist-satara) and fed with commercial pellet diet (Hindustan Lever Kolkata, India) and water *ad libitum* were used in this study. All procedures described were reviewed and approved by the IAEC, Yashoda Technical Campus faculty of Pharmacy, Satara. Dist.– Satara (Maharashtra)

2.2 Drugs and Chemicals

P C Chem provided Picrotoxin and pentylenetetrazole drug, Diazepam (valium tablet) was purchased from Abbott healthcare Pvt, Ltd and Verapamil (Calaptin) was purchased from Abbott Healthcare Pvt, Ltd, Sakshi corporation navi Mumbai provided ferulic acid.

2.3 Inclusion criteria

A majority of mice showed tonic clonic seizure, clonic seizure, myoclonic twitches, hind limb extentions & recovery. Only those rats. showing the convulsive responses were used for experiment.

2.4 Animal care

Swiss albino mice (18-22 g) were selected. Animals were housed under an alternative 12 h light/dark cycle in polypropylene cages with softwood granulate bedding. Three animals were housed in a single cage. Pelleted food and water were made available ad libitum. Animals used in these studies were maintained in facilities fully accredited by the CPCSEA and all experiments were performed under protocols approved by the Institutional Animal Ethics Committee (YSPM/YTC/Pharma /2021-2022/IAEC/003)

2.5 Induction of convulsion by picrotoxin

Picrotoxin (10mg/kg) was administrated and the animals were observed until occurrence of extension -flexion of forelimb and hind limb with falling on back sometimes with spasm of neck muscles (clonic tonic seizures). Latency period of seizure and number of convulsed/ all number of animals in each group were recorded.

2.6 Induction of convulsions by pentylenetetrazole

Pentylenetetrazole (PTZ) –induced seizures in mice is an accepted in-vivo model for the screening of antiepileptic drugs. Seizures are induced by the administration of 80 mg/kg, i.p PTZ and the mice are then observed for a 120 minute period. Mice were administered drugs for days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed.

2.7 Experimental groups

Anticonvulsant studies

2.7.1 Picrotoxin induced convulsion

In toxicant control (n=6), mice were injected with picrotoxin (10mg/kg), Standard group (n=6) mice were injected with Diazepam(4mg/kg) ,Test I -received picrotoxin(10mg/kg),Test II- received standard diazepam(4mg/kg),Test group III–received verapamil (20mg/kg) intraperitoneally; Test group IV– received Ferulic acid (75mg/kg) intraperitoneally, Test group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Test group VI- received ferulic acid +Diazepam (75mg/kg+2mg/kg).Thirty minutes after pretreatment,10mg/kg of picrotoxin was administered to each mice. They were then observed for tonic hind limb seizures for 30 min period.

2.7.2 Pentylenetetrazole induced convulsion

Animal were divided into VI groups, (n=6 mice of either sex in one group). Group I received pentylenetetrazole (80mg/kg),group II was allotted for Diazepam (4mg/kg) and Group III received verapamil (20mg/kg) intraperitoneally; Group IV– received Ferulic acid (75mg/kg) intraperitoneally, Group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Group VI-received ferulic acid +Diazepam (75mg/kg+2mg/kg). mice were administered drugs for seven days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed for (1) onset of convulsions, (2)incidence (number of mice showing convulsions and (3) mortality for the duration of 30 minutes.

2.7.3 Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunneet's multiple Comparison Test by Graph pad prism software (GraphPad software inc.., Version 5.0.0). The mean values ± SEM were calculated for each parameter. Level of significances was kept at $p< 0.05$.

3. RESULTS

In Pentylenetetrazole induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 1.

As seen in table No 1, group II (diazepam) showed complete abolition of convulsions, highly

significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decreased mortality percentage when compared to normal control group ($p < 0.05$)

Table no. 1: Effect of Verapamil and Ferulic acid alone and its combination with diazepam on pentylenetetrazole induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of Animals survived	Percentage protection (%)
Group I Toxicant (PTZ)	80 mg/kg i.P	1.08± 0.10	3.10±0.23	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	2.10±0.1	5.20±0.26	4/6	66%
Group IV (Ferulic acid)	75mg/kg i.p	1.20±0.10	13.10±1.10	2/6	33%
Group V (Diazepam +verapamil)	2mg/kg+20 mg/kg i.p	2.68±0.20	6.10 ±0.32	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+75 mg/kg i.p	2.10±0.16	7.18±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

In Picrotoxin induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 2.

As seen in table No 2, Group II (diazepam) showed complete abolition of convulsions, highly significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (Ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decrease mortality percentage when compared to normal control group ($p < 0.05$).

Table no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of animals survived	Percentage protection
Group I Toxicant (Picrotoxin)	10mg/kg i.p	2.07± 0.10	10.5 ±0.88	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	12.35±1.10	11.05±0.78	3/6	50%
Group IV (Ferulic acid)	75mg/kg i.p	18.17±1.50	13.73 ±1.01	0/6	0%
Group V (Diazepam +verapamil	2mg/kg+ 20mg/kg i.p	9.10±0.48	8.15±0.42	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+ 75mg/kg i.p	10.07±0.54	9.34 ±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

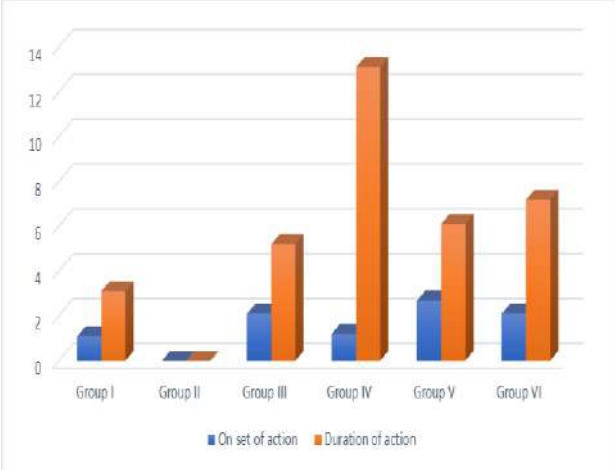

Fig. no. 1: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on pentylenetetrazole induced convulsions.

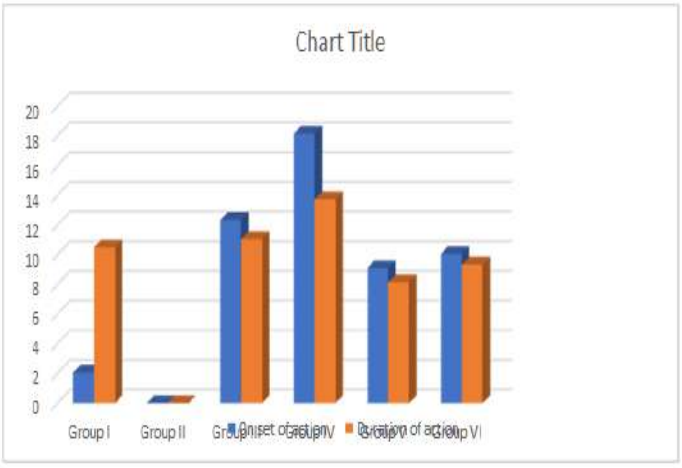

Fig. no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

4. DISCUSSION

Epilepsy is characterized by spontaneous recurrent seizures in which electrical activity in particular brain regions becomes over-excitable. Epilepsy is managed mainly with drugs; however, antiepileptic drugs currently in use neither provides a cure nor prevent relapse and are associated with many side effects such as fatigue, allergies, sedation, blood dyscrasias and teratogenesis, changes in mood and memory problems As a result of this, development of new, affordable and accessible pharmacological agents that can overcome these limitations has become a major goal in epilepsy research.[16]

PTZ may be exerting convulsant effect by inhibiting the activity of GABA receptors. GABA is a major inhibitory neurotransmitter in the brain and the inhibition of its neurotransmission promotes seizures.[17]

Antioxidant play an important role in anti seizure activity, it should be reduced the oxidative stress in epilepsy. Epilepsy is one of the most common neurological disorders. However, the Patho physiological mechanisms of epilepsy are not yet fully understood. Recent years have focused on the role of oxidative stress in seizures. There is emerging evidence that focuses on the role of oxidative stress and mitochondrial dysfunction both as a consequence and a cause of epileptic seizure. Experimental seizures are known to be associated with a massive release of reactive oxygen species.[18]

Ferulic acid [(E)-3-(4 hydroxy-3-methoxy-phenyl)prop-2-enoic acid) is an active phenolic constituent of many plant species which has shown a wide range of pharmacological

properties including the effects against the inflammation, oxidative stress, cancer, hepatotoxicity, diabetes, thrombosis. Moreover, isopentyl ferulate (an ester derivative of ferulic acid) has shown. anticonvulsant effect in mice.[19]

During seizures one can observe a decrease in the extracellular calcium concentrations prior to onset of seizure activity followed by an increase in the intracellular calcium concentrations. An important characteristic of all CCBs is their ability to inhibit the inward flow of calcium ions. CCBs depress the epileptic depolarization of neurons has shown the presence of specific binding sites of CCBs that enable them to cross the blood brain barrier. This gives important evidence for the presence of central effects of CCB.[20,21]

In our investigation, verapamil and ferulic acid in combination with diazepam was evaluated in behavioral study that gives good indication of protection and reduced latency of convulsions induced by pentylenetetrazole and picrotoxin. Verapamil and ferulic acid alone shows anticonvulsant effect also but verapamil and ferulic acid in combination with diazepam shows more significant effect than alone drugs treatment. Verapamil and ferulic acid potentiated antiepileptic effects of diazepam.

5. CONCLUSION

It was concluded that verapamil alone at the dose 75mg/kg i.p could significantly delayed onset of convulsion and increased percentage protection of animals from death. Ferulic acid did not possess anticonvulsant activity.

Diazepam at the dose 4mg/kg significantly abolish onset of convulsion and duration of convulsion at compare to toxicant control animals.

Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid. However it need further confirmation to establish clinical utility of verapamil and ferulic acid.

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POTENTIATION OF EFFECTS OF PROPRANOLOL AND HEPARIN BY ANTIOXIDANT IN ADRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Background: Myocardial Infarction also known as heart attack is still a major cause of morbidity and mortality around the world. Vitamin C is a powerful antioxidant that can strengthen body's natural defenses and propranolol and heparin are the drugs which affect the cardiovascular system. So there is need to see if Propranolol, heparin, vitamin C and combinations of vitamin C with low doses of propranolol and heparin can show cardioprotection against adrenaline induced myocardial infraction (MI) in rats. **Objective:** To study cardioprotective effect of Vitamin C and its combination with low dose of propranolol and heparin against adrenaline induced myocardial infarction (MI) in rats. **Materials and Methods:** Rats were divided randomly into seven groups (6 rats each); Group I-Normal control; Group II-Toxicant control (adrenaline treated group); Group III-rats were pre-treated with propranolol (10 mg/kg p.o) for 10 days (from day 12 to day 21); Group IV-rats were pre-treated with heparin (500 units/kg) for 2 days (on 20^{th} and 21^{th} day); Group V-rats were pretreated with vitamin C (40 mg/kg i.p) for 21 days; Group VI-rats were pretreated with vitamin C (40 mg/kg) and propranolol (5 mg/kg) for 21 days and from 12 to 21 days respectively; Group VII-rats were pretreated with vitamin C (40 mg/kg) and heparin (250 units/kg) for 21 days and on 20^{th} -21^{th} day respectively. The rat model of myocardial infarction was produced by injecting adrenaline (2 mg/kg b.w.) subcutaneously into all animals (except the control) twice at a 24-hour interval on days 20^{th} and 21^{th} day of the experiment. The cardiac markers tests were done at the conclusion of the trial. **Results:** Treatment with Adrenaline showed significantly increased in biochemical parameters (CK-MB, LDH, SGOT, Troponin-I). Prior treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin showed significant

alteration in these parameters. **Conclusion:** The results of present study highlights that propranolol, heparin and vitamin C when given alone showed cardioprotection but combination of vitamin C with Propranolol and Heparin with low doses has showed better cardioprotection based on the alterations of cardiac biomarkers.

KEYWORDS: Myocardial infarction, Adrenaline, Oxidative stress, Inflammation, Propranolol, Heparin, Vitamin C, Cardiac biomarkers.

1. INTRODUCTION

Cardiovascular Diseases CVDs, often known as silent killers, are the largest cause of disease burden and mortality around the world. Annual CVD deaths in India are expected to increase from 2.26 million in 1990 to 4.77 million in 2020, accounting for 29 percent of all deaths worldwide (17.9 million deaths), and by 2030, more than 23.3 million people would die from CVDs (WHO).[1] Coronary heart disease, cerebrovascular disease, congestive heart failure, and other heart and blood vessel illnesses are all classified as CVDs. Heart attacks account for more than four out of every five CVD deaths.

Myocardial infarction, often known as a heart attack, is a life-threatening disorder in which blood flow to the heart's coronary artery slows or ceases, causing damage to the heart muscle. It is the irreversible destruction of heart muscle caused by a lack of oxygen for an extended period of time. As a result, there is a mismatch between coronary blood supply and myocardial demand. It's linked to an inflammatory response and a change in the extracellular matrix as a result of free radical release.[2] Prolonged MI causes ischemia and cardiac cell death.[3] It has been well characterized that oxidative stress and inflammation are the main pathophysiological process involved in Myocardial Infarction.[4] The severity of cardiac lesions can be influenced by the inflammatory process. Anti-inflammatory medications may help to minimise the size of ischemic lesions in myocardial ischemia. Antioxidant therapy can also have cardioprotective effects by lowering oxidative stress during myocardial ischemia and reperfusion damage. The enzymes most widely used in the detection of MI are Troponin I, Creatine kinase-MB(CK-MB), Lactate dehydrogenase(LDH).[5]

Adrenaline, also known as epinephrine, is a stress hormone produced by the adrenal glands and released into the bloodstream. It is a component of the "fight or flight" reaction. Catecholamine is a naturally occurring substance. It also has medicinal uses in the treatment of cardiac arrest, allergic responses, and asthma, among other things. However, at doses

beyond physiological levels, it has been shown to increase the generation of reactive oxygen species (ROS) and tissue damage caused by reactive nitrogen species (RNS). Adrenaline-induced MI in rats is regarded as a reliable experimental model for studying medication cardioprotective effects. Adrenaline has been discovered to cause MI by promoting lipid peroxidation, which causes cellular antioxidants to be depleted.[6]

Vitamin C is a water-soluble antioxidant and cofactor for enzymes in both plants and animals.[7] It is a potent antioxidant that helps the body's natural defences against oxidative stress mediated by reactive oxygen species (ROS) by shielding cells from free radicals, which are damaging chemicals. Consuming extra vitamin C raises your blood antioxidant level, which aids the body's natural defences in fighting inflammation, according to studies.[8] Futhermore, vitamin C intake from food or suppliments can increase the bioavailability of iron by improving the absorption of the non-heme iron.

Propranolol is a nonselective beta-adrenoreceptor antagonist. It has a number of pharmacological properties that may be useful in MI and support its usage. It exerts its response by competitively blocking beta-1 and beta-2 adrenergic stimulation in the heart, which is typically induced by adrenaline.[9] It exerts its effects primarily by blocking the action of the endogenous catecholamines, epinephrine and norepinephrine.[10] antagonizes the action and relieve stress on the muscle. It has the property to decrease the workload of heart by slowing the heart rate and force of contraction and also decrease the demand of oxygen.

Heparin is an anticoagulant which is known as blood thinner, a chemical substance that prevent or reduce coagulation of blood prolonging the clotting time. It interacts with the naturally occuring plasma protein, Antithrombin III, to induce conformational change. It inhibit Factor Xa and thrombin (Factor II a). It also prevent the formation of a stable fibrin clot by inhibiting the activation of the fibrin stabilizing factor.[11] It blocks the activity of coagulation factor and is used for reinfarction and thromboembolism and can lead to reduction in dead.

In this study, we assess the cardioprotective effects of propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin in rats with adrenaline-induced myocardial infarction. Since propranolol and heparin are used to treat MI, but the study of low doses of these drugs has not been made and if this drugs are combined with antioxidant then enhancement in immune system will take place and can show advanced effect which can

enhance immune system and improve its function. This research also sheds light on how metabolic changes affect therapy efficacy.

2. MATERIALS AND METHODS

2.1. Experimental animals

The experiments were conducted according to ethical guidelines as approved, Wistar Albino rats were used in the present study. These rats were procured from registered breeder and was acquainted in the quarantine area for one week. After acquaintation, animals were transferred to the standard laboratory conditions of 22 ± 2ºC temperature, 50 ± 15% of relative humidity, 12 hr dark/12 hr light cycle and the animals received free access to pellet diet & water provided *ad libitum*. The study protocol was presented to the IAEC and was approved.

2.2. Drugs and Chemicals

Adrenaline was purchased from Aqua fine injecta pvt. ltd. pune, Propranolol and vitamin C were purchased from Sigma-Aldrich Chemical Co. (st. Louis, USA), Heparin from Pfizer Labs division of Pfizer Inc NY, NY 10017, CK-MB, LDH, SGOT and Troponin kits was purchased from Meril Diagnostics Pvt. Ltd, Gujarat, India.

2.3. Induction of experimental MI

Adrenaline (2 mg/kg body weight) was subcutaneously injected into all groups of rats except normal control group for 2 consecutive days at an interval of 24 h to induce experimental myocardial infarction.

2.4. Experimental protocol

A total of 42 wistar albino rats were used for this study. After acclimatization, they were randomly divided into seven groups, consisting of six rats per group.

2.4.1. Group I (Normal control)

Served as untreated normal control rats and received distilled water (1 ml p.o).

2.4.2. Group II (Toxicant control)

Animals received Adrenaline (2 mg/kg body weight s.c) on 20th and 21th day.

2.4.3. Group III (Propranolol + Adrenaline)

Animals received Propranolol (10mg /kg body weight p.o) for 10 days (from 12 to 21 days) and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.4. Group IV (Heparin + Adrenaline)

Animals received heparin (500 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.5. Group V (Vitamin C + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days from and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.6. Group VI (Vitamin C + Propranolol + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with Propranolol (5 mg/kg body weight p.o) for 10 days from 12th -21th and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.7. Group VII (Vitamin C + Heparin + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with heparin (250 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.5. Biochemical estimation

The blood samples were taken from the retro-orbital plexus and centrifuged to separate them. The serum of numerous experimental animals was collected and utilised to perform various biochemical analyses.

The activities of serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), Serum glutamic oxaloacetic Transaminase (SGOT) and Troponin-I were estimated using commercially available kits as per instructions.

2.6. Statistical analysis

Data were expressed as mean $\pm$ S.E.M (six rats per group). Groups of data were compared by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Values $p < 0.05$ were consider statistically significant. Statistical analysis was carried out using Graph Pad Prism 5.0 software (Graph Pad Software, San Diego, California, USA).

3. RESULTS

3.1. Effects of Vitamin C and its combination with low doses of Propranolol and Heparin against adrenaline induced MI in rats

The effects of Propranolol, Heparin, Vitamin C and its combination with propranolol and heparin on Cardiac biomarkers are seen in the table No:1. Rats treated with adrenaline (Group II) showed a significant increase in the level of serum LDH, CK-MB, SGOT and Troponin-I (Positive) as compared to the normal control (Group I). Pre-treatment with propranolol (10 mg/kg), heparin (500 units/kg) and vitamin C (40 mg/kg) when given alone reduced the elevated level of the cardiac markers induced by adrenaline. Prominent inhibition effect on cardiac markers was observed when animals were treated by combination of vitamin C with low dose of propranolol and heparin.

Table no. 1: Effects of vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

Groups	CK-MB(IU/L)	LDH(U/L)	SGOT(IU/L)	Troponin-I
Control	96.44±3.05	477.22±16.35	56.83±02.33	Negative
Adrenaline	273.77±12.09	749.55±33.13	219.44±07.33	Positive
Propranolol + Adrenaline	201.36±10.33	649.41±22.63	155.13±10.33	Negative
Heparin + Adrenaline	228.41±11.88	705.31±35.33	187.35±09.50	Negative
Vitamin C + Adrenaline	264.25±12.48	731.58±11.27	201.21±12.11	Negative
Vitamin C +Propranolol + Adrenaline	129.12±05.33	537.13±16.33	126.57±06.33	Negative
Vitamin C +Heparin + Adrenaline	162.51±8.33	613.24±25.13	141.29±10.48	Negative

Data are expressed as mean ±S.E.M (n= 6 animals in each group). Statistical analysis are carried out by one-way ANOVA followed by Dunnett's t-test.

Significance difference from normal group at $p < 0.05$.

Significance difference from control (MI) group at $p < 0.05$.

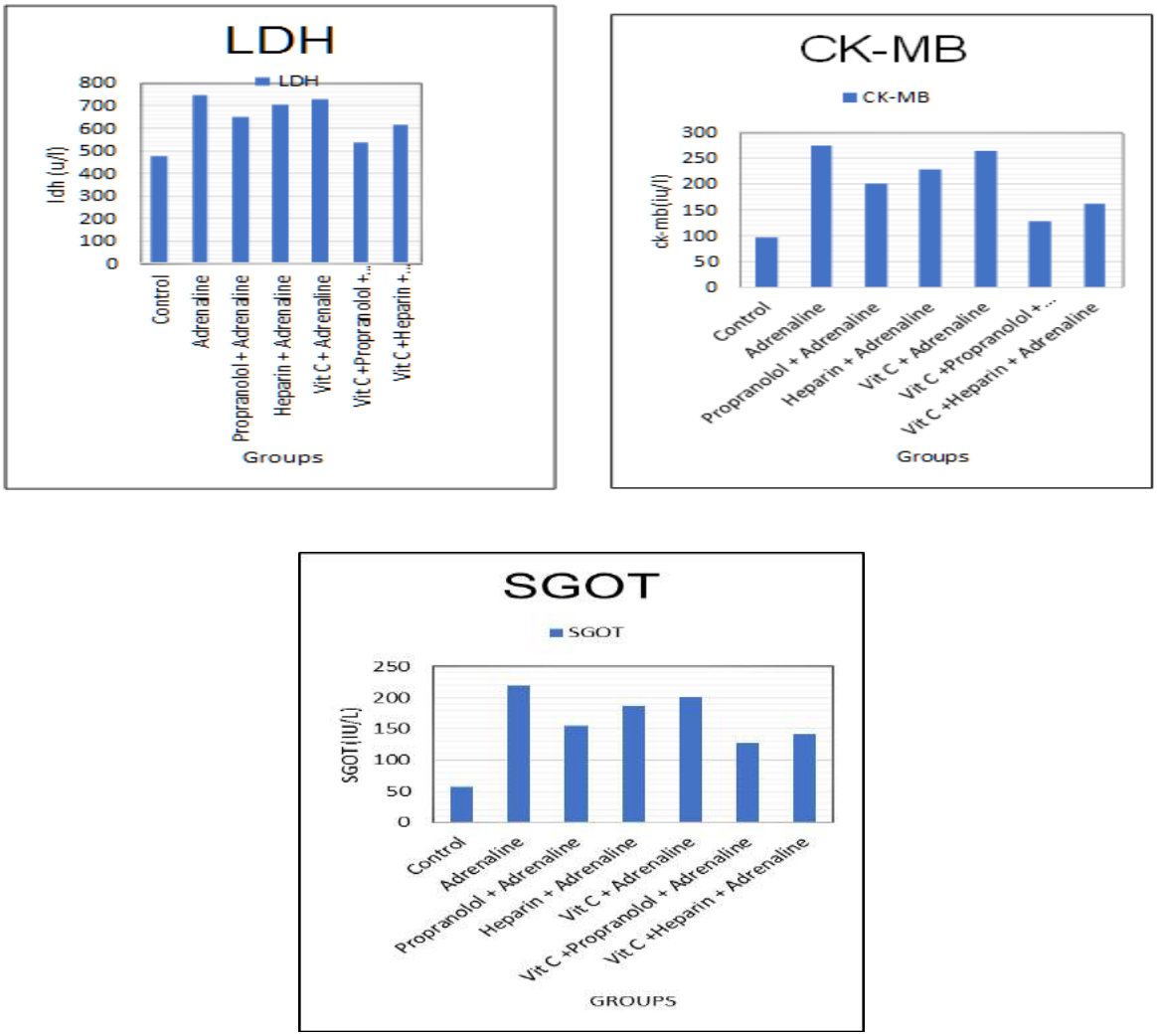

Fig. 1: Graphical representation of effects of Vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

4. DISCUSSION

In 2021, myocardial infarctions were on the rise, and there was no age limit. This year, young, healthy, fit persons with no medical history have suffered heart attacks, with some cases even resulting in death, contributing to an increase in mortality. The pandemic of COVID had also contributed in the increasing cases of heart attacks.

For a long time, myocardial cell protection and cell necrosis prevention have been therapeutic targets. Because present treatments have a limited influence on survival and annual expenditures, new therapeutics are needed to treat myocardial infarction.

The purpose of this investigation was to evaluate the potential cardioprotective role of propranolol, heparin, vitamin C and combination of vitamin C with low doses of propranolol and heparin in adrenaline induced MI in rats.

In this study, adrenaline was administered subcutaneously at a dose of 2 mg/kg for two days, resulting in a significant increase in blood levels of cardiac enzymes CK-MB, LDH, SGOT, and Troponin-I. This demonstrates that adrenaline injection causes myocardial injury and cardiac enzyme leakage into the circulation. This can take place due to increased in lipid peroxidation, inflammation or apoptosis.[12] Due to lipid peroxidation oxidative stress generates which can lead to the injury or damage to the cells which leads to the increase in the cardiac biomarkers. Our results supports the hypothesis that adrenaline has got the ability to produce free radicals and has caused myocardial infarctions.

Damage to the cell membranes, by production of large number of free radicals and ROS, generation of lipid peroxides, and lowering of antioxidative defense lines are major outcomes of MI.[13]

Since, ROS is important in the pathogenesis of MI, antioxidants and their combinations in the treatment of MI were the focus of research.

Pre-treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin has been observed. It was found that the dugs shows cardioprotective effects.

In recent years, long-term prevention of CVD is associated with consumption of fresh fruits, vegetables or plants rich in antioxidant. As a result there has been considerable interest in antioxidants.[14]

Vitamin C is a powerful dietary antioxidant. It is widely known vitamin in different kinds of fruits and is available as a supplement.[15] Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals.[16] It act by reducing the reactive oxygen species and leads in decrease in lipid peroxidation which is the main cause of depilation of cellular antioxidant and which leads to damage of cells and inflammation which leads to MI. Vitamin C act against it and showed mild effect by decreasing the levels of CK-MB, LDH, SGOT and Troponin-I helps in reducing the impact caused due to adrenaline.

Propranolol antagonizes the effect of adrenaline by lowering heart rate[17] and reducing the lipid peroxidation as it decreases the demand for oxygen, it also relaxes the blood vessels and improve blood flow with decrease in blood pressure therefore possesses the cardioprotective effect.

As the blood gets clots, when the tissue gets damaged due to the generation of oxidative stress the use of heparin helped in the anticoagulation process by inhibiting the enzymes due to which the clots are reduced. It also attenuates the rate of rise in blood pressure and prevents severe fibrinoid vascular lesions and thus shows cardioprotection.[18]

So, as the drugs showed positive effect there was need to see if the low doses of these drugs shows some advanced effect if combined with the antioxidant.

In this study, when the low doses of propranolol and heparin was combined with vitamin C, the combination with propranolol showed more advanced effect on oxidative stress which lead to lipid peroxidation due to its strong antioxidant property due to which the level of cardiac biomarkers were decreased as compared to that of the adrenaline treated animals (Toxicant group).

Similarly, when it was combined with heparin it showed advanced cardioprotection and lead to decreased inflammation, oxidative stress and blood clots which were observed by alteration of parameters which were caused by adrenaline induced MI in rats.

5. CONCLUSIONS

It can be concluded that propranolol, heparin and vitamin C when given alone improved adrenaline induced abnormal changes in the biomarkers and showed cardioprotective effect in rats after being exposed to adrenaline but the combined administration of vitamin C with low dose of propranolol and heparin produce a potentiation effect and showed better results possibly via reducing cardiac biomarkers associated with oxidative stress, inflammation, and apoptosis. More research is needed to determine the exact molecular pathways involved in the cardioprotective impact of medicines in MI rats.

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EVALUATION OF NEPHRO-PROTECTIVE EFFECT OF DPP4 INHIBITOR AND ANTIOXIDANT AGAINST GENTAMYCIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Background:- Nephrotoxicity is a global health challenge of vast proportion around the world. Recent studies demonstrated the reno-protective effects of two dipeptidyl peptidase-4 (DPP-4) inhibitors, saxagliptin and linagliptin, against gentamycin-induced renal injury. However, none of these studies investigated the combination of DPP 4 inhibitor and antioxidant. This prompted us to test this hypothesis and to assess, for the first time, the potential reno-protective effect of DPP-4 inhibitor and antioxidant. **Objective:-** This study aimed to investigate the potential protective effect of DPP-4 inhibitor and antioxidant on gentamycin-induced nephrotoxicity. **Method:-** Nephrotoxicity was induced in the rats with Gentamycin (100mg/kg). All animals except normal control were intraperitoneally administered with gentamycin at a dose of 100mg/kg once daily for 10 days. Respective treatment were started from day 2nd till day 14th (2 weeks). On the 15th day, blood samples were collected through retro-orbital plexus under anesthesia. Serum was separated to measure creatinine, BUN, uric acid, proteins & MDA (Malondialdehyde). Body weight were also recorded. **Result:-** Administration of combination of DDP-4 inhibitor and antioxidant ameliorated gentamycin induced renal injury and restored renal functional, oxidative, inflammatory, apoptotic & histopathological changes. **Coclusion:-** These findings suggest that combination of DDP-4 inhibitor and antioxidant treatment attenuate renal dysfunction and structural damage through the reduction of oxidative stress, mitochondrial dysfuction and apoptosis in the kidney.

KEYWORDS: Nephrotoxicity, Gentamycin, DDP-4 inhibitor, antioxidant, Renal Biomarkers.

1. INTRODUCTION

Nephrotoxicity can be defined as the adverse effect of substances on renal function.[1,3] These substances can include molds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine.[2,3] Due to relatively large blood flow (20 % of stroke volume) and the ability to extract and concentrate hydrosoluble toxic molecules, the kidney is prone to drug induced damage. The experimental data point to the fact that drug induced nephrotoxicity includes multiple mechanisms that can be classified as vascular, glomerular and tubular. The kidney damage is usually a consequence of tubular obsruction caused by cell swelling or debris deposition.[4,6] Toxic substances can damage various cell types in kidney. The most studied effect is necrosis of tubular epithelial cells.[5,6] One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or urine output.[2,3]

Aminoglycoside antibiotics are commonly used for the treatment of severe gram negative bacterial infections. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects.[7,9] The most widely used drug in this category is gentamycin.[8,9] Nephrotoxicity remains the major side effect hindering the clinical use of the aminoglycoside, gentamycin.[10,14] A small fraction of the administered dose preferentially accumulates in the proximal tubules, inducing oxidative stress, apoptosis, necrosis of renal cells and eventually acute renal injury and damage.[11,14] Therefore, many approaches were adopted to mitigate the progression of the renal injury as once-a-day administration regimen.[10,14] Neprotoxicity induced by GEN is a complex phenomenon characterised by an increase in blood urea nitrogen (BUN) and serum creatinine (Cr) concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure.[15,9]

Incretins; glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are gut hormones secreted from the intestinal cells in response to food intake. Once reaching the circulation, they potentiate the glucose dependent insulin secretion from pancreatic cells and inhibit glucagon secretion.[12,14] The incretin effect is significantly decreased in patients with type 2 diabetes (T2D) and contributes to impaired insulin secretion

and chronic hyperglycemia. GLP-1 and GIP are rapidly inactivated by the dipeptidyl peptidase-4 (DPP-4).[13,14]

Glutathione (GSH) is the most abundant nonprotein thiol and has many functions in vivo. The major role of GSH is the maintenance of cellular redox balance. It plays a role as a substrate of glutathione peroxidase, an antioxidative enzyme that scavenges various peroxides.[16] The physiological role of GSH as an antioxidant has been described and substantiated in studies of numerous disorders reflecting the increased oxidation is a result of abnormal GSH metabolism.[17,18] GSH is thought to be an important factor in cellular function and defense against oxidative stress, such as radiation and drug resistance. Many reports have demonstrated that GSH acts as an endogenous antioxidant.[19,20]

However, there have been no prior studies demonstrating a protective effect of sitagliptin (DPP-4 inhibitor) & GSH against the gentamycin induced nephrotoxicity. In this study, we demonstrated for the first time that combination of sitagilptin and GSH suppresses oxidative stress in vivo, and the impairment of renal function.

2. MATERIALS AND METHODS

2.1. Animals:- The study was approved by the Institutional ethics committee. 30 Wistar Albino rats (200-250gm) were selected for present study. The animals were housed at room temperature (22-28 ^{o}C) 12 hr dark and light cycle and given standard laboratory feed and water *ad - libitum*. Experiments were conducted in strict accordance with CPCSEA guidelines.

2.2. Drugs and Chemicals:- Gentamycin sulfate ampoules was obtained from Abbott (Mumbai), Sitagliptin was purchased from Sun Pharmaceutical Industries Ltd., Glutathione were purchased from HK Vitals, Chloroform were purchased from commercial vendors.

2.3. Experimental protocol:- Animals were divided into five groups, six animals each. Control Group received 2 ml/kg/day vehicle orally. Toxicant Control Group received gentamycin (100 mg/kg/day) intraperitoneally. Test I Group received sitagliptin (30 mg/kg/day) by oral gavage simultaneously with gentamycin. Test II Group received glutathione (300 mg/kg/day) intraperitoneally with gentamycin. Test III Group received combination of sitagliptin (30 mg/kg/day) and glutathione (300 mg/kg/day) with

gentamycin. Treatment continued for 14 days. Respective treatment were started from day 2nd till day 14th (2 weeks).

2.4. Sample collection:- On the 14th day, after the last gentamicin injection was applied, rats were placed in individual metabolic cages. On 15th day blood samples were collected through retro-orbital plexus under anaesthesia. Serum was separated to measure creatinine, BUN, urea, uric acid, MDA.

2.5. Biochemical determination:- The determination of serum creatinine (CliniQuant-FSR, Jaffe,s Method, Initial Rate, Creatinine assay kit), serum uric acid (CliniQuant-FSR, Uricase – Tinder, End Point, Uric acid assay kit), blood urea and blood urea nitrogen (CliniQuant-FSR, Urease – GLDH, Fixed Time, Urea (BUN) kit), MDA (abbexa MDA ELISA Kit) was done as instructed by manufacturer.

2.6. Data Analysis and Statistics:- Data were expressed as means ± SD. Statistical significance was tested with the one-way analysis of variance (ANOVA) followed by Bonferroni's Test as a post hoc test using GraphPad Prism version 5.00. Probability < 0.05 was considered significant.

3. RESULTS

Effect of sitagliptin, glutathione & combination of both on body weight

After two weeks of treatment, rats showed significant change in body weight because of inflammation of kidney. Final weight of rats in positive control group was significantly higher than initial weight of rats as compared to control group, whereas test group I, II & III showed reduction in gain of weight of rats than that of toxicant control group.

Effect of sitagliptin, Glutathione & Combination of both on renal biomarkers (Blood Urea, Blood Urea Nitrogen, Serum Creatinine, Serum Uric acid, MDA)

As shown in Table No. 1, Group II (Toxicant control) were injected with gentamycin (100 mg/kg). It showed significantly elevated levels of urea, BUN, creatinine, uric acid, MDA as compared to the control group.

Group III (Test I) were treated with sitagiptin (30 mg/kg). It showed slightly reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

Group IV (Test II) were treated with glutathione (300 mg/kg). It showed slightly readuction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

Group V (Test III) were treated with combination of sitagliptin (30 mg/kg) & glutathione (300 mg/kg). It showed significant reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group. There were no statistically difference between Group I and Group IV.

Effect of sitagliptin, glutathione & combination of both on serum proteins

There is no significant difference in the levels of serum proteins (albumin, globulin) in all gropus.

Table 1: Effect of the Test I, II & III group on body weight, Renal Biomarkers & Serum protein.

Parameter	Group I (Control)	Group II (Toxicant control)	Group III (Test I)	Group IV (Test II)	Group V (Test III)
Body Weight					
Initial Body Weight (gm)	223 ± 0.86	227* ± 0.71	234** ± 0.49	238** ± 0.32	231** ± 0.56
Final Body Weight (gm)	234 ± 0.61	256* ± 0.94	250** ± 0.72	249** ± 0.65	246** ± 0.69
Renal Biomarkers					
Blood Urea (mg/dl)	33.2 ± 0.94	179.9* ± 1.81	32.6** ± 0.87	34.7** ± 0.63	31.5** ± 0.79
Blood Urea Nitrogen (mg/dl)	15.5 ± 0.48	84.01* ±1.08	17.8** ± 0.37	16.5** ± 0.55	14.10** ± 0.61
Serum Creatinine (mg/dl)	0.50 ± 0.02	2.56* ± 0.05	0.62** ± 0.03	0.58** ± 0.04	0.55** ± 0.02
Serum Uric Acid (mg/dl)	4.7 ± 0.13	8.7* ± 0.37	5.1** ± 0.12	4.9** ± 0.15	4.6** ± 0.20
MDA Levels (µmol/L)	7.1 ± 0.37	11.4 ±0. 40	7.9 ±0. 39	7.8 ±0. 23	7.5 ±0. 18
Serum Proteins					
Serum Albumin (g/dl)	3.6 ± 0.14	3.4 ± 0.45	3.7 ± 0.96	3.8 ± 0.56	3.5 ± 0.35
Serum Globulin (g/dl)	-3.6 ± 0.25	-3.4 ± 0.52	-3.7 ± 0.38	-3.8 ± 0.69	-3.5 ± 0.58
A/G Ratio	3.6 : 1 ± 0.46	3.4 : 1 ± 0.71	3.7 : 1 ± 0.27	3.8 : 1 ± 0.21	3.5 : 1 ± 0.45

Data represent means ± SD of 6 rats in each group. *p < 0.05 compared with control group. **p < 0.05 compared with gentamycin group.

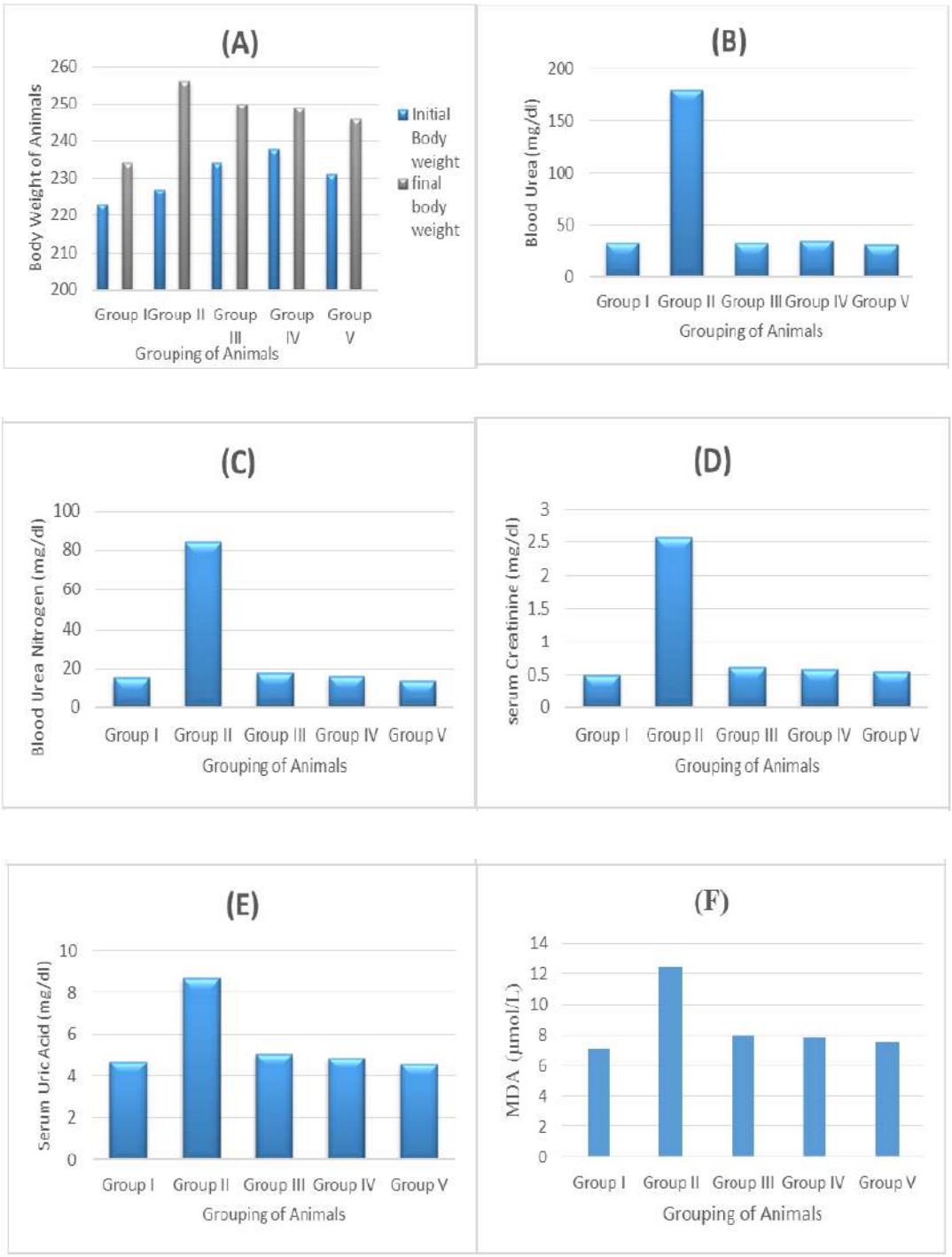

Fig. 1: Effect of sitagliptin, glutathione, combination of sitagliptin and glutathione on gentamycin – induced changes in renal biomarkers. A) Body weight B) Blood urea C) Blood urea nitrogen D) Serum creatinine E) Serum uric acid F) Malondialdehyde. Data represent means ± SD of 6 rats in each group.

4. DISCUSSION

Iatrogenic renal failure is commonly seen as a complication of many therapeutic agents. This can be likely explained by the capability of kidney to extract and concentrate toxic substances

and its high share of cardiac output. Thus, it is well-documented to be a target for many toxic xenobiotics.[21]

Nephrotoxicity as a side effects of all aminoglycosides, especially Gentamycin, limits its therapeutic use.[22] Aminoglycosides are not metabolized and are essentially eliminated by glomerular filtration. About 10% of the intravenously administered dose is accumulated in the kidney.[23] Gentamycin is largely accumulated in lysosomes, the Golgi and endoplasmic reticulum.[24,25] The serious effect occurs when the concentration of gentamycin inside the previously mentioned organelles exceeds a threshold followed by subsequent destabilization of their membranes with accumulation then release of gentamicin to cytosol.[26,27] Thus, the gentamycin in the cytosol will act on mitochondria and provoke the mitochondrial pathway of inducing oxidative stress, apoptosis and diminish the ATP.[28,29] Gentamycin induces similar morphological alterations in kidneys of both humans and experimental animal.[30] Gentamycin administration produced a elevation of kidney injury markers exhibited as a significant increase of serum BUN and creatinine levels.

In the present study, we aimed to investigate the effect of Sitagliptin and Glutathione against gentamycin-induced nephrotoxicity, hoping to achieve a new therapeutic approach that can protect or reverse gentamycin-induced nephrotoxicity. Sitagliptin significantly counteracted the nephrotoxic effects of gentamicin and retained all the injury markers near the normal levels.[31] Treatment with GM produces oxidative stress in tubular cells, both in vivo in rats[32] and in cultured tubular cells.[33] This oxidative stress is likely to be mediated by hydroxyl radicals, hydrogen peroxide and by superoxide anions[34,35] from mitochondrial origin.[36] GM directly increases the production of mitochondrial ROS from the respiratory chain.[28] The deleterious effect of overproduction of ROS and the process of lipid peroxidation, respectively, damage the protein molecules and degrade the membrane-bound phospholipids.[37] The decreased antioxidant activity in GM-induced nephrotoxicity can be explained by an increase in the generation of free radicals. This is followed by subsequent depletion of antioxidant enzymes during the process of counteracting oxidative stress.[38] Sitagliptin significantly ameliorated all these changes.[31]

Apoptosis contributes in the pathological process of different renal diseases and drug-induced nephrotoxicity.[39,40] About 20% of patients receiving GM treatment could be complicated by acute renal failure with evidence of acute tubular necrosis.[33] Experimental studies with GM revealed signs of apoptosis.[41] Earlier studies have pointed that attenuating of apoptosis

suppresses renal injury which focus on the importance of inhibition of apoptosis as a critical clinical target in renal diseases.[42,43] The intrinsic pathway of apoptosis is found to be initiated by mitochondrial dysfunction.[28] Briefly, GM promotes bax aggregation and translocation to the mitochondria, causing activation of caspase-9, which then activates caspase-3. These events lead to a loss of mitochondrial membrane potential and initiate apoptotic process.[39] Sitagliptin prevented renal tubular apoptosis induced by GM exhibited by a significant decrease in the number of positive brownish caspase-3 and bax immunoreactive cells in kidney sections. These results were supported with the previous reports confirming the anti-apoptotic effects of Sita.[44,45]

Protective roles for antioxidants in genral against free radicals have been demonstrated in a num. ber of in vitro and in vivo experiments. Among the species acting as scanegers, GSH's importance has been widely stressed, depletion of tissue GSH causing hypersusceptibility to some toxic chemicals and radication. Renal function, as indicated by glomerular filtration, etc., is also effected by depletion of GSH.[46] In mammalian cells and tissues, GSH is the most abundant nonprotein thiol; it is usually present in millimolar concentrations.[47,48] As the key intracellular antioxidant, GSH reacts with electrophilic compounds and serves as a reductant for eliminating hydrogen peroxide and lipid hydroperoxides.[49] The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the membrane's structure and permeability.[50]

5. CONCLUSION

The present study suggest that sitagliptin alone has renal beneficial effect & that it may serve as an adjuvant to reduce gentamycin induced renal injury in rats. This also suggest that glutathione alone acts as a pontetiate scavenger of free radicals to prevent the toxic effect of gentamycin. But the present study mainly suggest that the combination of boh sitagliptin and glutathione has a more nephropotective potential against gentamycin induced nephrotoxicity. This may be ascribed to their antioxidant, antimitochondrial dysfunction and anti – apoptotic effects. Nonetheless, further studies are needed to investigate different doses of the combination against gentamycin induced nephrotoxicity before safe application in humans.

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PHARMACOLOGICAL EVALUATION OF ANTIDEPRESSANT-LIKE EFFECT OF VITAMIN E AND ITS COMBINATION WITH AMITRIPTYLINE: AN ACUTE STUDY

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ABSTRACT

Aim: The aim of the study to evaluate anti-depressant like effect of Vitamin E and its combination with amitriptyline against reserpine-induced depression in rats. **Materials and Methods:** Reserpine was injected to induce depression in all rats except normal control group. All animals were divided randomly into six groups (6 rats each); Group-I received Vehicle; Group-II received Reserpine; Group-III received Amitriptyline; Group-IV received Vitamin E; Group-V and VI received combination of Amitriptyline and vitamin E. Forced swim test and Actophotometer were used to assess anti-depressant activity. **Result:** In locomotor activity testing, locomotor activity count was found to be increased, when animals were treated with a combination of amitriptyline and vitamin E i.e amitriptyline (5mg/kg) with vitamin E (50mg/kg) and amitriptyline (10mg/kg) with vitamin E (50mg/kg). In forced swim test, latency to immobility time and duration of immobility time was found to be increased and decreased respectively, when animals were treated with a combination of Amitriptyline (5mg/kg) with vitamin E (50mg/kg) as well as amitriptyline (10mg/kg) with vitamin E (50mg/kg). **Conclusion:** Vitamin E showed potentiation and synergistic anti-depressant effect with amitriptyline.

KEYWORDS: Depression, Antidepressant activity, Forced swim test, Reserpine, Vitamin E, Locomotor Activity.

1. INTRODUCTION

Depression is the most prevalent affective disease (defined as disruptions of mood rather than

thought or cognition); it can range from moderate depression to severe (psychotic) depression with hallucinations and delusions. It is a widespread and exhausting mental disorder that is characterized by low sensitivity, mood, sleeplessness and lack of appetite in enjoyable activities.[1] The Diagnostic and Statistical Manual of Mental Disorders describes the affective symptoms of depression as a loss of interest, which is used to estimate the lifetime incidence of major depression in people.[2]

A rauwolfia indole alkaloid that acts as a sympatholytic and sedative agent but research and clinical trials have shown that reserpine has depressive properties.[3][4] The mechanism of action of reserpine is depletion of monoamines neurotransmitters from nerve endings which may result in poor neuronal excitation and communication leading to depression.[5][6] Therefore, reserpine was used to induce depression in experimental animals.

Antidepressants are the drugs which can elevate mood in depressive illness. All these drugs affect monoaminergic transmission in the brain in one way or the other and have associated properties.[7]

Amitriptyline, a tricyclic antidepressant, is a structurally heterocyclic substance that works as a serotonin-norepinephrine reuptake inhibitor, raising the concentration of these neurotransmitters in the synapse and so successfully treating depression. It is widely accepted that monoamines reuptake inhibition is crucial for its action. By blocking the serotonin and noradrenaline transporters, amitriptyline increases the neurotransmitters in the synapse and hence enhances neurotransmission.[8] Because numerous adverse effects from continuous administration limit therapeutic therapy, it is vital to introduce new targeted medications with claims of improved tolerability and efficacy.[9]

Studies of antioxidant pathways have also suggested that when stress induces biochemical changes, antioxidants can neutralize free radicals and suppress the oxidative stress pathway, eliminating reactive oxygen and nitrogen species (ROS and RNS) that can injure brain neurons. As a result, anxiety and depression symptoms may be reduced as a result of this procedure.[10]

Vitamin E may have an antidepressant impact, according to other research. Vitamin E is a non-enzymatic antioxidant that works in tandem with enzymatic antioxidants like glutathione peroxidase and superoxide dismutase to reduce oxidative stress-related alterations.

Antioxidant levels in the blood, such as vitamin E, have been linked to sadness and anxiety. Nuts and vegetable oils are common natural sources of vitamin E.[11] Antioxidant supplement treatment has been demonstrated to be useful in individuals with anxiety and depression by increasing antioxidant defense in the biological system.[12][13] In addition, Vitamin E supplementation has few associated adverse events.[14]

The goal of this study was to see how vitamin E combined with amitriptyline delivered immediately affected animal behavior in the forced swim test (FST) and locomotor activity testing in rats. Vitamin E was thought to enhance the antidepressant properties of amitriptyline.

2. MATERIALS AND METHODS

2.1. Animals: This study employed adult male albino rats weighing 180-200 g. They were kept in groups of six at a constant temperature (25°C) and humidity (50%) with a 12-hour light/dark cycle (lights turned on at 7:00 a.m.) and free access to pellets and water. Each animal was utilized only once in each experiment. The institutional ethics committee authorized the experimental protocol.

2.2. Drugs and Treatment: Amitriptyline was procured from (Intas pharma) and vitamin E from MERCK. Reserpine was procured from Sigma-Aldrich. All of the medications were dissolved in RO water and tween 80 and administered one hour before the test. All test solutions were prepared fresh and given orally for 14 days in a volume of 10 mL/kg body weight. The animals were split into six groups (n = 6) at random. The dosages were chosen based on previous research findings.

Group I: received vehicle,

Group II: received reserpine 6 mg/kg, i.p

Group III: received amitriptyline 10mg/kg, p.o

Group IV: received vitamin E 50 mg/kg, p.o,

Group V: received vitamin E 50 mg/kg, p.o + amitriptyline 5 mg/kg, p.o

Group VI: received vitamin E 50mg/kg, p.o + amitriptyline 10mg/kg, p.o

1.1 Locomotor Activity Testing. The locomotor activity was determined. Photoelectric cells connected in a circuit with a counter powered the actophotometer. A count was taken when the animal blocked off the beam of light falling on the photocell. These cut-offs were

determined during a 10-minute period and the result was utilized as a measure of the animal's locomotor activity.[15]

1.2 Forced Swim Test (FST). The rats were put individually into container of water (usually cylindrical) filled with water according to Porsolt et al FST's procedure. After each test, the water was changed repeatedly to remove fur, urine, and faeces. When the rat stayed afloat in the water without straining and only made little limb movements they were thought to be immobile because they needed to keep their heads above the water's surface. During the 5-minute test, the total length of immobility was measured. The immobility period was estimated by subtracting total time (5 minutes) from time spent fleeing through activities like swimming and climbing. Climbing was defined as upward-directed forepaw motions by the side of the container, whereas swimming was described as movements throughout the container. Treatment with antidepressants shortened the time the animals were motionless and enhanced their escaping behavior.[16][17]

2.3 Statistical Analysis.

All results were presented as mean ± SEM and $p< 0.05$ were considered significant. Data were analysed by one-way ANOVA, followed by Dunnett's post hoc test using Graph Pad Prism version 9.3.1.

3. RESULTS

Locomotor Activity

The effect of Vitamin E and its combination with amitriptyline on locomotor activity was Observed in Table 1.

On day 1

Rats treated with reserpine (6 mg/ kg, i.p) showed a significant decreased in locomotor Activity count as compared to the normal control group. Group III and IV were treated with amitriptyline and Vitamin E respectively, showed a significant increase in locomotor activity, when compared to toxicant group (Group II). Combination of amitriptyline and vitamin E (10mg/kg, p.o & 50mg/kg, p.o) showed a significant increase in locomotor activity as compared to other groups. The results are tabulated in table 1.

Day 1st

Table 1: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Normal Control	340 ±17.12
II	Reserpine (6mg/kg)	142±9.12
III	Amitriptyline (10mg/kg)	180±12.33
IV	Vitamin E (50mg/kg)	154±5.26
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	230±14.33
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	243±11.33

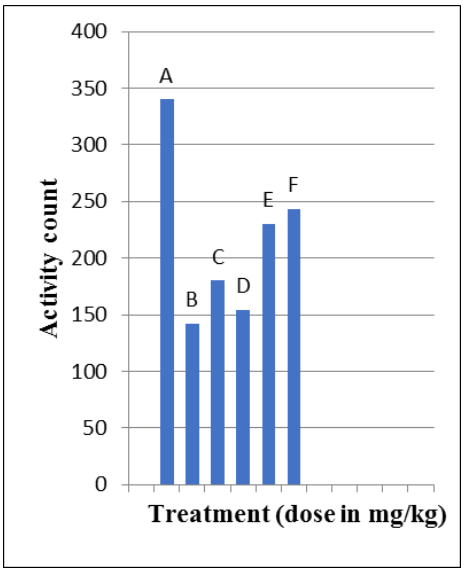

Figure 1: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 1st day. Values represent the mean ± SEM (n=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

values are expressed in mean ± SEM, where n=6

p<0.05, compared with normal control group

Day 7th

On day 7th, the locomotor activity count was found to be significantly decreased, when animals were treated with reserpine (6 mg/kg, i.p) as compared to normal control group. The effect of Amitriptyline and vitamin E alone showed significantly increase in locomotor activity count as compared to toxicant group (Group II). Better result were observed when Amitriptyline combined with vitamin E at doses of (10 mg/kg, p.o& 50 mg/kg, p.o) respectively. The results are tabulated in table 2.

Table 2: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Control	327±12.32
II	Reserpine (6mg/kg, i.p)	90±7.78
III	Amitriptyline (10mg/kg, p.o)	205±10.43
IV	Vitamin E (50mg/kg, p.o)	163±9.14
V	Amitriptyline + Vitamin E (5mg/kg, p.o+50mg/kg, p.o)	269±13.5
VI	Amitriptyline + Vitamin E (10mg/kg, p.o+50mg/kg, p.o)	274±7.6

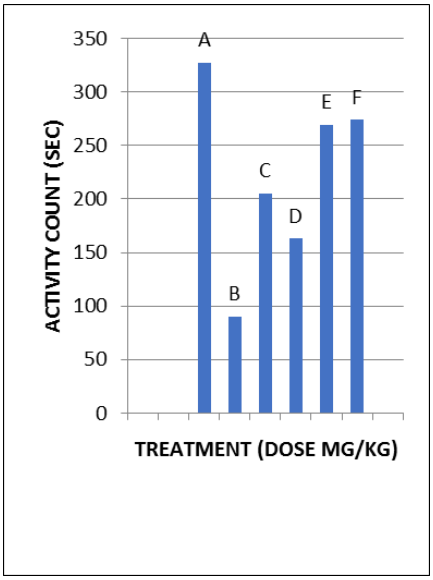

Figure 2: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 7th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group

On day 14

At the end of treatment phase, the group II animals injected by Reserpine showed significant decrease in locomotor activity as compared to normal control animals. The behavioural effect on day 14 was shown in table 3. Amitriptyline and vitamin E alone showed a significantly increase in locomotor activity count as compared to toxicant group (Group II). Co-administration of Amitriptyline and Vitamin E (10 mg/kg, p.o & 50mg/kg, p.o) showed a significant increased effect as compared to toxicant group (Group II).

Table 3: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Control	289±14.33
II	Reserpine (6mg/kg)	165±9.88
III	Amitriptyline (10mg/kg)	235±18.12
IV	Vitamin E (50mg/kg)	239±14.12
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	357±19.88
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	364±24.55

Day 14th graphical representation of (actophotometer)

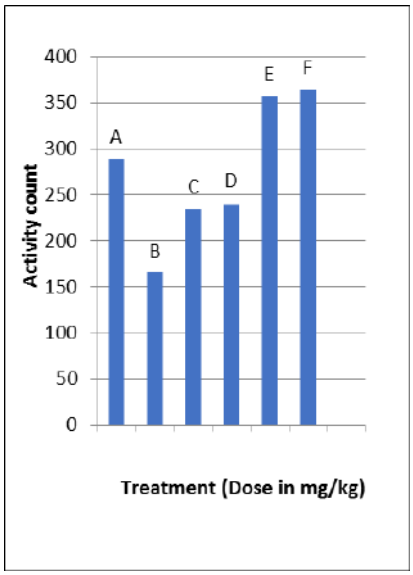

Figure 3: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 14th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group.

2. Forced swim test

Day-1st

Latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively as compared to normal control group, when animals treated with Reserpine (6mg/kg, i.p). Group III and IV animals were treated with Amitriptyline and Vitamin E showed significant increased latency to immobility time and decreased duration of immobility time. Combination of Amitrptyline with Vitamin E (10

mg/kg, p.o & 50 mg/kg, p.o) showed significant increased and decreased latency to immobility time and duration of immobility time respectively. The results are tabulated in table 4.

Table 4: Effects of Vitamin E and its.

combination with amitriptyline using forced swim test.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Duration of Immobility time (sec)
I	Control	35±2.33	67±5.33
II	Reserpine (6mg/kg)	22±1.25	102±9.33
III	Amitriptyline (10mg/kg)	37±2.45	85±7.58
IV	Vitamin E (50mg/kg)	41±4.12	93±8.85
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	30±1.23	82±5.26
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	39±2.56	77±7.33

Day 1st Graphical Representation (FST)

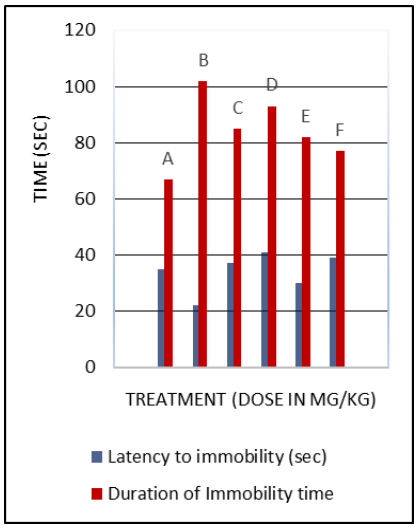

Figure 4: Effects of Vitamin E and its combination with amitriptyline using FST on1st day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group.

Day-7th

On day 7th, Latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively as compared to normal control group, when animals treated with Reserpine (6mg/kg, i.p). Group III and IV animals were treated

with Amitriptyline and Vitamin E showed significant increased latency to immobility time and decreased duration of immobility time. Better result were observed when Amitriptyline combined with vitamin E at doses of (10 mg/kg, p.o & 50 mg/kg, p.o) respectively. The results are tabulated in table 5.

Table 5: Effects of Vitamin E and its combination with amitriptyline using forced swim test activity.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Duration of Immobility time (sec)
I	Control	27±3.17	56±6.11
II	Reserpine (6mg/kg)	16±4.31	153±9.34
III	Amitriptyline (10mg/kg)	38±2.12	137±7.56
IV	Vitamin E (50mg/kg)	32±5.6	145±8.16
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	45±7.13	127±5.43
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	56±3.14	120±7.13

Day 7th Graphical representation (FST)

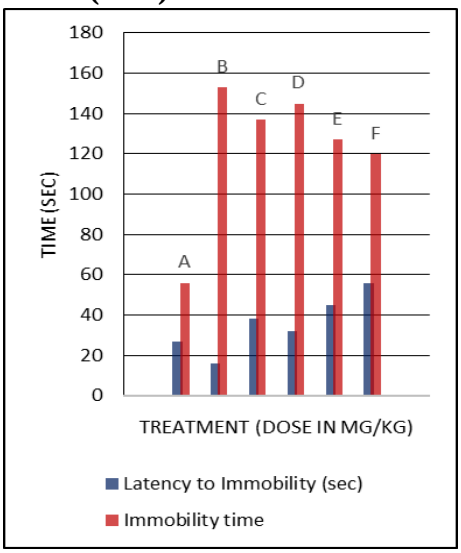

Figure 5: Effects of Vitamin E and its combination with amitriptyline using FST on 7th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group.

Day-14th

At the end, latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively, when animals were injected by Reserpine

(6mg/kg, i.p) as compared to normal control group. The effect of amitriptyline and vitamin E alone showed a significant increased latency to immobility time and decreased duration of immobility time as compared to toxicant group (Group II). The co-administration of amitriptyline and vitamin E (10 mg/kg, p.o and 50mg/kg, p.o) showed a prominent effect as compared to toxicant group (Group II).The results was found to be dose dependent and are tabulated in table 6.

Table 6: Effects of Vitamin E and its combination with amitriptyline using forced swim test.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Latency to Immobility time (sec)
I	Control	42±3.58	71±6.66
II	Reserpine (6mg/kg)	32±3.33	147±12.45
III	Amitriptyline (10mg/kg)	45±5.12	121±11.23
VI	Vitamin E (50mg/kg)	39±3.33	127±14.15
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	54±4.58	87±7.88
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	59±3.66	83±4.55

Day 14th Graphical representation (FST)

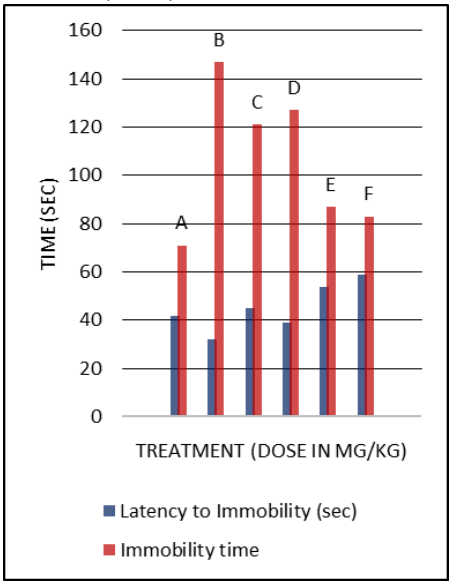

Figure 6: Effects Vitamin E and its combination with amitriptyline on FST on 14th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group.

4. DISCUSSION

The goal of this study was to see if vitamin E had antidepressant properties and to see how different dosages and combinations of amitriptyline medications affected reserpine-induced depression. The intraperitoneal administration of reserpine 6 mg/kg, i.p was proven to be a progressive model of depression and was employed to produce depression in our investigation.

Amitriptyline, a tricyclic antidepressant, is used as a standard medicine because it increases locomotor activity count in the actophotometer as well as increases latency to immobility time and increases duration of immobility time in the forced swim test, some studies have also demonstrated that amitriptyline reduces the immobility time in rodents on the first day of behavioural test.[18][19] Monoamine reuptake inhibition is thought to be essential for its effect. Amitriptyline increases neurotransmitters in the synapse and hence improves neurotransmission by inhibiting serotonin and noradrenaline transporters.[20]

Considering the role of oxidative and nitro-sative stress in the pathophysiology of depression and vitamin E's possible antioxidant properties, we tested vitamin E's antidepressant-like impact in an animal model of depressive-like behaviour generated by reserpine. Free radicals are extremely reactive chemical entities that are produced by inflammation and mitochondrial oxidative reactions. Reactive Oxygen Species (ROS) may react with macromolecules of the cell such as fatty acids, DNA, protein, and other macromolecules, causing damage to these macromolecules, when these radicals become excessive or when the antioxidant system is depleted.[21] ROS may have a role in the pathophysiology of depression through a variety of processes, including tissue damage, inflammation, neuro-degeneration, immunological responses triggered by tissue damage, and apoptosis.[22]

Clinical and preclinical research in cell and animal models provide support for oxidative stress in major depressive illness. These studies either look at oxidative homeostasis in patients with depressive disorder indirectly through an increase in neuronal damage caused by increased free radicals, such as lipid peroxidation and DNA strand-brakes.

All of the groups treated with reserpine 6 mg/kg experienced depression. On the first day of administration, all reserpine-treated groups showed a minor increase in immobility duration in FST and Actophotometer, but a considerable increase on the seventh day. In comparison to the toxicant group (Group II), those treated with amitriptyline, vitamin E, or a combination of

both showed meaningful impact. On fourteenth day it increases and decreases latency to immobility time, duration of immobility time respectively and also increases locomotor activity much more compared on first day of administration.

To our knowledge, this is the first study to show a potentiation and synergistic impact of vitamin E as an antidepressant medicine. Patients resistant to traditional therapies can be treated with vitamin E (50 mg/kg) and amitriptyline (10 mg/kg). Additionally, vitamin E (50 mg/kg) in conjunction with a sub-effective dosage of amitriptyline (5 mg/kg) may reduce antidepressant doses and hence adverse effects. This is critical in situations of infantile depression or postpartum depression, where drug safety is a concern.

5. CONCLUSION

In conclusion, the current investigation confirms that vitamin E potentiate anti-depressant effect of Amitriptyline in the forced swim test and Actophotometer. These findings might be useful in the development of novel therapeutic options as well as in clinical practice. The synergistic impact may result in a better response for those with treatment-resistant depression, as well as a reduction in the severity of adverse effects from low-dose antidepressants.

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Lipid Lowering Effect of Alpha Adrenoreceptor Blocker and Antidiabetic Drug in Experimental Animals

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Abstract: Lipid Lowering Effect of Alpha Adrenoreceptor Blocker and Antidiabetic Drug in Experimental Animals. **Methods:** Hyperlipidemia was induced by intraperitoneal injection of poloxamer 407 at a dose of 400mg/kg body weight in wistar albino rats. Drugs treatment were done by oral gavage for 3 days. At the end of the study, animals were kept fasted overnight and then blood sample was collected. The serum cholesterol (TC), triglycerides (TC), HDL, LDL, VLDL were calculated. **Results:** From the present investigation, it was observed that pioglitazone and terazosin drug have shown significant reduction in serum cholesterol, triglycerides, LDL, VLDL and increase in HDL level in p-407 induced hyperlipidemia.**Conclusion:** It is concluded that Pioglitazone and terazosin may possess antihyperlipidemic activity in Poloxamer 407 induced Hyperlipidemic Rats.
Keywords : Hyperlipidemia, Poloxamer 407, pioglitazone, Terazosin, Atorvastatin, lipid profile.

INTRODUCTION

Hyperlipidemia, also known as hyperlipoproteinemia, is characterised by unusually high amounts of lipids and lipoproteins in the blood.[1] Any aberrant lipid levels are included in this type of dyslipidaemia, which is the most frequent. Hyperlipidemia is basically divided into two types viz. primary and secondary type. [2,3] Primary hyperlipidemia is caused by genetic factors (for example, a mutation in a receptor protein), whereas secondary hyperlipidemia is caused by extrinsic factors such as diabetes. Because of their influence on atherosclerosis,

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lipid and lipoprotein abnormalities are frequent in the general population and are regarded as an unmodifiable risk factor for cardiovascular disease. Furthermore, some types may put you at risk for acute pancreatitis.[1]

According to the world health organisation (WHO), excessive blood cholesterol is responsible for around 56 percent of all occurrence of cardiovascular disease (CVD) worldwide, resulting in nearly 4.4 million death per year. When compared to 1990, it is estimated that more than 62.4 percent of person in India died from cardiovascular disease. [1,4]

Hyperlipidemia is a secondary metabolic disorder linked to diabetes that also increases the chance of developing the disease. Aside from the cause-effect link with diabetes, high levels of triglycerides, cholesterol, and low density lipoprotein in the blood are risk factors for cardiovascular disorders such as atherosclerosis, hypertension, and coronary heart disease.[1] Hyperlipidemia related to increased oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modification in LDL ,which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases.[5,6]

In addition, hyperlipidemia is induced by secondary effect of diabetes therefore by secondary effect of diabetes therefore, the agent having some antidiabetic effect also showed favourable effect to hyperlipidemia. [7,8] Type 2 diabetes is treated with thiazolidinediones (TZD), pioglitazone, and rosiglitazone. Pioglitazone is a less strong agonist of the peroxisome proliferator-activated receptor gamma (PPAR-) than rosiglitazone, but it is also beneficial in lowering fasting blood glucose and HbA1c levels. Pioglitazone, a commonly used antidiabetic, has been proven to improve HDL and decrease LDL and TG in diabetics, in addition to improving glycemic control. All anti-diabetic medicines affect lipid profiles differently, however pioglitazone has a better lipid-lowering effect than other anti-diabetic treatments, including rosiglitazone. In type 2 diabetic individuals, pioglitazone has been proven to have better cardiovascular advantages than rosiglitazone and glimipride. When compared to atorvastatin alone, co-administration of pioglitazone with atorvastatin improved the lipid profile in non-diabetic patients with high cardiovascular risk. Pioglitazone has a minor PPAR alpha (PPAR-) agonist effect in addition to PPAR-, which may be responsible for improved lipid and cardiovascular profiles.[9]

Alpha-blocking drugs are used as first-line treatment for benign prostatic hyperplasia (BPH), one of the most common causes of consultation for obstructive or irritative urological problems in middle-aged and elderly men. They are also used as second-line treatment for uncontrolled arterial hypertension, in monotherapy or in combination.[10]

In vitro investigations have shown that terazosin metabolites have antioxidant characteristics, which could be effective in preventing atherosclerosis in hypertensive patients, especially when other comorbidities like dyslipidaemia and diabetes are present. Then there's evidence of doxazosin's hypocholesterolaemia and antioxidant properties, which haven't been established with other alpha-blockers but suggest a possible benefit against endothelial dysfunction in a variety of situations.[10]

Poloxamer 407(P-407) is a non-ionic surfactant made up of polyoxyethylene and polyoxypropylene units in a block copolymer. It’s known for its biocompatibility and capacity to administer medications for a variety of diseases, and it works as a barrier against post-surgical adhesion. P-407 possesses remarkable thermo-reversible characteristics, in that it is liquid at room temperature but aggregates and forms a gel at body temperature before producing micelles. This temperature-dependent micelle and gel formation ability makes them commercially beneficially in personal care products including mouthwashes, deodorants and skin care products, as well as an inactive substance that can be used as a vehicle or media for a range of medicinal preparations [11]

In the present investigation we determined the lipid lowering effect of the alpha-blocking drugs terazosin and antidiabetic drug i.e. pioglitazone in hyperlipidemia model.

MATERIALS AND METHODS:

Ethics approval: The study was approved by institutional ethics committee of YSPM’s YTC, Satara MH India.

Drug and Chemical: Terazosin tablets (Intas Pharmaceutical Ltd), Pioglitazone tablets (Ontop Pharmaceutical PVT.Ltd), Atorvastatin tablets (Emcure Pharmaceutical Ltd), Poloxamer 407 (Ozone Pharmaceutical Ltd) were used for these research study.

Animals:

Inbred 30 Wistar albino rats (150–220 gm) were selected for present study. The animals were housed at room temperature (22-28 ºC) 12 hr dark and light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (25/12/2017/CPCSEA). Animals were maintained as per committee for the purpose of control and supervision of experiments on animals guidelines. [12,13]

Induction of hyperlipidemia:

The inducing agent was poloxamer 407. Before administration, P-407 was completely dissolved in water and refrigerated overnight to aid its complete dissolution. The syringe and needle to be used for the induction was cooled to avoid gelation within the syringe during injection.[11]

Experimental procedures:

Poloxamer 407-induced hyperlipidemic model

To render the animals hyperlipidemic, the rats were subjected to a 6 h-fast. Next, the rats were administered an intraperitoneal injection (i.p.) of a 400 mg/kg dose of poloxamer 407. [14,15] It had been prepared by combining the agent with saline or water for injection and then refrigerated over-night to facilitate dissolution of poloxamer 407. Starting two hours after the administration of the poloxamer 407, the rats were treated with prepared samples once daily for 3 days by oral gavage.[15]

Animals grouping and treatment:

A total of 30 rats were used. The rats were randomly divided into 5 groups. Each group contained 6 rats.

Group I: Normal Control rats fed with normal chow and distilled water (NC)

Group II: Hyperlipidemic Control rats induced without treatment (HC)

Group III: Hyperlipidemic rats treated with the standard drug (Atorvastatin 10mg/kg p.o.)

Group IV: Hyperlipidemic rats treated with the antidiabetic drug (pioglitazone 10mg/kg p.o.)

Group V: Hyperlipidemic rats treated with the alpha blocker drug (terazosin 5mg/kg p.o.)

Biochemical estimations:

Blood was collected by retro-orbital sinus puncture, under mild ether anaesthesia in the experimental models. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analysed for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low-Density Lipoprotein Cholesterol (VLDL-C).[12]

Statistical analysis:

The data was statistically analysed using one-way ANOVA followed by Tukey's multiple test. The results were expressed as Mean ± SEM (n=6). A value $P < 0.05$ was considered to be significance. [16]

RESULTS AND DISCUSSION

Table 1: Effect of Pioglitazone and Terazosin on lipid profile of P-407 induce hyperlipidemia

Group	Cholesterol (mg/kg)	Triglycerides (mg/kg)	HDL (mg/kg)	LDL (mg/kg)	VLDL (mg/kg)
Normal control	142 ±13.78	99 ±5.80	44 ±3.12	86 ±7.66	26 ±1.66
Toxicant control (Poloxamer 407)	322 ±22.11	198 ±12.33	24.00 ±1.44	185 ±12.86	56 ±4.33
Standard (Atorvastatin)	158 ±14.33	122 ±14.18	41 ±3.22	91 ±8.63	27 ±1.18
Test I(Pioglitazone)	202 ±18.22	143 ±12.44	33 ±2.11	134 ±11.12	41 ±3.33
Test II (Terazosin)	283 ±17.33	167 ±15.33	29 ±2.33	165 ±14.33	51 ±4.12

The values are expressed as a mean ± SEM, n=6, $p<0.05$ when compare to normal control and hyperlipidemic control.

Graphical Representation:

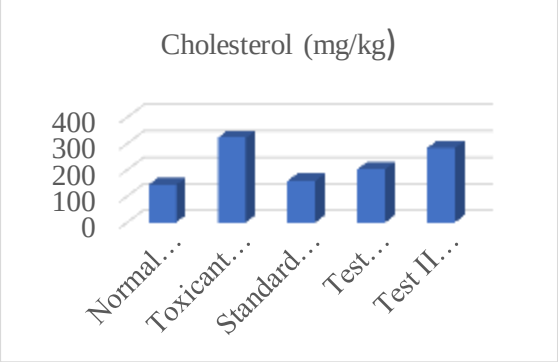


Fig.No.1 Effect of Pioglitazone and Terazosin on Total Cholesterol

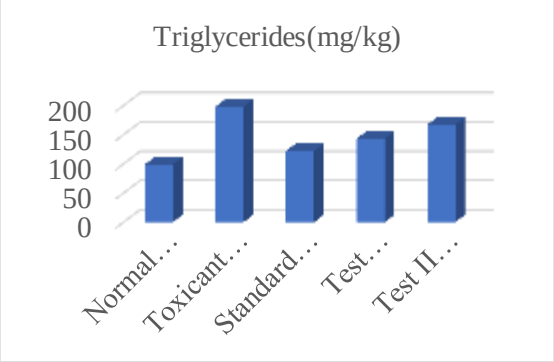


Fig.No.2 Effect of Pioglitazone and Terazosin on Triglycerides

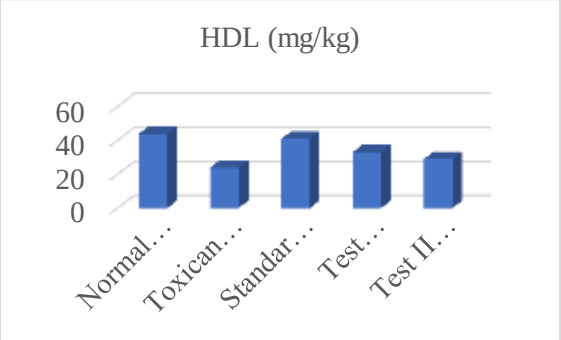


Fig.No.3 Effect of Pioglitazone and Terazosin on HDL

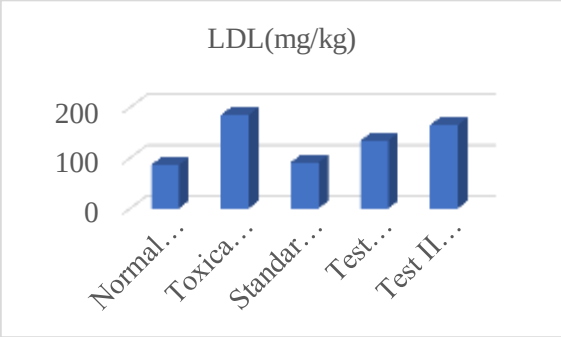
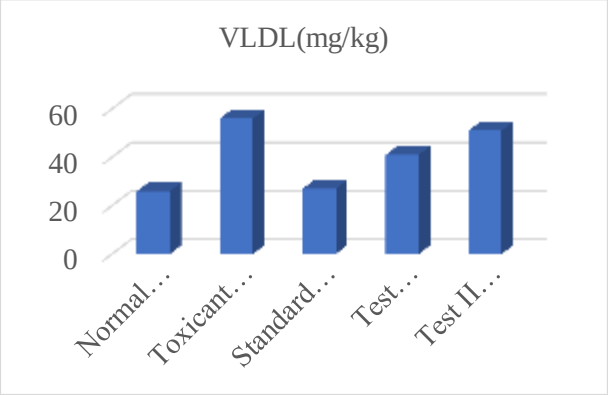


Fig.No.4 Effect of Pioglitazone and Terazosin on LDL



Lipids are water-insoluble organic molecules that are soluble in organic solvents. Lipids provide a variety of tasks, including chemical messengers, energy storage and provision, temperature regulation, and membrane lipid layer development. Hyperlipidemia is defined as an unusually high level of lipids, such as total cholesterol (TC), triglycerides (TG), and lipoproteins (lipoproteins) [17]. Hyperlipidemia-related diseases are substantial risk factors for the development of cardiovascular disease (CVD) [18].

Hyperlipidemia is a risk factor for atherosclerosis beginning and progression [19] as well as a high-risk factor for coronary heart disease development. As a result, the causal hyperlipidemia can be targeted for prevention or therapy of such illness. The abnormal high concentration of serum lipid is mainly due to increase in the mobilization of free fatty acids from the peripheral depots. [20,21]

Poloxamer 407 is non-ionic surfactant and is nontoxic to cellular membrane, was used successfully to induce hyperlipidemia in previous studies it causes effects by activating HMG CoA enzyme A. Poloxamer 407 a block copolymer composed of a hydrophobe that is flanked on each side with hydrophillic polyoxyethylene units. Our previous findings demonstrated that elevation in plasma TG was more sensitive than elevation in total plasma cholesterol following P-407 administration.[22]

Pioglitazone is a glucose-lowering medication that works as an agonist of peroxisome proliferator–activated receptor gamma.[13] All anti-diabetic drugs have varying effect on lipid profile but overall pioglitazone has shown more favourable lipid-lowering effect in comparison to other antidiabetics.[23]

Terazosin, which is structurally similar to prazosin, is a novel selective alpha 1- adrenoceptor antagonist.[24] The association between thyroid hormone imbalance and blood lipids encompasses processes such as beta oxidation at the muscle and liver level, as well as increasing the turnover of LDL, which could explain the decrease in cholesterol levels. Terazosin would cause a change in thyroid hormone levels, allowing us to notice a reduction in cholesterol and triglyceride levels.[10]

Group I administered with saline or water considered as normal control group. Group II administered with poloxamer 407 showed significant increase in lipid profile level except HDL level as compared to normal control group. Group III administered with atorvastatin showed significant decrease lipid profile except HDL level which is good cholesterol as compared toxicant control group. Group IV treated with pioglitazone significantly decreased TC, TG, LDL, VLDL and increased HDL level as compared to hyperlipidemic control group. Group V treated with terazosin significantly decreased TC, TG, VLDL, LDL and increased HDL level as compared to toxicant control group.

Conclusion:

In conclusion the present study has demonstrated that pioglitazone and terazosin are found to be of potential anti-hyperlipidemic activity in poloxamer-407 induced hyperlipidemia in wistar rats and it is observed that significant reduction of cholesterol, triglycerides, LDL, VLDL and increases HDL cholesterol level. According

to above study we can conclude that pioglitazone showed more significant effect as compared to terazosin. So pioglitazone is beneficial in preventing atherosclerotic cardiovascular diseases.

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HEPATOPROTECTIVE EFFECT OF LYCOPENE AGAINST PARACETAMOL-INDUCED HEPATIC DAMAGE IN ALBINO RATS

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ABSTRACT

Aim: The hepatoprotective effect of Lycopene was evaluated against paracetamol induced hepatic damage in albino rats. **Materials and Methods:** Liver function tests and biochemical parameters were estimated usingstandard kits. Livers were quickly removed and fixed in 10% formalin and subjected to histopathological studies. **Results:** There was a significant ($p < 0.05$) reduction in serum bilirubin levels with silymarin and lycopene 10mg/kg treated groups signifying protection against hepatic damage, lycopene 5mg/kg treated groups also showed significant change in bilirubin level. Similarly, significant ($p < 0.05$) reduction in the levels of serum transaminases were observed with all the treatment groups though more evident in the positive control and lycopene 10mg/kg treated groups. **Conclusion:** The results of this study strongly indicate that Lycopene may possess hepatoprotective action against paracetamol induced hepatic damage in rats.

KEYWORDS: Paracetamol, Lycopene, Silymarin, Heptoprotective.

INTRODUCTION

The liver is of vital importance in intermediary metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, eventually leading to diseases like hepatitis or cirrhosis.[1]

Paracetamol's hepatotoxicity is caused by its reactive metabolite. N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol

toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450.[2] Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity.[3,4,5] In spite of tremendous strides in modern medicine, the treatment of liver disorders is inadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage.[6] Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem.[7]

Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects.[8] In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials.[9]

The main objective of this study is to further understand the mechanism of lycopene's antioxidant action by evaluating the protective effect of orally administered lycopene pre-treatment on paracetamol induced rats. paracetamol is a hepatotoxic agent used to induce liver injury in experimental animals to check the efficiency of potential hepatoprotective agents.[10] The present study investigates the activity of the Lycopene against paracetamol-induced toxicity in comparison with silymarin a well-known antihepatotoxic agent.

MATERIALS AND METHODS

Experimental design

This experimental study was carried in models of paracetamol induced hepatotoxicity in albino rats. Lycopene was evaluated in paracetamol induced hepatic damage. The effects of lycopene was compared with silymarin, a proven hepatoprotective agent in this model of hepatotoxicity.

The study was conducted in strict accordance with the study protocol and CPCSEA guidelines Study animals were housed in the Central Animal House of our Institute, in an air-conditioned area with 12-15 filtered fresh air changes, temperature 22-30°C, relative humidity 30-70% six rats per cage were housed in polypropylene cages having husk paddy as the bedding, during the study. Twelve hourly light and dark cycles were maintained.

The model was standardized and hepatic damage was confirmed in the model. The effects of lycopene were evaluated in experimental models of paracetamol induced hepatic damage 30 albino rats of either sex weighing between 150-200 grams were used for the entire study. Lycopene was used in two doses of 5mg/kg and 10mg/kg based on the dose animal studies of lycopene in previous as hepatoprotective. Lycopene was administered orally. Daily, suspended in 0.5% CMC. Silymarin was administered in the dose of 50mg/kg, orally.

The effects of lycopene were evaluated in paracetamol induced hepatic damage, using silymarin as positive control. 24 Wistar rats were randomly allocated into four group's namely toxicant control (paracetamol 2gm/kg), silymarin (50mg/kg), lycopene (5mg/kg) and lycopene. (10mg/kg), each group containing 6 rats. The study drugs were administered for a duration of 7 days. On the 8th day, Induction of hepatic damage was carried out with paracetamol given orally in the single dose of 2g/kg. 24 hours following the administration of paracetamol, 2ml of blood was collected by puncturing the retro-orbital sinus and biochemical investigations was performed. Then the rats were euthanized by administering ketamine intraperitoneally. The liver was dissected out, washed in cold saline and blotted dry by placing it on tissue paper Weight and volume of liver was measured and processed further for histopathological examination.

Assessment of liver function parameters

At the end of the experimental period, animals were sacrificed by cervical decapitation under mild ketamine anesthesia, blood was collected and the serum was separated by centrifuging at 300 rpm for 10 min. The collected serum was used for the assay of marker enzymes. The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel.[12] Alkaline phosphatase (ALP) was determined by the method of Kind and King.[13]

Assessment of biochemical parameters

The total bilirubin was estimated by Method of Malloy and Evelyn.[16] Immediately after sacrificing the animal, the liver was excised from the animals, washed in ice-cold saline, and the weight of the liver was recorded.

Histological studies

Livers were quickly removed and fixed in 10% formalin, dehydrated in gradual ethanol (50%–100%), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and

then stained with hematoxylin and eosin dye for photo microscopic observations of the liver histologic architecture of the control and treated rats.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D). Differences in liver function parameters and biochemical parameters were determined by factorial one-way ANOVA. Individual groups were compared using Tukey's test. Differences with $P<0.05$ were considered statistically significant.

RESULTS

There were no macroscopic changes observed in the liver any of the study groups. There was statically significant decrease in the liver weight and volume observed with grips that received silymarin 50mg/kg and lycopene 10mg/kg when compared with toxicant control. (Table 1)

Table 1: Effect of lycopene on liver Weight and Volume in rat model of paracetamol induced hepatotoxicity.

Groups	Liver weight (gm/100gm body weight)	Liver volume (ml/100gm body weight)
Normal control	4.82±0.11	5.62±0.24
Toxical control	5.81±0.12	9.62±0.17
Standard (Silymarin 50mg/kg)	3.81±0.12	5.64±0.17
Test Ⅰ (Lycopene 5mg/kg)	4.62±0.43	5.13±0.22
Test Ⅱ (Lycopene (10mg/kg)	3.95±0.41	5.90±0.18

All values represent Mean ±SD (n=6).

Biochemical parameters

There was a significant ($p<0.05$) reduction in serum bilirubin levels with silymarin and both doses of lycopene 5mg /kg and 10mg/kg treated groups signifying protection against hepatic damage. Similarly significant ($p<0.05$) reduction in the levels of serum transaminases were observed with positive control and lycopene 5 and 10mg /kg treated groups. (Table 2)

Table 2: Effect of lycopene on biochemical parameters in rat model of paracetamol induced hepatotoxicity.

Groups	Serum bilirubin (mg/dl)	Aspartate transaminase (IU/ml)	Alanine transaminase (IU/ml)	Alkaline phosphatase(IU/ml)
Normal control	0.53±0.03	81.10±8.52	75.43±8.33	90.96±6.66
Toxicant control	0.93±0.08	308.40±17.10	149.05±11.43	248.01±22.23
Standard (Silymarin 50mg/kg)	0.28±0.04	140.02±12.35	62.53±7.10	126.02±10.40
Test Ⅰ (Lycopene 5mg/kg)	0.48±0.03	198.26±12.16	68.32±05.28	180.03±15.33
Test Ⅱ (Lycopene 10mg/kg)	0.36±0.06	137.24±13.39	66.42±06.32	122.01±11.30

All values represent Mean ±SD (n=6) $p<0.05$ using one way ANOVA with post hoc Tukey's test (versus toxicalnt control)

Table 3: Histopathological changes.

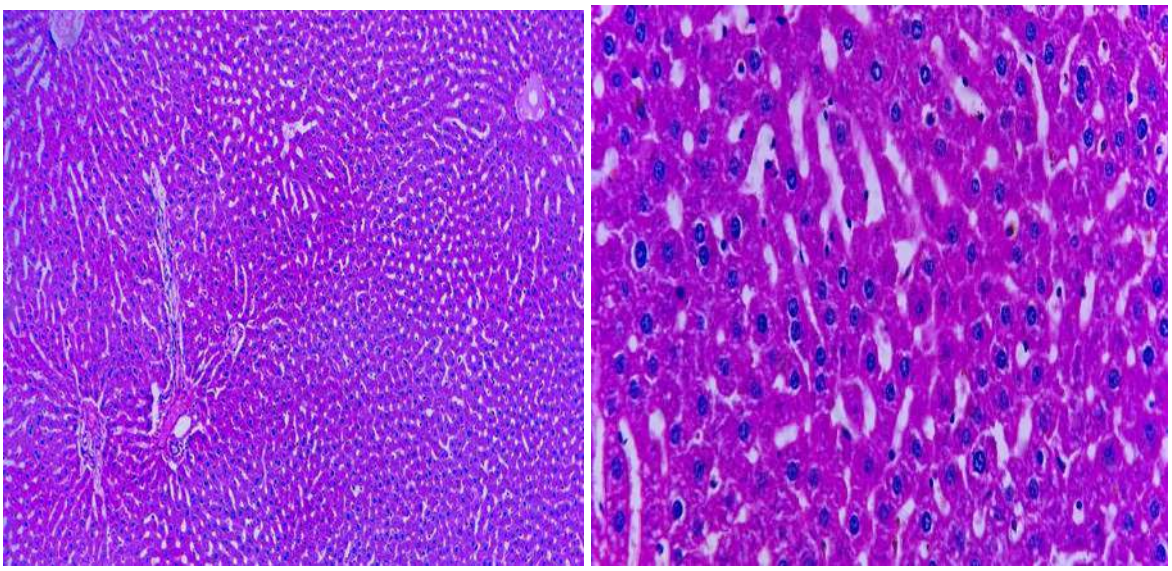
Groups	No of animals showing						
	Degeneration				Necrosis		
	0	Ⅰ	Ⅱ	Ⅲ	0	Ⅰ	Ⅱ
Normal control	0	4	2	0	1	5	0
Standard (Silymarin 50mg/kg)	1	5	0	0	4	2	0
Test Ⅰ (Lycopene 5mg/kg)	0	4	2	0	1	5	0
Test Ⅱ (Lycopene 10mg/kg)	1	4	1	0	4	2	0

Out of the six animals in the normal group four showed grade I degeneration while the remaining, two showed grade II degeneration. Five animals showed presence of 1-2 necrotic cells per high power field (Grade 1) while one animal showed no necrosis. Similar changes were seen in the group that received lycopene in the dose of 5mg/kg with regard to the number of animals.

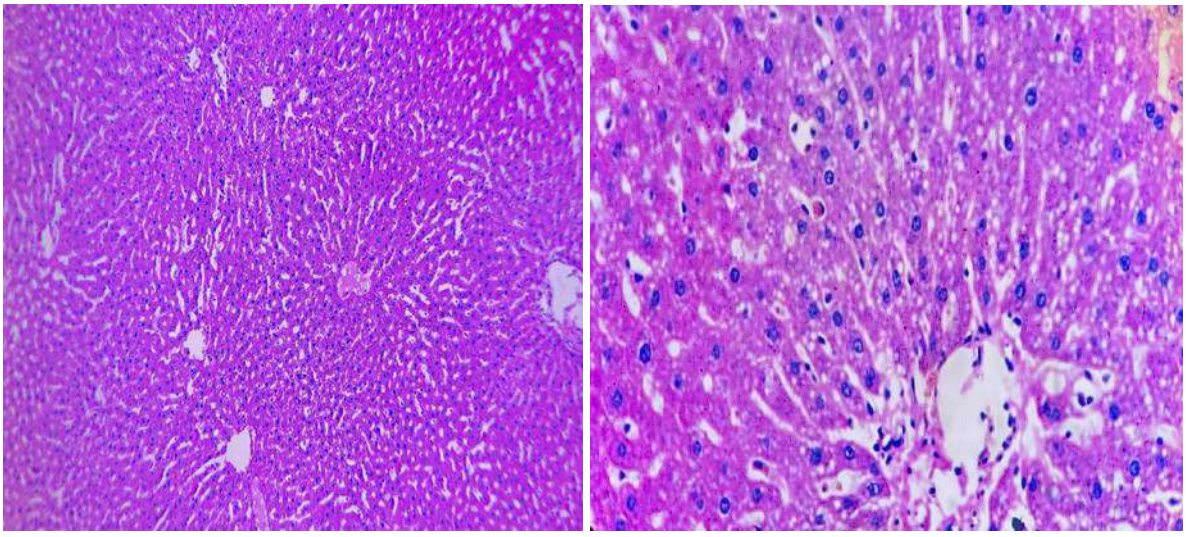
In the silymarin treated group, 4 animals showed no necrosis while two animal showed necrosis and Minimal degenerative changes were seen in 5 animals.

In lycopene 10mg/kg treated group, one animal showed no degeneration and grade II degeneration each, with remaining 4 animals showing grade I degeneration. Two animal in the lycopene 10mg/kg treated group showed necrosis while 4 showed near normal hepatic

parenchyma. (Table 3, Figure 1).



1. Normal control **2. Standard (Silymarin 50mg/kg)**



3. Test Ⅰ (Lycopene 5mg/kg) **4. Test Ⅱ (Lycopene 10mg/kg)**

Figure 1: (1 to 4) effects of study drugs on histopathology in rat model of paracetamol induced hepatic damage.

DISCUSSION

While standardizing this model, Serum bilirubin, AST, ALT and ALP as biochemical parameters were chosen. Morphological parameters such as liver weight and liver volume were measured to find changes in liver morphology. Structural alterations the liver due to ongoing insults was confirmed by doing histopathological examination of liver at the end of study.

During standardization of our study, none of the study animals died during the study duration. Paracetamol produced significant ($p < 0.05$) elevation in serum bilirubin AST and ALT levels, compared to the toxicant control. Lycopene has caught the attention of investigators as a potential hepatoprotective due to its antioxidant, anti-inflammatory and anti-proliferative properties.[30]

Liver is the chief target organ of lycopene accumulation in the body. After oral administration, lycopene is rapidly absorbed and gets accumulated in the liver, with a lesser amount going to the spleen. Safety of lycopene has been proved beyond doubt in multiple toxicity studies. No significant toxic effects were observed with lycopene up to 2000mg/kg body weight when administered orally. Animals were observed for 24 hours.

There is a need of evaluation of lycopene in other models of hepatotoxicity with higher doses given its wide safety margin. Further studies are essential to expatiate its mechanism of action. In future, lycopene would be a potential hepatoprotective agent against drug induced hepatotoxicity in clinical use. Especially in the prevention/treatment of paracetamol induced hepatotoxicity.[32,33]

CONCLUSION

It is concluded that lycopene emerge hepatoprotective effect against paracetamol induced hepatic damage in rats. Lycopene needs to be evaluated in other models of hepatotoxicity and further studies are required to delineate its mechanism of action. Lycopene might be a potential hepatoprotective for clinical use in future.

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CURCUMIN POTENTIATES THERAPEUTIC EFFICACY OF VOGLIBOSE

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ABSTRACT

Introduction: Herbal antidiabetic preparations are often used as an add-on therapy in diabetes and such herbal preparations often contain curcumin. Hence, in the present investigation the combine effects of curcumin and voglibose in normal as well as diabetic rats was studied. **Methods:** Streptozotocin (60 mg/kg i.p.) diabetic rats was treated for 14 days with curcumin (30 mg/kg p.o) and voglibose (0.06 mg/kg p.o). After treatments, the blood glucose level was assessed. Data was analyzed using one-way analysis of variance (ANOVA) followed by post hoc Scheffe's test. **Results:** Treatment of diabetic rats with curcumin or voglibose alone decreased the blood glucose level. The combination of voglibose with curcumin further decreased blood glucose levels in diabetic rats, indicating synergestic effect. **Conclusion:** The results highlights that curcumin and voglibose given alone showed effect against STZ induced Hyperglycemia but the combination of curcumin with voglibose showed better and synergistic effect. Therefore, it might be a promising strategy for combating diabetic complications.

KEYWORDS: Hyperglycemia; Curcumin; Voglibose; Streptozotocin; Antidiabetic activity.

1. INTRODUCTION

In today's society, diabetes is a serious metabolic disease. Its frequency has risen considerably in recent years, causing the World Health Organization to classify it as a major public health crisis.[1] The International Diabetes Federation's specialists estimate that 193 million people worldwide have undiagnosed diabetes and are at risk of developing chronic complications. According to estimations, there will be 629 million persons with diabetes worldwide in 2045.[2] It is a chronic metabolic condition characterised by a loss of glucose

homeostasis as well as changes in lipid and protein metabolism due to abnormalities in insulin secretion, action, or both. Insulin is a protein (hormone) produced by beta cells in the pancreas in response to a variety of stimuli, including glucose, sulphonylureas, and arginine, but glucose is the most important factor.[3]

Analyzing blood sugar levels can be used to diagnose diabetes. On fasting, blood sugar level is 80 mg/dl, and in the postprandial stage, it can reach 160 mg/dl. Finger prick blood sugar test, fasting blood sugar test and glucose tolerance diagnostic test are some of the laboratory tests used to diagnose diabetes.[4]

Impaired insulin secretion, tissue insulin resistance, or a combination of the two are thought to be the most popular factors that contribute to the pathogenic mechanisms of T2DM, a disease spectrum that starts with tissue insulin resistance and progresses to a state marked by complete loss of pancreatic beta cell secretory activity.[5] The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissue.[6]

Streptozotocin (STZ) is a synthetic nitrosoureido glucopyranose derivative isolated from Streptomyces achromogenes fermentations that is utilised as an antitumor antibiotic and is chemically linked to other nitrosureas used in cancer therapy.[7] It produces β-cell toxicity, which leads to insulin insufficiency, and is easily carried into pancreatic β-cells by GLUT-2. The β-cell O-GlcNAcase enzyme, which is in charge of removing O-GlcNAcase from protein, is specifically inhibited by STZ. This leads in β-cell death and irreversible O-glycosylation of intracellular proteins.[8]

When taken alongside standard medications, a combination of herbal pharmaceuticals (or isolated phytochemicals) has been demonstrated to be effective in the treatment of some disorders.[9] Among all of the available medical systems in the world, Indian traditional medicine is one of the most comprehensive.[10]

Turmeric contains curcumin, a yellow pigment derived from *Curcuma longa* that has anticarcinogenic and anti-inflammatory characteristics, including an inhibitory action on TNF-a. Curcumin has also been found to inhibit NO synthesis and scavenge nitrite and peroxynitrite radicals released by macrophages, which helps to lower blood glucose levels and improve the antioxidant capacity of pancreatic β-cells.[11] The anti-inflammatory,

antioxidant, antiviral, antifibrotic, anticoagulant, and glucose-regulating properties of curcumin are its distinguishing features.[12]

In the treatment of diabetes, alpha-glucosidase inhibitors and fast-acting, short-duration insulin secretagogues are widely utilized. In the final step of carbohydrate digestion, voglibose, alpha -glucosidase inhibitor, reduces the breakdown of disaccharides into monosaccharides by acting competitively on the activities of disaccharidase (alpha -glucosidase). This decreases glucose breakdown and absorption, preventing postprandial hyperglycemia.[13]

As a result, examining these interactions is crucial for illness treatment that is both safe and effective. Despite the fact that Curcumin nanoparticles have been shown to have anti-diabetic properties, the interaction between Curcumin with Voglibose has yet to be studied.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Streptozotocin sterile powder 1gm was purchased from Teva Parenteral Medicines Inc. Irvine.

Curcumin was purchased from Yarrow chem Products Ghatkopar (west) Mumbai, India.

Voglibose was purchased from Discovery Mankind Pharma Ltd. New Delhi.

2.2 Maintenance of animals

Albino rats of Wistar strain weighing 180-200g were used for the studies after obtaining the permission from institutional animal ethical committee. The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light/dark cycle; at an ambient temperature of 25 ± 5 °C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*.

2.3 Oral glucose tolerance test

For Oral Glucose Tolerance Test, rats was divided into four groups (n = 6). Medications were given orally to overnight fasted animals.

After half an hour of test medication administration, a glucose solution (2.5g/kg body weight) was given orally in a volume of 1 ml, and blood glucose levels were monitored at 0, 30, 60, 90, and 120 minutes using a Glucopoint glucometer.

2.4 Hypoglycaemic study

For hypoglycaemic study, rats were divided into four groups (n = 6) and were administered vehicle (1ml), curcumin(30mg), voglibose (0.06mg), curcumin(30mg) and voglibose(0.06mg) respectively. The blood glucose levels were estimated on days 0, 7 and 14.

2.5 Induction of Hyperglycemia

Diabetes was induced using streptozotocin (STZ). The animals fasted overnight and diabetes was induced by way of a single intra peritoneal injection of a freshly prepared solution of STZ (60 mg/kg b.w.) in a 0.1 M citrate buffer (pH 4.5). On the third day of STZ-injection, the animals with fasting glycaemia higher than 200 mg/dL and with signs of polyuria and polydipisia were considered to be diabetic and included in the study.

2.6 Experimental design

The diabetic animals, divided into four groups (n = 6) were administered vehicle, curcumin (30 mg/kg), voglibose (0.06 mg/kg), curcumin (30mg) and voglibose (0.06 mg/kg), respectively, for 14 days. The fasting blood glucose levels were estimated on days 0, 7 and 14.

At the end of the treatments, the blood samples were collected for the analysis of blood glucose level. The experimental procedures were approved by the Institutional Animal Ethics Committee.

2.7 Statistical analysis

Using the 7.5 version of SPSS computer programme, data were statistically examined using one way ANOVA, followed by a post hoc Scheffe's test. When the p-value was less than 0.05, the results were considered significant.

3. RESULTS

3.1 Effect of curcumin, voglibose and its combination in oral glucose tolerance test

The curcumin, voglibose and its combination showed a significant reduction in blood glucose levels from 30 min onwards in oral glucose tolerance test as compared to normal group. The results are represented in table 1.

Table 1: Effect of curcumin, voglibose and its combination in oral glucose tolerance test.

Group	Treatment	Blood-Glucose Level (mg/dl)				
		0min	30min	60min	90min	120min
I	Normal Control (Glucose 2.5g/kg)	90.6±9.2	106.7±10.6	157.4±15.7	166.7±16.6	250.1±10.10
II	Curcumin (30mg/kg p.o) + Glucose (2.5g/kg)	83.8±8.3	110.5±11.20	99.8±9.9	94.2±9.4	91.1±9.2
III	Voglibose (0.06mg/kg p.o) + Glucose (2.5g/kg)	82.4±8.4	95.4±9.5	81.2±8.1	80.8±7.9	79.1±7.6
IV	Curcumin (30mg/kg p.o) + voglibose (0.06mg/kg p.o) + Glucose (2.5g/kg)	81.7±7.9	91.7±9.1	89.5±8.7	82.8±8.1	76.7±7.6

Each value represent mean ± S.E.M., *n*=6

3.2 Effect of curcumin, voglibose and its combination in normal animals

In normal animals, curcumin did not significantly reduced blood glucose level on 0^{th}, 7^{th} and 14^{th} day as compared to normal control group. Voglibose significantly reduced blood glucose level on 0^{th}, 7^{th} and 14^{th} day as compared to normal control group. Combination of curcumin and voglibose significantly reduced blood glucose level on 0^{th}, 7^{th} and 14^{th} day as compared to normal control group. The results are represented in table 2.

Table 2: Effect of curcumin, voglibose and its combination in normal animals.

Group	Treatment	Blood-Glucose Level (mg/dl)		
		Day 0	Day 7	Day 14
I	Normal Control	75.4±7.8	75.8±7.8	76.4±7.4
II	Curcumin (30mg/kg p.o)	74.5±7.9	74.6±7.6	73.7±7.3
III	Voglibose (0.06mg/kg p.o)	72.8±7.1	61.9±6.6	61.1±6.2
IV	Curcumin (30mg/kg p.o) + Voglibose (0.06mg/kg p.o)	70.5±6.9	57.9±5.8	53.8±5.3

Each value represent mean ± S.E.M., *n*=6

3.3 Effect of curcumin, voglibose and its combination in diabetic animals

In diabetic animals, curcumin did not significantly reduced blood glucose level on 0^{th}, 7^{th} and 14^{th} day as compared to normal control group. Voglibose significantly reduced blood glucose

level on 0th, 7th and 14th day as compared to normal control group. Combination of curcumin and voglibose significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. The results are represented in table 3.

Table 3: Effect of curcumin, voglibose and its combination in diabetic animals.

Group	Treatment	Blood-Glucose Level (mg/dl)		
		Day 0	Day 7	Day 14
I	Diabetic Control STZ (60mg/kg i.p)	381.2±38.1	398.1±39.7	391.7±38.1
II	Curcumin (30mg/kg p.o)	370.5±37.9	360.1±5.1	359.5±5.7
III	Voglibose (0.06mg/kg p.o)	356.1±36.3	149.6±15.1	140.9±14.10
IV	Curcumin (30mg/kg p.o) + Voglibose (0.06mg/kg p.o)	331.2±33.3	116.8±9.66	110.2±12.44

Each value represent mean ± S.E.M., *n*=6

4. DISCUSSION

A series of metabolic illnesses known as diabetes mellitus are characterised by chronic hyperglycemia carried on by deficiencies in insulin secretion, insulin action, or both. The significance of insulin as an anabolic hormone leads to metabolic irregularities in carbohydrates, lipids, and proteins. These metabolic abnormalities are introduced on by insufficient insulin levels to start producing an adequate response and insulin resistance of target tissues, primarily skeletal muscles, adipose tissue, liver, at the level of insulin receptors, signal transduction system, and effector enzymes or genes. The kind and length of diabetes affect the severity of symptoms. Some people with diabetes have no symptoms, especially those who have type 2 diabetes in its early stages. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome.[14]

Most diabetic complications are caused by persistent hyperglycemia, a typical symptom of diabetes. Treatment should seek to lower blood glucose levels to near-normal levels in all individuals. Oral hypoglycemic medications are currently available for Hyperglycemia treatment. The majority of medications have failed due to ineffectiveness or side effects. There is no cure for diabetes. This problem has highlighted the need for more better, safer, and less expensive diabetes management techniques. Alternative therapies must be discovered in order to solve these challenges and give better therapeutic management. An excellent method to treat hyperglycemia and other DM problems is to combine the actual antidiabetic medications with phytochemicals.[15]

Streptozotocin is a deoxy-s [(methyl-nitrosoamino) carbonyl)-amino]-D gluco pyranose molecule that causes Hyperglycemia in most laboratory animals. Streptozotocin and other beta cell toxins in high dosages cause insulin insufficiency and Hyperglycemia. Although streptozotocin is favoured because of its more selective beta cell cytotoxicity, its sensitivity varies by species, strain, sex, and nutritional condition, and there are batch variances in activity.[16]

In the search for alternatives to current medication for diabetes mellitus, curcumin has gained attention in the last decade for its antidiabetic properties.[17] Curcumin also reported to have beneficial effects on various diseases, like multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease.[18] In addition, it could delay development of T2DM, improve β-cell functions, prevent β-cell death, and reduce insulin resistance in animals.[19]

Voglibose is alpha -glucosidase inhibitor that also stimulates GLP-1 secretion.[20] Inhibition of enzymes in the digestive organs, such as alpha-glucosidase, can be used to prolong glucose absorption as a treatment for diabetes. Alpha-Glucosidase (α-d-glucoside glucohydrolase) is an exo-type carbohydrase that catalyses the liberation of α-glucose from the non-reducing end of the substrate in microbes, plants, and animal tissues. The blocking of this enzyme decreases the rise in blood sugar after a carbohydrate meal.[21] It slows and reduces the absorption of monosaccharides by preventing the intestinal breakdown of complex carbohydrates into simple sugars.[22]

In our Investigation, the oral glucose tolerance test studies revealed that curcumin, voglibose and combination of curcumin and voglibose has the capacity to lower blood glucose.

Hypoglycemic studies experiments conducted for our investigation showed that curcumin, voglibose, and combinations of curcumin and voglibose had the ability to reduced blood glucose.

In our present antidiabetic study, group II animals treated by curcumin did not significantly reduced blood glucose level. Group III animals treated by voglibose could significantly reduced blood glucose level as compared to toxicant group. Group IV animals treated by its combination could significantly reduced blood glucose level as compared to toxicant group.

5. CONCLUSION

Results obtained from the present study proved that curcumin when given alone did not show effect against STZ induced Hyperglycemia in rats and voglibose when given alone could significantly reduced blood glucose level but more prominent effect was observed when combination of curcumin and voglibose was given. Hence combination of these drugs showed synergestic effect.

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DESIGN, DEVELOPMENT AND EVALUATION OF TRADITIONAL POLYHERBAL FORMULATION TO CURE DENGUE AND CHIKUNGUNYA

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ABSTRACT
Herbal medicine is the most traditional method of treatment that has been used throughout history and is utilised in all cultures. The present study was focused on development and evaluation of polyherbal syrup to cure dengue and chikungunya. Dengue and chikungunya viruses are caused by an arbovirus. Both viruses are arthropod-borne viruses sharing a common vector. These two diseases are transmitted from infected person to the healthy person through bite of virus carrying female mosquito. There is no specific treatment for dengue and chikungunya. Generally, the doctors priscribes papaya leaves extract and NSAID drugs to treat the symptoms of dengue and chikungunya. Keeping our hopes up, we took steps to locate a remedy using all natural herbs that have been documented centuries ago and came up with a comparatively safer solution for this ailment. In present research some natural herbs are used to develop safe cost-effective syrup for dengue and chikungunya. The aqueous extracts of selected herbs were formulated in particular ratio to form herbal syrup. The plants chosen were *carica papaya, aloe vera, tinospora cordifolia* and *ocimum tenuiflorum.* The raw materials were collected, authenticated accordance with WHO guidelines. Several experimental batches were created by adjusting the percentage of simple syrup. This batches were tested for a variety of assessment factors. This formulation's accelerated stability was also studied. The formulation complies with all the phytochemical and physicochemical parameters, therefore it is concluded that polyherbal syrup was found to be safe.

KEYWORDS: Dengue, Chikungunya, Polyherbal syrup, Phytochemical screening, Accelerated stability studies.

INTRODUCTION
Dengue and chikungunya are two mosquito-borne viral diseases of great public health concern in India. Dengue virus (DENV) and chikungunya virus (CHIKV) are transmitted by the same species of mosquito, Aedes aegypti and share spatiotemporal territories. DENV belongs to the Flaviviridae family and CHIKV belongs to the genus Alphavirus of Togaviridae.[1] DENV and CHIKV typically incubate for 4-7 days and 3-7 days, respectively. Patients infected with either virus often have an initial onset of fever, myalgia, and headache, with some developing a maculopapular rash and/or gastrointestinal symptoms.[2] Mild dengue fever to severe dengue hemorrhagic fever and/or dengue shock syndrome are the clinical symptoms.[3] The symptoms of dengue are thrombocytopenia, high fever, severe headache, muscle and joint pains, nausea and vomiting, mild pain in throat and extreme weakness.[4] Chikungunya virus (CHIKV) is an arbovirus spread by mosquitos. When symptomatic (85 to 95% of cases), CHIKV infection causes an acute fever–arthralgia syndrome that can evolve into chronic inflammatory rheumatism.[3] The symptoms of chikungunya are polyarthralgia, sudden high fever, joint pains and muscle pains, diarrhoea, abdominal pain and fatigue.[5]

Till date, there are no specific globally accepted treatments for dengue fever and chikungunya fever in any system of medicine. DENV and CHIKV does not cause very high mortality. Traditionally large numbers of plants are reported for their use against contagious diseases, including infection caused by viruses.[6] During the critical phase of dengue, malaria, chikungunya that thrombocytopenia is characterized by a decrease in platelet count below 100000 perm3 from the baseline.[7]

Herbal formulation
Herbal medicine are treated as traditional medicines since they were extensively used in traditional system of medicine like Ayurveda, siddha, Unani.[8] The majority of herbal syrup was obtained from plants, and herbal medicine refers to the use of extract for therapeutic purposes. Herbal medications are also available in syrup form, in addition to conventional dosage forms.[9]

Following are the ingredients used in formulation

1. **Papaya leaves:** *Carica papaya* (papaya, paptia, paw paw) is an herbaceous plant belonging to the family

Caricaceae. Tradtionally the *Carica papaya* leaf extract used in dengue and chikungunya fever patients with thrombocytopenia; it accelerates the increase in the platelet count.[10,11,12]

2. **Aloe vera:** It consist of fresh leaves of *Aloe barbadensis* belonging to family *Liliaceae*. Aloe vera reduces inflammation and arthritis caused by adjuvants.[13,14,15]
3. **Gulvel:** *Tinospora cordifolia*, also known as "Guduchi" in Sanskrit. It is a significant medicinal herb used in Ayurvedic medicine to treat polyarthralgia, osteoporosis, colds, fevers, diabetes, and even rheumatoid arthritis.[16,17,18,19,20]
4. **Tulsi:** Tulsi is an aromatic plant belonging to the family *Lamiaceae*. It is useful in dengue, chikungunya and malarial fever.[21,22,23,24]

MATERIALS AND METHODS

Collection and Authentication of plant material

Leaves of *Carica papaya, Aloe barbadensis, Tinospora cordifolia, Ocimum tenuiflorum* were collected from the herbal supplier. All the plant material were authenticated by Y. C. College, Department of Botany, Satara.

Preparation and Phytochemical evaluation of extracts

The plant material was washed thoroughly with running tap water, more than five times. The main stems of the leaves was removed using a scissor. The material was cut in to pieces and washed it well with boiled cool water. Choped into even smaller pieces. The pieces were grinded well for about 15 minutes with 50mL boiled cold water till a uniform pulp is formed. The pulp was placed into the juice extractor and squeezed it till get the pure extract. The phytochemical evaluation of extracts was done individually.

Preparation of simple syrup

The 66.67gm of sugar was weighed and added to purified water and heated until it dissolve with occasional stirring. Sufficient boiling water was added upto 100ml.

Preparation and Phytochemical evaluation of polyherbal syrup

The simple syrup (66.67%w/v) was prepared as per Indian pharmacopoeia. The extracts were added into simple syrup I.P. and the volume was made upto 100ml. The 3 trial batches were prepared by varying the concentration of simple syrup.

Formulation table

Composition of polyherbal syrup

Ingredients	Quantity			Activity
	A	B	C	
Papaya Leaf Juice	25 ml	25 ml	25 ml	Platelet increasing agent
Aloe vera Juice	18 ml	18 ml	18 ml	Anti-inflammatory agent
Gulvel Juice	8 ml	8 ml	8 ml	Anti-arthritic agent
Tulsi Juice	10 ml	10 ml	10 ml	Analgesic agent
Simple Syrup	46.66%	56.66%	66.66%	Base, Viacosity modifies

Evaluation parameters

a. Colour, Odour, Taste

The syrup's colour, odour and taste were examined.[25]

b. Determination of pH

The 10ml of final syrup was taken in to the volumetric flask and filled the volume upto 100ml with distilled water. The pH paper was used to measure the Ph.[25,26]

c. Determination of viscosity

The viscosity of syrup was measured using an ostwald viscometer. Firstly the ostwald viscometer was cleaned with warm chromic acid or acetone. The water was filled up to the mark "G" in the dry viscometer and placed the viscometer vertically on a suitable platform. The time was noted while water was flowing from mark A to mark B. This operation was done at least three times and recorded the time to acquire reliable readings. Then the viscometer was rinsed and filled it with test liquid (syup) till mark A, then the time was calculated to takes for the liquid to flow up to mark B.[26]

Formula for viscosity

$$\text{Viscosity} = \frac{\text{Density of test liquid} \times \text{Time required to flow test liquid} \times \text{Viscosity of water}}{\text{Density of water} \times \text{time required to flow water}}$$

d. Determination of density

The density of syrup was calculated using the beaker. The beaker was cleaned with chromic acid or nitric acid. The beaker was rinsed two to three times with distilled water. The weight of the empty dry beaker was noted. The beaker was filled with test liquid, and the excess liquid out of the beaker was wiped. Finally, calculated the weight in grams of a liquid.[9]

Formula for density

$$\text{Density of liquid under test (syrup)} = \frac{\text{Weight of liquid under test}}{\text{Volume of liquid under test}}$$

e. Determination of specific gravity

After cleaning with chromic acid or nitric acid, the bottle was rinsed with filtered water two to three times. The weight of an empty dry bottle was taken with a capillary tube stopper (w1). The bottle was filled with distilled water, screw on the stopper, and the liquid from the outside of the bottle was wiped. And using an analytical balance, weighed the bottle with distilled water (w2). After emptying and drying, the procedure was repeated by replacing water with the liquid under test (syrup). The container was weighed with the stopper and the liquid under test on an analytical balance (w3).[9,27]

Formula for specific gravity

$$\text{Specific gravity of liquid} = \frac{\text{Weight of liquid under test}}{\text{Weight of water.}} \text{ under test (syrup)}$$

Accelerated stability study

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters. The produced polyherbal syrup was evaluated to an accelerated stability investigation for three months. The syrup was maintained at room temperature and was kept in an amber-colored container. Every month, pH, viscosity, density, and specific gravity were measured. The phytochemical analysis was done at the end of every month.[28]

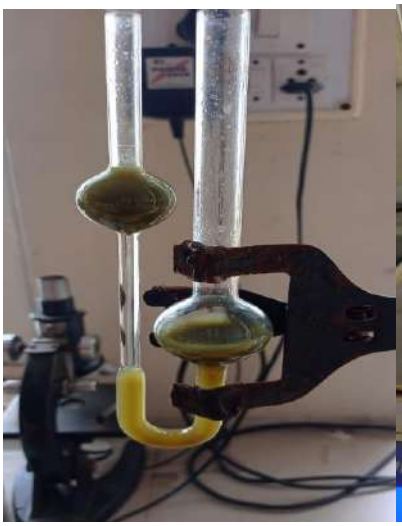

Fig. 1: Viscosity.

Fig. 2: Density.

Fig. 3: Specific gravity.

RESULT AND DISCUSSION

Raw material analysis

The phytochemical evaluation of individual herbs are given in table:

Components	Papaya	Aloe vera	Gulvel	Tulsi
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	-	+
Phenol	+	-	-	+
Carbohydrate	-	+	+	-
Glycoside	+	-	+	-
Terpenoid	-	-	-	+
Protein	+	+	+	+
Steroid	+	-	+	+

Evaluation of trial batches

The trial batches were evaluated for physical parameters such as colour, odour, taste, pH, viscosity, density and specific

gravity etc. The results are given in table:

Sr. No.	Parameters	Batch A	Batch B	Batch C
1	Colour	Greenish Brown	Greenish Brown	Greenish Brown
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Bitter	Bitter	Bitter
4	pH	6	6.1	6.2
5	Viscosity	0.0153	0.01625	0.018
6	Density	1.05	1.06	1.07
7	Specific gravity	1.432	1.424	1.424

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters.

Phytochemical analysis of polyherbal syrup for batch C

Chemical constituents	Results
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Phenol	+
Carbohydrate	+
Glycoside	+
Terpenoid	+
Protein	+
Steroid	+

Accelerated stability study for batch C

Physical Parameters of Polyherbal syrup:

Sr. No.	Parameters	Initial study	First month	Second month	Third month
1.	Colour	Greenish Brown	Greenish Brown	Greenish Brown	Greenish Brown
2.	Odour	Characteristic	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter	Bitter
4.	pH	6.2±0.03	6.0±0.02	6.1±0.02	6.1±0.04
5.	Viscosity	0.018±0.03	0.018±0.03	0.017±0.04	0.016±0.05
6.	Density	1.06±0.05	1.07±0.04	1.07±0.03	1.06±0.06
7.	Specific gravity	1.424±0.01	1.432±0.02	1.424±0.02	1.433±0.03

Phytochemical analysis of polyherbal syrup for batch C

Chemical constituents	Initial study	First month	Second month	Third month
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	+	+
Phenol	+	+	+	+
Carbohydrate	+	+	+	+
Glycoside	+	+	+	+
Terpenoid	+	+	+	+
Protein	+	+	+	+
Steroid	+	+	+	+

CONCLUSION

The polyherbal syrup consisting of four herbs which folklore claim of being used in dengue and chikungunya and these were evaluated and standardized. Also the physicochemical properties of prepared syrup like colour, odour, taste, pH, viscosity, density and specific gravity were satisfactory and the formulation was within the all specification. The phytochemical analysis was done of prepared syrup for three months. The accelerated stability study for three months indicates that the formulation is stable under room temperature.

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Evaluation of protective role of a Ferulic acid on Letrozole induced polycystic ovarian syndrome in female rats

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ABSTRACT: Background: *Ferulic (*hydroxycinnamic) *acid* is an antioxidant of phenolic phytochemical group used for the skin care product. Polycystic Ovarian Syndrome (PCOS) is a state of hormonal disorder causing an enlarged ovary with small cysts at the outer edges. Aim: The study was designed to investigate the protective effect of ferulic acid (3-methoxy-4-hydroxycinnamic acid) in letrozole induced polycystic ovarian syndrome in rats (PCOS). Methods: All the experimental animals except control group were orally administered with Letrozole (1mg/kg) dissolved in 0.5 % w/v Carboxymethyl cellulose (CMC) solution per oral route for 21 days to induce PCOS. Followed by a dose of ferulic acid (10, 20, and 40 mg/kg p.o.) for 15 days using water as vehicle. Results: The PCOS was confirmed in the letrozole induced rats with increased concentration of androgen, abnormal lipid levels, glucose, glycosylated haemoglobin and also depletion of antioxidants. The administrated of letrozole cause to abnormalities in serum hormone profile, lipid profile, blood glucose levels and increases body weight and ovary weight. Ferulic acid successfully exerted its protective effect by restoring all the parameters to normalize and improving or disappearance of ovarian cysts. Histopathological observations showed a remarkable recovery of the ovarian tissue and the presence of normalized structure of antral follicle. Conclusion: Ferulic acid showed protective effects in letrozole induced PCOS in rats. Biological effects of ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS conditions.

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Keywords: PCOS, Fertility; Ovulation, Letrozole Ferulic acid, Cysts.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is a common and complex female endocrine disorder in women of reproductive age [1,2] with an estimated prevalence of 6 to 10 % [3]. Clinical manifestation of PCOS amenorrhea, abdominal obesity, hirsutism, and androgen excess (Hyperandrogenism), infertility, and expanded ovaries with multiple cysts. Women with PCOS are at increased risk for diabetes, dyslipidemia, atherosclerosis,

bleeding, hypertension, cardiovascular disease as well as endometrial carcinoma [4]. It is also related with psychological impairments like depression and related mood disorders.

Lipid imbalance, insulin resistance, oxidative stress, and genetics are some of the contributing factors of PCOS [5]. Currently, many therapies are available to induce ovulation and manage PCOS, but it is associated with mild to severe side effects, like; arthritis, hot flushes, muscle or joint pain and psychological side effects like, mood swings, depression, irritability, and bloating. Therefore now-a-days focus is being laid on natural source herbal medicinal plants that have been utilized for the treatment of the various disorders related to the reproductive system due to the lesser or no side effects [3].

Ferulic acid(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) is water soluble, phenolic compound found in active chemical constituent in Chinese medicine herbs such as female ginseng ,and many staple foods, like; fruits, cereals, vegetables and coffee [6,7]. Ferulic acid has been reported to possess a wide variety of biological effects like Antioxidant, anti-inflammatory, hypoglycaemic, and Hyperlipidemic activities [8]. In this study we evaluated that Ferulic acid (3-methoxy-4-hydroxycinnamic acid) may be beneficial in management of PCOS induced by Letrozole due to the reported activity.

MATERIALS:

Drugs and reagents:

Letrozole and Clomiphene citrate were purchased from retail Shop Satara, India. Ferulic acid was obtained from Dolphin Pharmacy Instruments, Pvt., Ltd. Mumbai.

METHODS:

In this study the experimental models used is Letrozole induced PCOS models. The model was widely used accepted for assessing PCOS activity. All animals were selected and divided into six groups and housed eight female rats per cage. All animals in five groups except control group were orally administered with Letrozole for 21 days.

Two animals from each group were scarified by using CO_2 chamber. Ovaries was removed and observed for presence of cysts. On 22nd day, Test group I, II, and III was administered with Ferulic acid for 15 days, whereas standard group was dosed with Clomiphene citrate for 15 days per oral route [9-11].

Animals:

This prospective comparative study was conducted at Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, and Maharashtra, India. Healthy, Virgin, cyclic and adult female wistar rats (150 to 200 g) were used in the present study. These animals were procured from registered breeder and acquainted in the quarantine area for one week.

Housing of animals:

The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions of 22 ± 2°C temperatures, 50 ± 15 % of relative humidity, 12 h dark/ 12 h light cycle with free access to pellet diet and water provided *ad libitum*. The study protocol was approved form institutional animal ethic committee. The experiments were performed as per as guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Governments of India. The Institutional Animal Ethics Committee approved the study protocol YSPM/YTC/PHARMA-IAEC/48/2020.

PCOS induction:

All the experimental animals except control group were orally administered with letrozole (1 mg/kg) dissolved in 0.5 % w/v CMC solution per oral route for 21 days to induce PCOS. Vaginal smear checked or examined daily and the animals in regular estrous phase were selected for study. Vaginal smears were collected and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS. Two animals from each group were scarified by using CO_2 chamber. Ovaries were removed and observed for presence of cysts [11,12]. In female rats, the estrous cycle characterized by proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrusII) in normal animals. During estrus cyclic differences in vaginal cytology occurs in response to the morphological changes and continuous changes in cell types (leukocytes, nucleated epithelial and cornified epithelial) occurs in PCOS induced animals [8,9].

Treatment groups:

Animals were randomly assigned into six group (Table 1) and adequate supply food and drinking water.

Study design:

The study consisted of 48 female Albino Wistar rats equally divided into 6 groups as group 1 (control

group), group 2 (PCOS induced group), group 3 (Standard group), group 4, 5, and 6 as treatment groups. Following Letrozole administration, standard group was administered with Clomiphene citrate at a dose of 1mg/kg in 0.5 % CMC per oral and treatment group 4, 5, and 6 were administered Ferulic acid with the dose of 10, 20, and 40 mg/kg of body weight respectively in water per oral for 15 days. After 21 days, PCOS control group and after 36 days, animals from other groups were fasted overnight and blood was collected by retro orbital puncture then serum was separated and was used for estimation of hormones, lipid parameters and glucose. Body weight was measured at the end of study (On day 36th) animals were then sacrificed and ovaries were excised, cleaned of fat and weighed [11].

Table 1. Treatment Groups.

Group 1: Control	Healthy rats were administered vehicle (10 ml/kg)
Group 2: Negative control	Animals were administered with Letrozole (1 mg/kg)
Group 3: Positive control	Animals were administered with Letrozole (1 mg/kg) + Clomiphene citrate (1 mg/kg)
Group 4: Test group with low dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (10 mg/kg)
Group 5: Test group with intermediate dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (20 mg/kg)
Group 6: Test group with high dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (40 mg/kg)

Biochemical estimation:

***Measurement of fasting blood glucose*:**

Blood glucose level was measured by using Accu-cheak active glucometer (Roche Diabetes care GmbH Sandhofer Strasse11668305 Mannheim, Germany).

***Hormonal assay*:**

Blood samples were collected by retro-orbital puncture; serum was used for hormonal estimation (FSH, LH and Testosterone). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone was measured via Enzyme Linked Immunosorbent Assay (ELISA) with the help of commercial kits (ELISA kit).

***Lipid profile*:**

The lipid profile (LDL, HDL, Total cholesterol, Triglycerides) was estimated at the end of the study. Lipid profile (LDL, HDL, Total cholesterol, Triglycerides) were quantified by using enzymatic kits procured from Aspen Laboratories pvt, Ltd

***Histopathology*:**

The excised ovaries were fixed in 10 % v/v formalin solution. According to histological procedure, they were subjected to tissue processing by washing with water which was followed by dehydration through ascending grades of alcohol then cleared through xylene. Then paraffin embedding method was used. The blocks were sectioned by using microtome and were placed on slides. These sections were stained with hematoxylene-eosin (HE), dehydrate, cleared and mounted on DPX mount under glass cover slips. The light microscope was used for observation which was connected to a camera to capture image.

Statistical analysis:

The statistical analysis was done by using Graph pad software version 5.0 and results were compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. The results were analysed by Two-way analysis of variance followed by Bonferroni posttests. A p value <0.05 was considered as statistically significant.

RESULTS:

Examination of oestrus cycle:

Fig 1. showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research, Fig 2 demonstrated not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

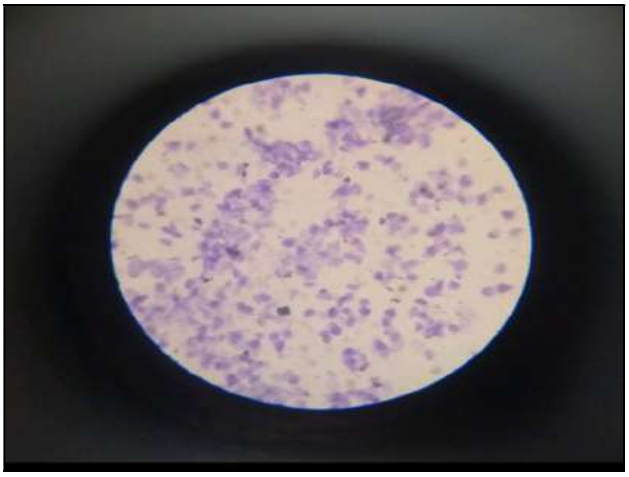

Fig 1. Smear with cornified squamous epithelial cells (Normal animals).

Showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research.

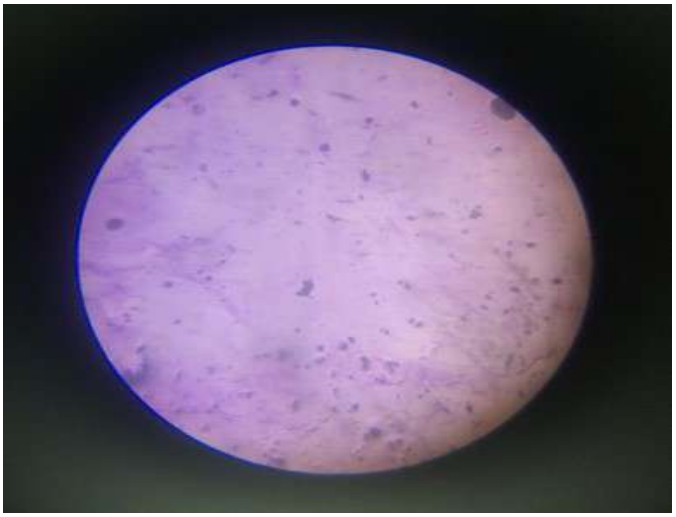

Fig 2. Examination of oestrus cycle (PCOS induced animals).
Not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

Morphology of ovary:

Fig 3 shows Normal ovary structure, where as Fig 4 shows Fluid filled cysts in PCOS induced group.

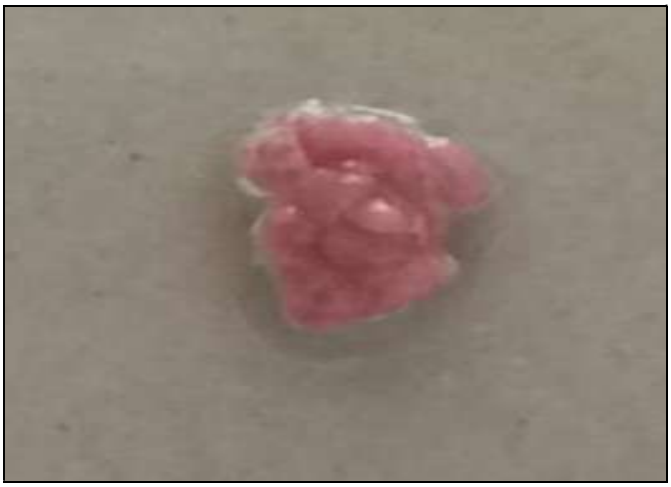

Fig 3. Morphology of ovary (Normal ovary).

Fig 4. Morphology of ovary (Fluid filled cysts in PCOS induced group).

Body weight:

The effect of Ferulic acid on body weight was represented in Fig 5. Letrozole treatment to a significantly increase in body weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.001$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the body weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decreased ($P<0.001$) body weight when compared to Negative control rats.

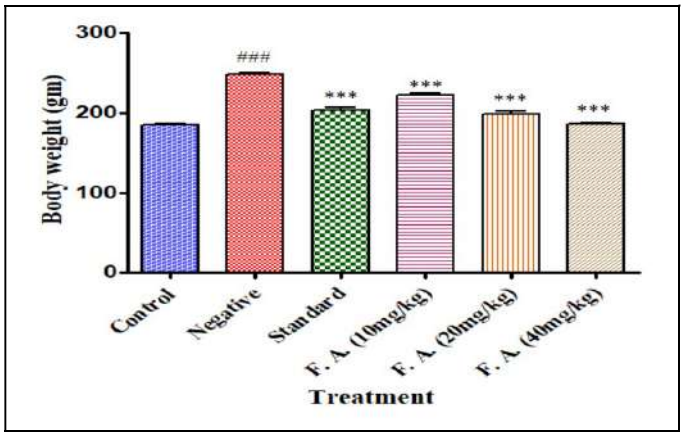

Fig 5. The effect of Ferulic acid on body weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Organ weight:

Letrozole treatment to a significantly increase in ovarian weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.01$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the ovary weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decrease ($P<0.001$) ovary weight when compared to Negative control rats as given in Fig 6.

Serum hormonal profile:

The serum levels of Testosterone and luteinizing hormone (LH) were increased in PCOS induced group ($p<0.001$, $p<0.001$; respectively) while follicle stimulating hormone significantly decreased ($p<0.001$) in comparison to the control group. A significant fall ($p<0.001$) in testosterone levels was observed in standard, low dose, intermediate dose and high dose groups. Treatment with at dose of Ferulic acid 10, 20, 40 mg/kg and standard ($P<0.01$, $p<0.01$, $p<0.001$, and

Table 2. The effect of Ferulic acid on serum hormonal level.

Groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control	0.092 ± 0.003	12.17 ± 0.70	25.67 ± 2.72
Negative	0.140 ± 0.003$^{###}$	19.33 ± 1.25$^{###}$	10.50 ± 0.99$^{###}$
Standard	0.112 ± 0.001***	11.17 ± 0.60***	21.67 ± 0.80***
F. A. (10 mg/kg)	0.119 ± 0.002***	15.0 ± 0.68**	15.33 ± 1.11
F. A. (20 mg/kg)	0.092 ± 0.002***	14.50 ± 0.76**	17.50 ± 0.99*
F. A. (40 mg/kg)	0.083 ± 0.002***	11.17± 0.60***	20.17 ± 0.60***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control. LH and FSH are luteinizing and follicular stimulating hormone.

Table 3. The effect of Ferulic acid on lipid profile.

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
Control	61 ± 1.65	26 ± 1.18	22.17 ± 1.30	82.50 ± 1.97
Negative	102 ± 2.58$^{###}$	14.67 ± 0.66$^{###}$	51.17 ± 2.10$^{###}$	132.80 ± 2.82$^{###}$
Standard	76.67 ± 1.74***	22.67 ± 0.88***	38.67 ± 0.88***	90.83 ± 2.57***
F. A. (10mg/kg)	90.67 ± 1.97**	19.17 ± 1.07*	41.50 ± 0.76**	109.70 ± 2.48***
F. A. (20mg/kg)	71.17 ± 1.35***	21.50 ± 0.76***	37.17 ± 1.32***	90.67 ± 1.97***
F. A. (40mg/kg)	62.50 ± 1.89***	27.67 ± 0.88***	26.67 ± 2.33***	75.67 ± 2.96***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control.

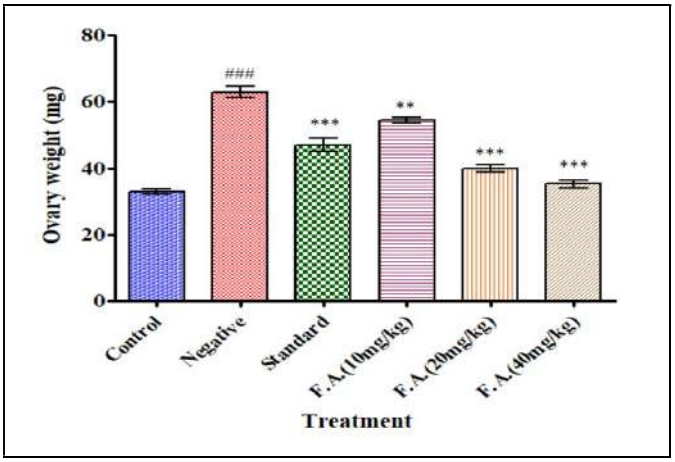

Fig 6. The effect of Ferulic acid on ovarian weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. **p<0.01, ***p<0.001; when compared with negative control.

p<0.001; respectively) produced a significant decreased in Luteinizing hormone levels when compared with Negative group. Animals treated with at dose of Ferulic acid 20, 40 mg/kg and standard produced a significant increase (p<0.05, p<0.05, and P<0.001; respectively) in FSH levels when compared with Negative group (Table 2).

Ferulic acid reduces blood glucose level:

The effect of Ferulic acid on blood glucose levels was represented in Fig 7. Letrozole treatment to a significantly increase in blood glucose levels (p<0.001) as compared to control group. Oral treatment with at dose of Ferulic acid 10, 20, 40 mg/kg, for 2 weeks (P<0.001, P<0.001 and P<0.001; respectively) significantly decreased the blood glucose levels in experimental animals while treatment with Clomiphene citrate (1mg/kg) significantly decrease (P<0.001) blood glucose levels when compared to Negative control rats.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed

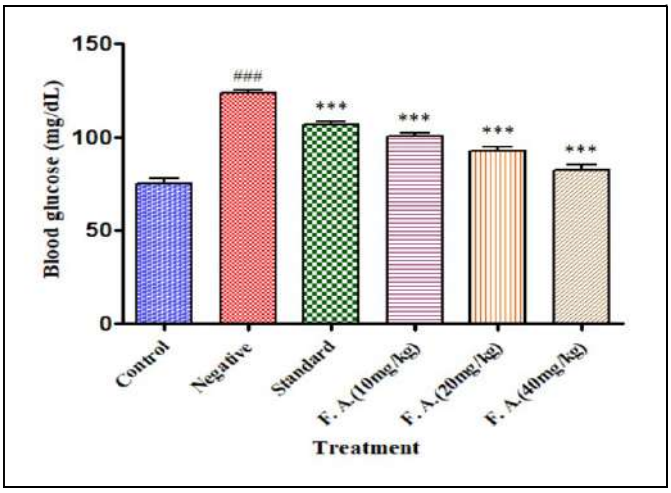

Fig 7. Ferulic acid reduces blood glucose level.
Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed significant changes in serum lipid as compared to control. Cholesterol, LDL and triglyceride were greatly increased as p<0.001, p<0.001 and p<0.001 respectively while HDL levels were decreased (p<0.001) in PCOS induced group (Negative group). Clomiphene treatment significantly decreased Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001) levels when compared to PCOS induced group. While HDL levels significantly increased (p<0.001) when compared to PCOS induced group. Low dose of Ferulic acid (10 mg/kg) decreased the levels of Cholesterol (p<0.01), LDL (p<0.01) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.05) in comparison to negative group. Intermediate dose of Ferulic acid (20 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group. High dose of Ferulic acid (40 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group.

Histomorphological changes

Histopathological examination of stained sections of ovary showed ovarian changes and ovarian follicular cysts (Fig 8). Yellow coloured arrow showing numbers of ovarian follicular cysts. Negative group showing multiple numbers of ovarian follicular cysts compared to normal control group. Oral administration of Clomiphene citrate (1 mg/kg), low dose of Ferulic acid (10 mg/kg), Intermediate dose of Ferulic acid (20 mg/kg), and high dose of Ferulic acid (40 mg/kg) significantly improved or disappearance the number of ovarian follicular cysts compared to negative group.

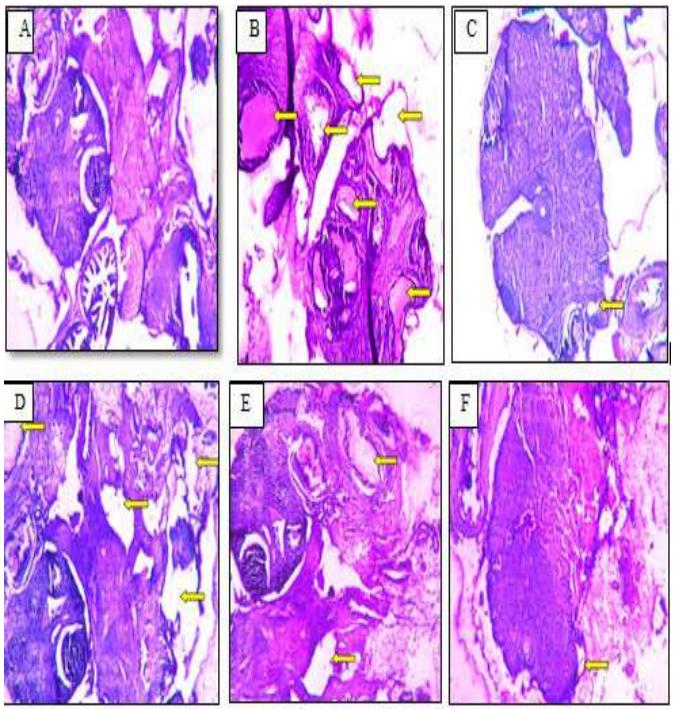

Fig 8. Effect of Ferulic acid in HE-stained ovary tissue (40X).
A. Normal control: showing normal histology of ovary. B. PCOS control: showing large numbers of ovarian follicular cysts. Yellow arrow indicates cysts. C. Letrozole + Clomiphene citrate showing less numbers of cysts. Yellow arrow indicates cysts. D. Letrozole + Ferulic acid (10 mg/kg) showing fewer moderate numbers of cysts. Yellow arrow indicates cysts. E. Letrozole + Ferulic acid (20 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts. F. Letrozole + Ferulic acid (40 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts.

DISCUSSION:

Polycystic ovarian syndrome (PCOS) is major female health problem. It is a chronic metabolic disorder characterized by hyperglycaemia, obesity, excess androgen level, hyperlipidaemia, and decrease FSH level. The World Health Organization estimates that it affects 116 million women worldwide as of 2012 [13]. Various experimental models for PCOS have been developed in rats like administration of testosterone propionate (TP), dehydroepiandrosterone (DHEA), and 5a-dihydrotestoterone (DHT) and Estradiol valerate (EV). It is models fully convincing and identify with the

condition of human PCOS completely [14]. Letrozole is a non-steroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting increased testosterone and decreased E2 production and stimulate PCOS like condition by causing circulating hyperandrogenism, hormonal imbalance, and intra ovarian androgen excess leading to appearance of polycystic ovary. Letrozole induced PCOS was causehyperglycaemic condition which may contribute to insulin resistance, hyperlipidaemia leading to metabolic syndrome [10-15]. Letrozole induce animal model causes polycystic ovarian syndrome in our research study. It is PCOS rat model characterized by an increase in androgen biosynthesis. P450 aromatase enzyme is responsible converting testosterone and androstenedione to estradiol and estrone. This enzyme inhibits activity led to enhance ovarian androgen production or concentration and resulted in PCOS disorder. Due to inhibit of aromatase enzyme activity increases ovarian androgen secretion and resulted into increase level or concentration of testosterone, LH, and FSH, Letrozole treatment showed some metabolic feature, like increased body fat, triglycerides, cholesterol and body weight [10,14]. Ferulic acid showed marked significantly decreased body weight and ovary weight in PCOS rats that may be responsible for reduced the fatty formation, decreasing follicular cysts (follicular fluid). The body weight was considerably reduced by treatment with Ferulic acid (20 and 40 mg/kg). The weight of ovaries in the negative control group was greater than that of normal control group rats. Ferulic acid (20 and 40 mg/kg) treatment significantly decreased ovaries weights which matched to those in control group animals. Type-2 diabetic mellitus and insulin resistant hyperglycaemia are inter-linked with PCOS. Altered insulin levels which can directly stimulate ovarian androgen production in PCOS Insulin stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotrophic hormone (ACTH) and increase pituitary LH release. Increase androgen level cause ovarian cyst. FA improves altered insulin levels, impaired glucose homeostasis and insulin sensitivity [15]. PCOS induced rats showed marked rise in blood glucose level relative to control group. Oral administration of Ferulic acid significantly reduced the increased blood sugar levels, and indicating the beneficial impact of Ferulic acid on insulin resistance and diabetic condition. Women with PCOS are hyperandrogenemic which is associated with alteration in circulating lipoprotein and lipid level resulting in dyslipidemia. Regulation of carbohydrate metabolism, insulin plays important role in the metabolism of lipids. Insulin is inhibitor of lipolysis, since it inhibits the activity of the hormone-sensitive lipases in adipose tissue and increased FFA concentration into the circulation. Increased FFA concentration also raises β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol. FA decreased the levels of FFA, TG, Cholesterol and phospholipids in plasma [16-19]. Characteristically PCOS patient have increased cholesterol level. The women with PCOS tend to be obese probably due to high cholesterol and lipid content. The same effect was seen in current research work after PCOS induction. In comparison with the normal control group, the negative control group reported significantly enhanced LDL, Cholesterol, triglycerides concentration and lowered HDL concentration. Ferulic acid (10, 20, and 40 mg/kg) decreased significantly LDL, cholesterol, triglycerides levels and enhanced HDL level. Ferulic acid displayed beneficial outcome against hyperlipidaemia. In this research, non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This lead in testosterone and LH level increased while FSH level decreased. This imbalanced hormonal level leads to inconsistent oestrus cycle [20,21]. The similar condition has been noted in our research. Letrozole induced rats showed considerably increased levels of testosterone, LH and decreased FSH levels compared to control. Standard drug Clomiphene citrate (1 mg/kg), and Ferulic acid (20 and 40 mg/kg) treated rats showed significantly decreased testosterone, LH level and FSH level increased. The Histopathological report of Letrozole induced rats indicated the existence of polycysts in the ovary. Negative group showed large numbers of ovarian follicular cysts. After treatment with Ferulic acid (20 and 40 mg/kg), decreased or improved numbers of ovarian follicular cysts. All the biochemical and Histopathological parameters in our results advocate the Ferulic acid is most constructive treatment against PCOS.

CONCLUSION:

Treating the various parameters in PCOS induced rats, the impact of Ferulic acid treatment with intermediate (20 mg/kg) and high (40 mg/kg) dose was observed to be similar with standard treatment (Clomiphene citrate). In Letrozole induced PCOS animals, Ferulic acid restored the lipid profile, hormone and glycemic status

as well as ovarian morphology. Ferulic acid might be beneficial in managing PCOS condition due to multiple pharmacological actions like hypoglycemic effects, antihyperlipidemic, anti-inflammatory, protective action against obesity, phytoestrogenic and antioxidant activity. Biological effects of Ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS condition.

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REVIEW ARTICLE

A Review on *in situ* Gel of Gastro Retentive Drug Delivery System

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ABSTRACT:
The 'in situ gel' system has appeared as one of the most effective drug delivery systems. Its specific distinguishing feature of 'Sol to Gel' transition aids in the continuous and controlled release of medicines. These systems have a number of advantages, including simple production, convenience of use, enhanced adherence, and patient comfort by lowering drug delivery frequency due to their distinctive sol to gel transition characteristics. An in-situ gelling system is a formulation that is in solution form before penetrating the body but transforms to gel form under specified physiological conditions. This review mainly focused on introduction, Advantages and Disadvantages of in situ gel, mechanism, types of Polymers used, Evaluation of in situ gel and its applications.

KEYWORDS: In situ gel, Sol to gel, Polymer.

INTRODUCTION:
Over the last 30 years'the development of regulated and long-lasting medication delivery methods has received more attention. The design of polymeric drug delivery systems has been the subject of substantial investigation. The development of in situ gel systems has received a lot of attention in recent years[1]. In the last several years, a growing number of in situ gel forming systems have been studied, and numerous patents for their use in a variety of biological applications, including drug administration, have been published. In situ gel formulations offer an intriguing alternative to establishing systemic therapeutic effects through parenteral methods, which can result in incredibly low solubility and transit by hepatic first-pass metabolism, particularly for proteins and peptides. Because of its unique 'Sol to Gel' transition, the in-situgelling technology aids in the continuous and regulated release of medication, as well as increased patient compliance and comfort.

Gastro retentive in situ gelling systems, also known as stomach-specific systems, have the capacity to give regulated medication delivery with improved gastro retention within the stomach. When in interaction with body fluids or a change in pH, in situ gelling systems are liquid at ambient temperature but gel when exposed to them[2]. Because the gel formed by the in-situ gelling system is brighter than gastric fluids, it floats above the contents of the stomach or adheres to the stomach mucosa because of bioadhesive nature of the polymer, resulting in dosage form retention and increased gastric residence time, resulting in prolonged drug delivery in the digestive tract[3,4]. A formulation that is in solution form before going the body, but changes to gel form below certain physiological conditions, is known as in situ gelling system. Temperature, pH change, solvent exchange, UV radiation, and the existence of certain molecules or ions all influence the sol to gel transition. Various natural and semi-synthetic polymers are gelled in situ and could be utilized for oral, ophthalmic, transdermal, buccal, intra peritoneal, parenteral, injectable, rectal, and vaginal administration. Pectin, gellan gum, chitosan, alginic acid, Carbopol, xyloglucan, xanthan gum, hydroxy propyl methyl cellulose, poloxamer, and other natural polymers are employed in the creation about in situ gelling systems[5-8].

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***In situ* gel system advantages**[9,10]

- It aids in the administration of drugs.
- Its unique 'Sol Gel transition' aids in the regulated and prolonged release of the medication.
- The bioavailability of the drug will be higher.
- It aids in the reduction of drug administration frequency in the body.
- It can be administered to unconscious and old patients.
- There will be increased residence time of the drug due to gel formation.

***In situ* gel systemdisadvantages**[11,12]

- The drug's sol form is more sensitive to deterioration.
- Only minimum dose can be given.
- It needs a large number of fluids.
- After drug administration, eating and drinking limited for a few hours.

Ideal Properties of polymers[13]

- It must be compatible and non-toxic.
- It should act in a Pseudoplastic manner.
- It should influence the tear behavior.
- The polymer must be able to stick to the mucosal membrane.
- The polymer should be able to reduce viscosity by increasing shear rate.

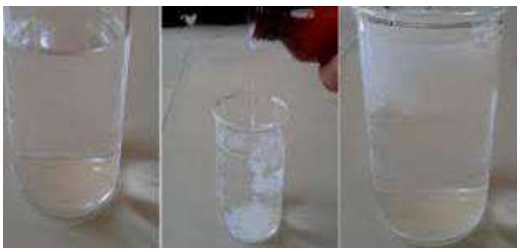

Fig No.1: *Insitu* Gel

***In Situ* Gel Mechanism:**

Physical and chemical mechanisms are used to generate the in-situ gel system.

Physical Mechanism:

- **Diffusion:**

In situ gels are made using a type of physical process called diffusion. In this method, the polymer matrix is precipitated or solidified as the solvent from the polymer solution diffuses into the surrounding tissue. N-methyl pyrrolidone (NMP) is a polymer that is extensively utilized in the development of in situ gelling systems[14].

- **Swelling:**

In situ formulation uses a type of physical method called swelling. In this procedure, the polymer capsule is surrounded by fluids from the outside environment, which inflate from the outside to the inside, progressively releasing the medication. When glycerol (glycerol monooleate) is exposed to water, it expands and forms Lyotropic liquid crystalline phase structures. This material is bio adhesive and can be degraded in vivo by enzymes.

Chemical Mechanism[15]**:**

- **Ionic cross-linking:**

The ion sensitive polymer is used in this approach. Ion sensitive polymers may undergo phase transition in the presence of various ions such as Na+, K+, Ca+, and Mg+. Ion-sensitive polysaccharides are a type of polysaccharide. In the presence of a little amount of K+, k-carrageenan forms hard, brittle gels, whereas in the presence of Ca2+, I-carrageenan forms elastic gels. Gellan gum is commonly referred to as Gelrite. It's an anionic polysaccharide that gels in place when monovalent and divalent cations are present[16,17].

- **Enzymatic cross-linking:**

Enzymatic cross linking is the most appropriate method used in formation of in situ gelling system. In this process, gel is created by cross linking with the enzymes which are present in body fluids. In situ formation induce by natural enzymes and that are not been investigated widely but appears to have certain advantages over chemical and photochemical approaches. An enzymatic method, for example, can handle efficacy in physiological conditions without the use of potentially harmful chemicals like monomers and initiators. Hydrogels have been studied for application in intelligent stimuli-responsive insulin delivery devices. Modify the enzyme amount while maintaining an appropriate mechanism for managing the gel formation rate, which admits the mixes to be injected before gel formation.

- **Photo-polymerization:**

During the development of a system for in-situ gelling, electromagnetic radiations are utilized in the photo-polymerization procedure. A solution containing reactive macromeres or monomers, as well as invader, can be injected into a tissue location, and the gel can then be formed using electromagnetic radiation. The most ideal polymers for photo polymerization are those that dissociate by polymerisable functional groups in the appearance of a photo initiator such as acrylate or similar monomers and macromers, which are commonly utilized at long wavelength UV and visible wavelengths. Short wavelength UV is rarely employed since it penetrates tissue poorly and is biologically harmful. The initiator for UV photo-polymerization in this process is a ketone, like 2,2 dimethoxy-2-phenyl acetophenone. Camphorquinone and ethyl eosin initiators are used in visible light systems[18].

Approaches of *In Situ* Gelation:

- **Temperature dependent in situ gel:**

In insitu gelling formulation, in ecologically sensitive polymer systems, temperature is the most commonly used stimulus. Both in vitro and in vivo, temperature changes are easy to manage and administer. Body warmth causes gelation in this technique therefore no external heat is required. These hydrogels are liquid at ambient temperature (20–25°C), but gel when they come into touch with body fluids (35–37°C). There are three types of temperature-induced systems. They are negatively thermos sensitive type example: Poly (Nisopropylacrylamide) Polyacrylic acid is a positively thermosensitive type; poloxamer, pluronics, and Tetronics are thermally reversible types. Thermo responsive or temperature responsive polymers are used in this system because they demonstrate a dramatic and discontinuous change in their physical characteristics as a function of temperature. These polymers exhibit a miscibility gap at high and low temperatures, indicating the presence of an upper and bottom essential solution temperature.

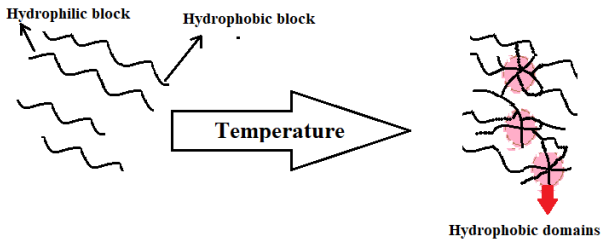

Fig no 2: Mechanism of Temperature dependent in situ gel system

- **pH dependent in situ gelation:**

In this system gel is formed due to pH changes. In this method pH sensitive polymers or pH responsive are used. pH sensitive polymers feature acidic or basic groups on their surface that can receive and released protons in accordance to changes in the overall pH[19]. Poly electrolytes are massive polymers with ionizable groups. The presence of poly electrolytes in the formulation produces an increase in external pH, causing the hydrogel to enlarge and form an in-situ gel. Polymers with anionic groups are suited for this method.CAP (cellulose acetate phthalate), carbomer and its derivatives, PEG (polyethylene glycol), pseudo latexes, and PMC (poly methacrylic acid) are a few examples.

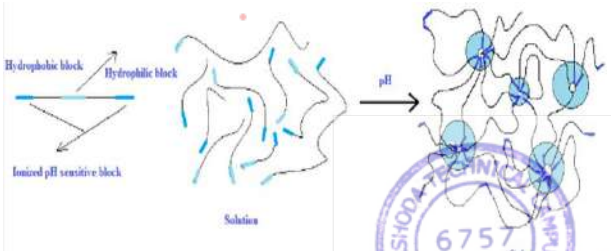

Fig no 3: Mechanism of pH dependent in situ gel system

- **Ion activated in situ gelation**

In this method, gelling of the solution instilled is triggered by change in the ionic strength. The amount of gelation is thought to be influenced by the osmotic gradient across the gel's surface. Gelrite or Gellan gum, Hyaluronic acid, and Alginates are examples of polymers that exhibit osmotically induced gelation[20,21].

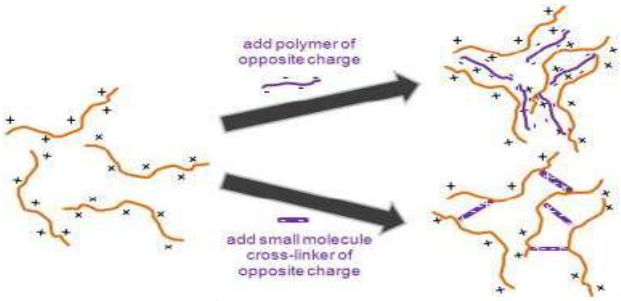

Fig no 4: Mechanism of ion activated in situ gel system

Polymeric System of *In Situ* Gel Classification:

- **Natural polymers:**
  Example: Pectin, Chitosan, Alginic acid, Gellan gum, Xanthan gum, Gaur gum, sodium hyaluronate, Carbopoletc.
- **Synthetic or semi-synthetic polymers:**
  Example: Hydroxypropyl methylcellulose, Cellulose acetate phthalate, methylcellulose, Poloxamer, Polyacrylic acid etc.

Natural Polymers:

- **Pectin:**

Pectinis a type of polysaccharide in which the majority of the polymer is make up of α-(1-4)-D galacturonic acid residues. In the egg-box model, less methoxy pectin (degree of esterification 50%) produce gels quickly in aqueous solution when free calcium ions interlink the galacturonic acid chains. The gelation of pectin, a source of monovalent, divalent, and trivalent ions, chance in the existence of H+ ions. Pectin can be used in these formulations without using organic solvents because it is water soluble. Divalent cations in the stomach aid in the transformation of pectin to a gel state when taken orally. Pectin is divided into two types:a) Low methoxy pectin:below than 50% of the carboxyl groups methylate the pectin. b) High methoxy pectin: greater than 50% of the carboxyl groups methylate the pectin[22].

- **Chitosan:**

Biodegradable, biocompatible, thermosensitive, pH dependent, cationic amino polysaccharide is produced by alkaline deacetylation of chitin. pH and temperature fluctuations cause chitosan to gel. It has good mucoadhesive properties because of the electrostatic interaction between cationic chitosan and anionic mucosal surfaces. Because of their availability, nontoxicity, and low cost, displaying polymers are employed to gel chitosan at higher critical solution temperatures[23,24].

- **Sodium Alginate:**

Alginic acid is a linear block copolymer polysaccharide made up of 1,4-glycosidic links connecting β-D-mannuronic acid and α-L-glucuronic acid residues. The percentage of each block and the order in which the blocks are arranged along the molecule differ depending on the algal source. When divalent or trivalent metal ions are added to dilute aqueous alginates solutions, a cooperative mechanism involving sequential glucuronic remains in the α-L-glucuronic acid blocks of the alginate chain form solid gels. Alginic acid formulations were investigated for a longer precorneal stay, not only due to its ability to gel in the eye, but also due to its mucoadhesive properties[25,26].

- **Carbopol:**

Carbopol is a well-known pH-dependent polymer that remains in solution at acidic pH but gels at alkaline pH with a low viscosity. HPMC is used in conjunction with Carbopol to give the Carbopol solution viscosity while also lowering the acidity. pH-induced in-situ precipitating polymeric systems include a variety of water-soluble polymers such as the Carbopol system-hydroxypropyl methylcellulose system and poly (methacrylic acid)-poly (ethylene glycol). conceived and developed a pH-induced in-situ precipitating polymeric system (an aqueous solution of Carbopol-HPMC system) for plasmid DNA delivery[27].

- **Gellan gum:**

Gellan gum is an anionic hetero polysaccharide, secreted by microbe Sphingomonas elodea. It is produced from glucose, rhamnose, and glucuronic acid, which are joined to form a tetra saccharide molecule. Gelrite is deacetylated Gellan gum that has had the acetyl group in the molecule removed by alkali treatment. Gellan gum is used as a suspending and stabilizing agent in the food business[28].

- **Xanthan gum:**

Xanthan gum is a high molecular weight extracellular polymer generated by the gram-negative bacterium Xanthomonas campestis during fermentation. A cellulosic backbone (β-D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid α-D-mannose connected to alternating glucose residues of the main chain make up the major structure of this naturally generated cellulose derivative. Xanthan gum is dissolvable in both hot and cold fluid and is stable in acidic and alkaline environments. It is anionic because it contains both glucuronic and pyruvic acid groups[29,30].

- **Sodium hyaluronate:**

It is a water-soluble form of the sodium salt of hyaluronic acid. It's a natural, endogenous carbohydrate that helps the body produce collagen and keep its flexibility. It also increases formulation stability and reduces the portability of oxidation[31,32].

Synthetic or Semi- Synthetic Polymer:

- **HydroxypropylMethylCellulose (HPMC):**

The glucan chain in cellulose is prepared fromβ-(1, 4)-D-glucopyranose units that are repeated. Temperature sensitive sol-gel phase transition is observed in some natural polymers, such as HPMC, MC, and EC. When the temperature drops, cellulose material increases its viscosity, while its derivatives, such as HPMC and MC, increase their viscosity when the temperature rises. MC is a natural polymer made consisting of native cellulose chains with an alternative methyl substitution group. The solution is liquid at low temperatures (300˚C), but as the temperature increases (40-500˚C), it gels[33].

- **Cellulose acetate phthalate:**

Pseudo latex is cellulose acetate phthalate(CAP), which is a kind of cellulose acetate phthalate. It's a synthetic latex generated by dispersing a pre-existing polymer in water. Latex is a pH-sensitive, cross-linked polyacrylic polymer with potentially helpful qualities for sustained medicine distribution to the eye because it is a free-running solution with a pH of 4.4 that is raised to pH 7.4 by clotting tear fluid. The ocular duration of an ophthalmic preparation-scintigraphy is monitored using CAP, which does not require the use of an organic solvent[34].

- **Methylcellulose (Mc):**

Methylcellulose is a cellulose derivative that's employed as a gelling polymer in situ. At low temperatures, several cellulose derivatives remain liquid, but when heated, they turn into gels. The aqueous solutions of MC and HPMC, for example, at 40-50 °C and 75-90 °C, phase transition into gels respectively. The hydrophobic interactions between molecules containing methoxy groups cause HPMC and MC solutions to gel. Due to hydration at a lower temperature, macromolecules come into contact with each other. When the heat is increased, the hydration is gradually lost, resulting in a lesser viscosity[35,36,37].

- **Poloxamer:**

Poloxamer is a three-block copolymer that is water soluble. Poloxamer is sold as Pluronic and has a good thermal setting characteristic as well as a longer drug residence period. It's most commonly employed as a gelling, emulsifying, and solubilizing agent. Poloxamer produces a clear, colourless gel. Based on the ratio and distribution of hydrophilic and hydrophobic chains, many molecular weights are accessible, each with a distinct gelling behavior[38].

- **Polyacrylic acid (PAA):**

PAA is commercially known to be Carbopol.It is commonly used in ophthalmology for increasing pre-corneal retention. It can exhibit excellent mucoadhesive properties to compare with another cellulose derivative[39].

Evaluation of *In* Situ Gelling System:

- **Clarity:**

Visual inspection against a black and white background can be used to check the clarity of prepared solutions.

- **Measurement of pH:**

The pH of each of the formulation was measured using a calibrated digital pH meter

- **Viscosity:**

The viscosities of the produced formulations were measured using a Brook field viscometer. it was sheared at 50 and 60 rpm using spindle number 63. The viscosity of each sample was measured three times.

- **Sol to gel time:**

Using a USP (Type II) dissolution equipment containing 500mL of 0.1N HCl (pH 1.2) at 370.50˚C. the in vitro gelation time was calculated. The gelling time is the amount of time it takes for an in-situ gelling system to gel for the first time. The gel floated on the buffer solution in a matter of seconds.

- ***In vitro* buoyancy study:**

The time it takes for the gel to rise to the top of the dissolution flask from the bottom is known as the floating lag time andthe floating period is the amount of time it takes for the generated gel to float on top of the dissolution liquid's surface is known as floating duration. In a USP type II dissolution test apparatus containing 500 ml of 0.1 N HCl (pH 1.2) at 370.50˚C.

- **Gel-Strength:**

The gel is made from the sol form in a beaker. This gel-filled beaker is elevated at a set rate, allowing a rheometer probe to gently pass through the gel. It can be determined by observing variations in probe load as a function of probe depth of immersion below the gel surface.

- ***In-vitro* drug release studies**

The plastic dialysis cell is used to conduct medication release experiments. The cell is made up of two half cells, a donor partition, and a receptor partition. The formulation's sol form is deposited in the donor compartment. In an incubator, the constructed cell is shaken horizontally. The entire volume of a receptor solution can be removed and replaced with new media at regular intervals. Analytical techniques are used to examine this receptor solution for drug release.

Application of *In situ* Polymeric Drug Delivery System:

- **Oral drug delivery:**

Natural polymers including pectin, xyloglucan, and gellan gum are employed to build oral medication delivery systems in situ. An in-situ gelling pectin preparation administered orally has been found to provide paracetamol for a long duration. The main advantage of using pectin in these formulations is that it is soluble in water, thus no organic solvents are required. According to the study, theophylline was administered orally using an in-situ gelling gellan formulation. The formulation included a gellan solution containing calcium chloride and sodium citrate complex. When calcium ions are given orally, they are discharged into the stomach's acidic environment, causing gellan to gel and create a gel in situ[40].

- **Ocular drug delivery system:**

Ocular delivery techniques frequently use natural polymers such as alginic acid, inulin, and xyloglucan. To release visual ocular tension in glaucoma, diverse chemicals such as autonomic medicines, anti-inflammatory agents, and antibacterial agents are employed in a local ophthalmic administration system. Because conventional administration systems generally result in poor availability and therapeutic response due to fast tear fluid turn over and dynamics, which leads to rapid drug removal from the eye, ocular in-situ gels were created to alleviate the bioavailability problem. Viscosity enhancers such as Carboxy Methyl Cellulose, HPMC, Carbomers, and Poly Vinyl Alcohol are used to increase viscosity in formulations to extend precorneal residence time and increase bioavailability while being simple to produce[41].

- **Nasal Drug Delivery Systems:**

In-situ gel was shown to reduce the increase in nasal symptoms when contrasted to the commercial formulation Nasonex (mometasone furoate suspension 0.05%). The presence of intact ciliated respiratory epithelium and usual goblet cell morphology in the rat nasal cavity indicated that these formulations were safe for nasal administration. Wu et al. Combining N- [(2-hydroxy-3methyl trimethyl ammonium) propyl] chitosan chloride and poly (ethylene glycol) with a small amount of – glycerol phosphate, researchers developed a novel thermos sensitive hydrogel for insulin delivery in the nose. At room temperature, the formulation was in solution form, but when stored at 37 ℃, it converted into a gel form. As a result, these methods are suitable for the nasal administration of protein and peptide medicines[42].

- **Rectal and vaginal drug delivery system:**

Many types of medications can be delivered via the rectal route, including liquid, semisolid (ointments,

creams, and foams), and solid dose forms (suppositories). Acetaminophen, an anti-inflammatory medicine, was formulated as a rectal in situ gel by employing polycarbophil, poloxamer F188, and poloxamer 407 as synthetic polymers generating in situ gelling liquid suppository, which is regarded to be an excellent way for increasing bioavailability. To improve therapeutic effects and patient compliance, a mucoadhesive, thermosensitive, prolonged release vaginal gel containing the clotrimazole-cyclodextrin complex was developed[43,44].

- **Injectable drug delivery system:**

Injectable in situ gel is mostly made up of synthetic polymers and block copolymers. A novel injectable thermosensitive in situ gelling hydrogel has been created for tumor treatment. The drug-loaded chitosan solution was neutralized with glycerol phosphate in this hydrogel. EMT-6 tumor implanted subcutaneously on albino mice were used to examine local delivery ofpaclitaxel from the intratumoral injected formulation. Ito et al. One example of inflammatory drug is Bupivacaine which is formulated as injectable in situ gel using poly(D,L-lactide), poly (D,L-lactide coglycolide) and PLGA as polymer shows prolong action drug in gel conditions[45,46].

CONCLUSION:

According to the current study, the 'in situ gel' system has appeared as one of the most effective drug delivery systems. The in-situ gel preparation was developed to improve patient compliance, comfort and lowering dose frequency. This approach increased residence and continuous release. It worked for both systemic and localization at the site of action. A variety of physiological parameterslike pH, temperature and ionic state which influence the gel's growth. In situ gel formation is used to generate a variety of natural, synthetic, and semi-synthetic polymers that could be utilized for oral, ophthalmic, nasal, rectal and vaginal and injectable drug delivery system.

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The Monkeypox Virus, methods to prevent the re-emergence of the Virus

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ABSTRACT

In Central and West Africa, the monkey-pox is a new and re-emerging zoonosis that occasionally results in fatal illnesses. The etiological agent of the complaint is monkey-pox contagion, a virus belonging to the family of orthopoxviruses. The State Serum Institute in Copenhagen initially identified monkey-pox infection in laboratory monkeys in 1958, and the Democratic Republic of the Congo reported the first fatal case of the disease in 1970. The primary spreaders of monkey-pox are African rodents.

The most frequent routes of infection for mortal beings are respiratory, percutaneous, and permucosal exposures to infected monkeys, zoo animals, champaign kids, and people. The incubation phase of the complaint lasts between 5 and 21 days, however it often lasts between 6 and 13. Most instances begin with a classic prodromal sickness that lasts for two days and includes fever, malaise, and lymphadenopathy. The face, triumphs of the hands, and soles of the bases are significantly affected by the rashes. The majority of instances are seen in people who have had direct contact with animals. However, monkey-pox can be predicted if the recognizable skin lesions are present and there is a history of exposure. Culture in the lab and polymerase chain reaction (PCR), Electron microscopy and immunohistochemistry are the instruments available for corroboration of the claim. In immunocompromised patients, the prognosis of the complaint is dismal. Care should be made to treat and cover fractures in the skin as a normal preventative measure while working with non-human primates or other mammals. Infection control measures, including as good hygiene, frequent hand washing, disinfection of shells and clothing, and the use of specific protective clothing (PPE), are crucial during trade with monkey-pox-affected animals.

Keywords- Animal mortality, Emerging, Monkey pox, Prevention, Zoonotic disease

INTRODUCTION

Emerging and re-emerging zoo-noses with various etiologies are important contributors to morbidity and death in both people and animals [1-3]. Several viral zoo-noses have attracted public health authorities' attention [1-6]. There are a number of zoonotic infections, such as cowpox, buffalo-pox, goat-pox, monkey-pox, and camel-pox that can infect both animals and people in different parts of the world [1]. In non-human primates, the pox-viruses cause four diseases, with monkey-pox being the most prevalent [7]. A sylvatic zoonosis called monkey-pox is infrequent and fatal in Central and West African wooded regions [8].

An important zoonotic disease that affects public health is monkey-pox [9, 10]. A smallpox vaccination can prevent monkey-pox in people since it is linked to smallpox in humans [7]. When two spurts of a complaint like a spell swept through monkey exploratory colonies in 1958, the term "monkey-pox" was coined [11].

In the Democratic Republic of the Congo, fatal monkey-pox was first identified in a human being in 1970. Since then, pastoral regions in Western Africa and the Congo Basin, particularly the Democratic Republic of the Congo, have demonstrated the instances' maturity. A significant epidemic occurred in the Democratic Republic of the Congo in 1996–1997. In the spring of 2003, instances of monkey-pox were confirmed in the Midwest of

the United States of America, marking the disease's first known occurrence outside of Africa [8].

Epithelial pustule and vesicular lesions are brought on by the infection in both New and Old World monkeys and hams. Both the owner and the animal must have vaccinations in order to be protected [7]. The potential for monkey-pox to transmit from person to person and be exploited in bio-terrorism exists [10]. This mini-main review's goal is to outline the monkey-pox outbreak's emerging position as a zoonotic disease with public health implications.

PATHOGENESIS

The MPXV, an orthopoxviruses belonging to the family of monkey-pox viruses, is the culprit behind the disease [11, 12]. Other orthopoxviruses that cause diseases in humans include camel-pox, cowpox, vaccinia (used in the smallpox vaccine), and variola (smallpox). According to genome sequencing, there are two monkey-pox clades: Congo Basin and West African, and variations in lethal pathogenicity and mortality have been demonstrated in the two geographical locations [8]. The infection is an envelope, double-stranded DNA infection with a slightly pleomorphic core and side bodies, as well as the swine flu, West Nile fever, contagious ecthyma, Ebola hemorrhagic fever, Hantavirus infection, Rift Valley fever, Hendra hemorrhagic fever, Nipah hemorrhagic fever, and raspberry flu, have emerged from various world regions and drawn attention with a size of 140–260 nm in the perimeter and 220–450 nm in length [13]. It is resistant to phenolic detergents, but polar lipophilic detergents like chloroform and low pH render it inactive. The similarly related vaccinia contagion completely inactivates at 60°C in 2-3 hours, or twinkles at 22°C following exposure to 20nM caprylate [14, 15].

TRANSMISSION

Humans can get monkey-pox by being bitten by an infected animal or by coming into direct touch with the animal's lesions, blood, or bodily fluids [1, 4, 8, 11, 12]. Even if the complaint is less communicable than smallpox, it still has the potential to spread. The disease is thought to be spread by respiratory driblets during prolonged, direct face-to-face contact. Direct contact with an infected person's bodily fluids or contagious items, such as bedding or clothing, can also spread monkey-pox [11]. Another method of complaint transmission is vaccination or transmission via the placenta (natural monkey-pox) [8]. Direct contact with animals, especially rodents, is thought to be the most common way for complaints to spread [11].

EPIDEMIOLOGY

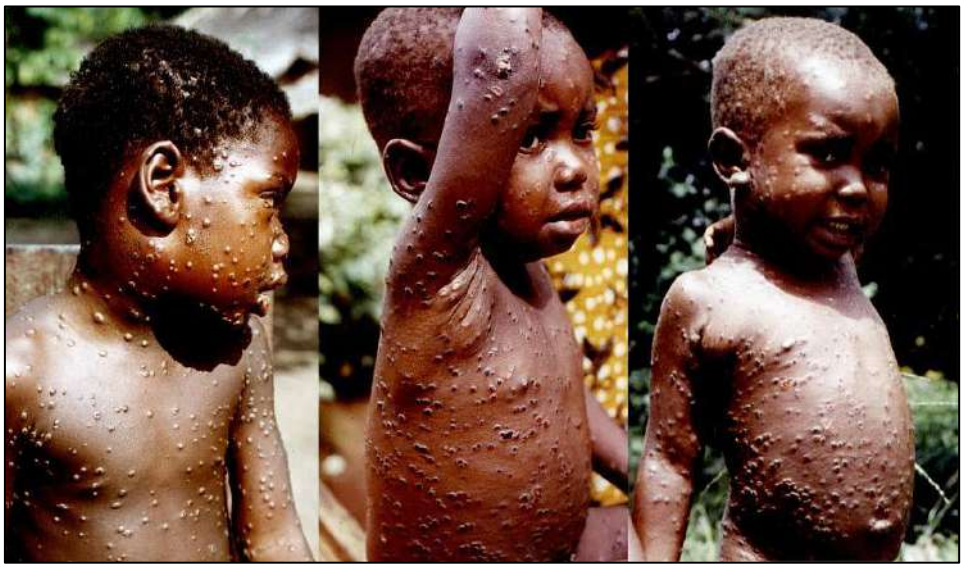
In 1958, monkey-pox infection was first connected to a spell infection in incarcerated monkeys [11]. However, the first case of fatal monkey-pox was reported in a 9-year-old kid from the Democratic Republic of the Congo in 1970. [8, 12]. Considering that fatal monkey-pox cases have also been recorded from a number of African nations, including Benin, Cameroon, Central African Republic, Cote d'Ivoire, Democratic Republic of the Congo, Gabon, Liberia, Nigeria, Sierra Leone, and South Sudan [8, 11]. In 2017, there was a severe human monkey-pox epidemic in Nigeria [8]. The 2003 epidemic of monkey-pox in the Midwest of the United States was the first known case of the disease outside of Africa. Prison pets like Champaign Tykes and other tiny animals, others who have come into contact with ill children from the Champaign region have estimated the maturity of cases around the country [8].

NATIVE HOST

A wide range of African life forms, including rope squirrels, tree squirrels, Gambian mice, banded mice, dormice, and primates, have been shown to carry the monkey-pox virus. Further research is necessary to ascertain the actual force of the infection and how it is sustained in nature. There are still unanswered issues regarding the contagion's natural history [8]. Funisciurus and Heliosciurus squirrels have received awards for their work as hosts and budgets [16]. According to American opinion, the disease was transmitted from several non-African animals (such champaign tykes) that the African creatures shared space with [8]. In laboratory experiments, the disease has been induced to infect mice, rats and rabbits [11].

MEDICAL SYMPTOMS

The incubation period for monkey-pox is typically 7–14 days, although it may also be 5–21 days [8]. There are two phases to the illness. The irruption stage is marked by fever, intense headache, lymphadenitis, back pain, muscle pain, and extreme delicacy (lack of vitality) (0- 5 days), the time frame in which eruptions are occurring simultaneously on the face (in 95 of the instances), the palms of the hands and the soles of the feet (in 75 of the cases), and the torso. The progression of the rash includes the creation of crusts as well as macules, pustules, vesicles, and papules. Children who had monkey pox had rashes on several body parts (Fig. 1). [8]. Generalized exanthema and a severe systemic sickness with a homicidal expansion can develop in those who are immunocompromised or who have cellular susceptible response impairment. A hemorrhagic form of monkey-pox has never been transmitted to humans [17]. For fatal monkey-pox, the case-fatality rate is less than 10. Monkeypox-related losses mature in young cases, although [8]. In this setting, Jezzek and co-investigators [17] observed that 19 of the unvaccinated monkey-pox individuals had subsequent bacterial skin infections.



(Source: DRC, 1970-1977. -WHO /Mark V. Szczeniowski).

Figure 1: *A 7-year-old Zairian girl with monkey-pox in the acute stage, day 7 of rash, and monkey-pox in a 3-year-old Zairian boy with rash in the scabbing stage.*

A tone-restricting rash known as monkey-pox affects nonhuman monkeys. The earliest symptoms include fever and 1 to 4 mm dermatological pustules, which later turn into papules and crust over. A typical monkey-pox lesion has an epidermal hyperplasia ring around its red, septic, and depressed centre. Although these "papules" can be seen on the face, limbs, triumphs, soles, and tail, certain areas appear to have more of them [18]. Some species only have skin lesions. In severe cases, symptoms including coughing, sinus drainage, dyspnea, anorexia, facial edoema, mouth ulcers, or lymphadenitis maybe present. Circulated infection with deeper lesions is unusual in robotic infections. When exposed to an aerosol, monkeys only get pneumonia. Naturally infected creatures mature; nonetheless, losses can occur, particularly in stimulated monkeys. , infections might be asymptomatic [19].

OPINION

To create a clear opinion on monkey-pox, laboratory techniques such as contagion insulating, electron microscopy, immunohistochemical, and PCR are essential. The clinical manifestation of the complaint isn't really typical to make the opinion [4, 13]. Among the most prevalent discriminatory evaluations are those based on smallpox, chickenpox, measles, bacterial skin disorders, scabies, cure disinclinations, syphilis, and varicella infection [8, 13]. In clinical, veterinary,

and monkey-pox virus-infected cell cultures, RT-PCR is frequently employed to detect monkey-pox infection DNA [20]. Immunoreactivity is used to describe viral antigens, and the enzyme-linked immunosorbent assay (ELISA) is used to detect IgG and IgM antibodies [21, 22]. The Polymerase Chain Response (PCR) System is regarded as Dependence on Laboratory Opinion because to the Delicacy and Perceptivity [8].

TREATMENT

Beast investigation proved that antiviral therapy with associated with negative effects. The treatment of Variola virus-induced human smallpox illness in adults and children with tecovirimat (also known as TPOXX or ST-246) is permitted by the FDA. The FDA does not, however, approve of its usage for diseases caused by other orthopoxviruses, such as monkey-pox. Animal mortality can be reduced more effectively by antiviral drugs than by administering the smallpox vaccination as a preventative measure [8, 22]. The way a matter is handled as it matures is indicative. The CDC advises that all animals with suspected monkey-pox be put to death to prevent the disease from spreading, even though many animals recover on their own and antiretroviral medications have been found to be beneficial in experimental infections. Isolation of the patient, protection of the skin and mucous membranes, Nutritional assistance, symptom relief, monitoring, and treatment of problems, as well as re-hydration therapy such symptomatic treatments are useful in monkey-pox infection [11].

CONTROL AND PREVENTION

Several precautions may be done to avoid contracting the monkey-pox virus. Avoid handling any materials that have come into contact with a sick animal, such as a coverlet. Separate diseased cases from those who could get infected. After coming into contact with infected individuals or animals, thoroughly wash your hands [13]. In the case of a monkey-pox pandemic, the animals can be immunized using the disease itself as a prophylactic measure [18]. The American Food and Drug Administration have granted ACAM2000 a licence to be used for smallpox vaccination in those who have been shown to be at a high risk of contracting the illness. Under an Expanded Access Investigational New Drug application, it has been made accessible for the treatment of monkey-pox illness (EA-IND). For those aged 1 and older who have been shown to be at high risk for infection to avoid monkey-pox illness, the CDC suggests that immunization with ACAM2000 might be taken into consideration. The U.S. Food and Drug Administration has authorized JYNNEOS™ (also known as Imvamune or Imvanex), a reduced live virus vaccine, for the prevention of monkey-pox (Fig. 2) [11].

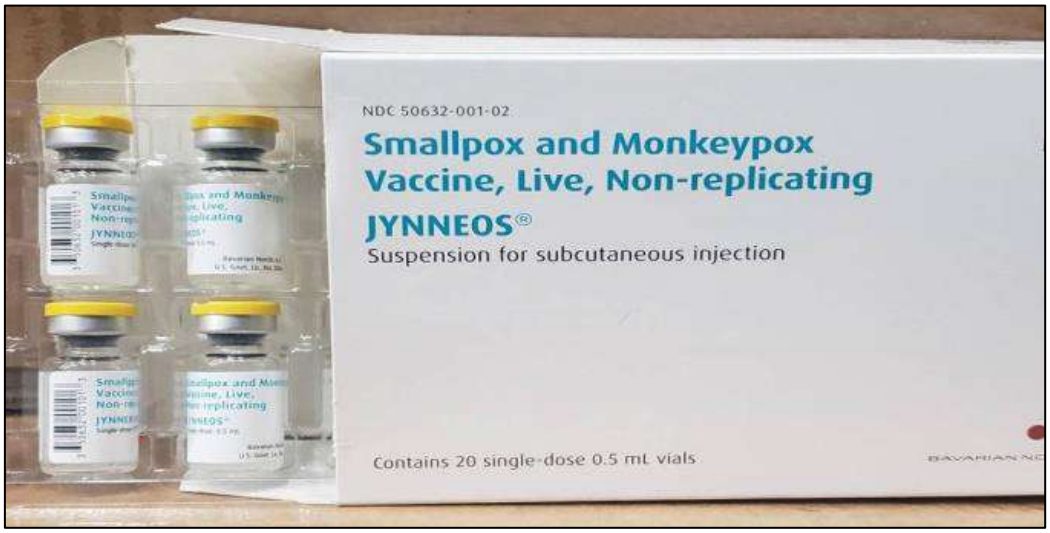


Figure 2: *Vaccines licensed by the U.S. Food and Drug Administration (FDA) are available for preventing monkey-pox infection – JYNNEOS (also known as Imvamune or Imvanex), Source: U.S. CDC 30 June 2022 [23].*

As with restricting or banning the movement of small African animals and monkeys, preventing the transmission of monkey-pox through the pet trade has been effective in halting the disease's spread outside of Africa. Animals kept inside should not receive the smallpox vaccination. Instead, sick animals should be taken out of the herd and put in a counter blockade down low. Any animals that may have come into touch with the diseased animals should be quarantined for 30 days and checked for symptoms of monkey-pox. The only option to prevent infection in the absence of a specialized medication or vaccine is to increase public awareness of the risk factors and inform people of the steps they may take to reduce their exposure to the disease [8].

CONCLUSION

The monkey-pox virus is an emerging infectious illness that spreads from animals to people. The complaint frequently manifests clinically as a fever, rash, and blown lymph nodules. There may have been an immunological and ecological niche created for monkey-pox to reappear due to increased mortal-beast interaction as a result of climate change and deforestation, back country meat consumption, poor health, and inadequate exploratory structure, among other causes. Monkeypox is no longer just seen in native communities. As a result, monkey-pox infection is a risky, global resurgence of a disease. , there is no proven or secure method of treating a monkey-pox infection. The FDA has authorized JYNNEOS, a novel smallpox vaccine, to prevent both monkey-pox and smallpox. The need of practicing good hand hygiene is highlighted in order to prevent the transmission of illness after handling sick animals and contaminated natural objects. More research should be done on the severity, molecular epidemiology, and treatment of monkey-pox.

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REVIEW ARTICLE

Pulsatile Delivery of Drug for a Range of Diseases

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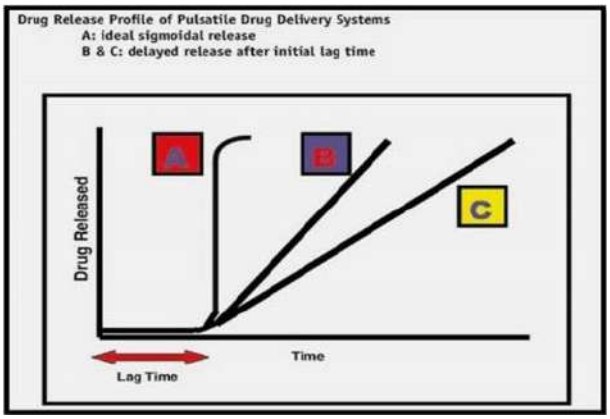
ABSTRACT:
Pulsatile delivery helps in providing the drug at an exact moment based on the disease's pathophysiology that cause improved patient therapeutic efficacy. Drug release rapidly after specific lag time this are advantage for many diseases condition. This system is designed according the body's circadian rhythm and after a lag time, the medication is released fast and totally as a pulse. Pulsatile delivery systems helpful in disease include asthma, arthritis, attention deficient syndrome, peptic ulcer and hypercholesterolemia. These systems are useful for diseases with chrono pharmacological behavior that necessitate nighttime administration, medications with a high first pass effect or GIT site specific absorption, and drugs with a high risk of toxicity or tolerance. By reducing dosing frequency, these devices help increase patient compliance. The foundation for this article is the disease that treat by pulsatile delivery system with drug used for specific disease condition and chrono pharmacology of all disease that mention in article.

KEYWORDS: Pulsatile delivery system, Lag time, Circadian rhythm, Chrono pharmacology.

INTRODUCTION:
In the new technologies the released of drug in immediate or extended fashion. Depending on the disease condition the development of drug release technology is modified.[1,2] PDDS is also part of that development in which it delivers the drug according to chrono pharmacotherapy of disease. This situation necessitates the release of the medicine after a period of time has passed. This situation may be produced using a pulsatile delivery system, which is described as the quick and transitory release of a certain amount of molecule in a short time period following a pre-set off release period (lag time). A pulse must be developed in this manner that the medicine is released completely and quickly. After the lag period, the medicine is released in sync with the body's circadian cycle.[3]



Were,
A] After a lag interval, a sigmoidal release occurs.
B] After a lag time, the release is delayed.
C] After a lag time, there is a sustained release.
Figure 1. Drug release profile of pulsatile drug delivery system.(2)

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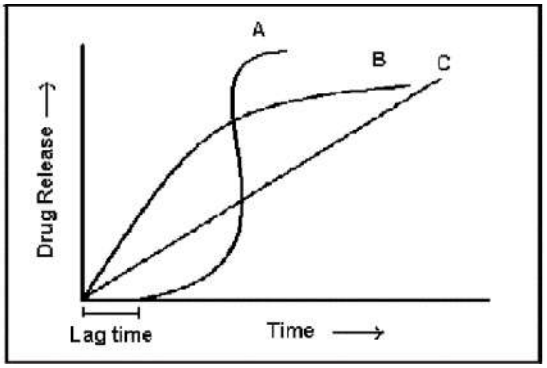

A] Pulsatile delivery system
B] Conventional drug delivery system
C] Extended drug release.

Figure 2. Drug release profile with compare to other drug delivery system and pulsatile delivery system (3)

In this graph 1Following the lag time, indicate sigmoidal release This is how pulsatile medication delivery systems will look in the future. When a medicine is released after a lag period, the drug is released all at once.[4]

Chrono Pharmacology:

It is a branch of research concerned with optimizing medicinal effectiveness and minimizing side effects by scheduling drugs in respect to biological rhythms.[5] The aim is to have a better knowledge of the periodic and thus predictable variations in medication's desired effects and tolerance. Chrono pharmacology is the branch of medicine that studies how drugs affect people's biological clocks.[6]

Rhythms in Human Physiology:

Rhythms in Human physiology are self-sustaining cycles that are innately determined by the length of time between subsequent repetitionsunder normal conditions. Within 24 hours, 100 different measured human body markers show cyclic variations.[6]

In medicine, circadian rhythms are particularly essential. Circadian rhythms (circa about dies, day, or about 24 hour) A physiological day lasts around 25 hours. The environment, night and day social schedules, reset the clock every day. [6] The brain's circadian clock regulates daily physiological cycles such as sleep/wake, digestion, temperature, and hormones. Endogenous circadian rhythms are biological rhythms. Free-running rhythms result from a lack of external synchronizers. The duration of free-running rhythms varies per species and can be longer or less than 24 hours. Our internal clocks are determined by our genes. In mammals, the suprachiasmatic nucleus of the hypothalamus (SCN) houses an internal biological clock that sends time signals throughout the body.[7] Circadian rhythms and annual/seasonal rhythms are controlled by it. To send out its time-of-day message, The SCN makes advantage of its autonomic nervous system connections, either by altering the endocrine glands' sensitivity or by directly influencing the endocrine output of the pineal gland (i.e., melatonin synthesis)

Rhythms of Different Systems:

- **Respiratory system:**

Greater bronchoconstriction at night due to increased parasympathetic tone, lower adrenaline and cortisol levels at midnight, and increased sensitivity to irritants and allergens at night.[7]

- **Gastrointestinal tract:**

Between 10 p.m. and 2 a.m., secretion of acid 2-3 times greater.[8]

- **Cardiovascular system:**

When comparing diastolic and systolic blood pressure, the amplitude of the 24-hour change is greater for diastolic blood pressure. At 9-11 a.m. and 6-7 p.m., blood pressure displays two peaks. Blood pressure drops (somewhat) in the afternoon and started decreasing at night.[9]

- **Endocrine system:**

Cortisol secretion is highest soon before awakening and lowest at mid-night in the morning. Growth hormone is at its highest during sleep. Early in the morning, testosterone peaks. 5-10 times increase in insulin Following the absorption of meals, the level rises.

- **Plasma protein binding:**

During nocturnal sleep, albumin and acid glycoprotein are at their lowest and highest levels, respectively. As a result, during night, medicines linked to plasma protein, such as lignocaine, carbamazepine, diazepam, valproic acid, and prednisolone, exhibit an increase in free fraction.

- **Liver enzymes:**

In the middle of the (nocturnal) activity span, oxidative reactions reach a high. UDP-glucuronyl transferees catalyze more conjugation during activity than at rest. During the rest period, sulphate conjugation is faster than during activity.

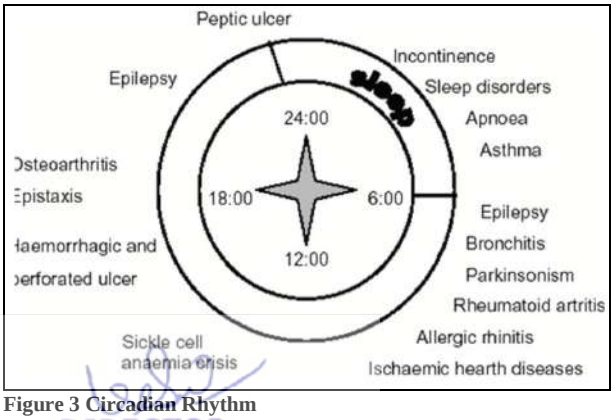

Figure 3 Circadian Rhythm

Diseases and Chronotherapeutic:

24-hour cycle Up to now, the homeostatic hypothesis has guided the development of medication delivery methods. This notion is based on the idea that biological functions are consistent across time. Circadian rhythms have been identified for practically all human processes, including heart rate, blood pressure, body temperature, plasma concentrations of numerous hormones, stomach pH, and renal function, according to chronobiological research.[11] The importance of rhythmic processes in the therapy of human illnesses has become clear. Circadian rhythms exist in pathological stages of disease, just as they do in physiological processes. Epidemiological studies have shown that there is a higher chance of illness symptoms at this time.[12]

In the therapy of a variety of disorders, the potential benefits of chronotherapeutic have been proven. Patients with allergic rhinitis, rheumatoid arthritis and associated disorders, asthma, cancer, cardiovascular disease, and peptic ulcer disease are all interested in learning more about how chronotherapy can help them.[13] Patients with allergic rhinitis frequently report that their worst symptoms occur first thing in the morning. Patients taking a long-acting antihistamine at night instead of in the morning, as is typically suggested, may have better results in controlling morning pain.[14]

Diseases Required Pulsatile Delivery System:

1. Asthma
2. Peptic Ulcer
3. Cadiovascular Disease
4. Diebeties Mellitus
5. Hypercholestrolemia
6. Parkinson's Disease

Asthma:

Asthma is common chronic inflammatory disease of the airways characterized by hyperresponsive to variety of stimuli. Resistant airway in asthmatic patients, bronchoconstriction and symptom aggravation grow gradually during the night and early morning. Circadian alterations in lung function occur. For example, expiratory flow rates are the highest at 4 p.m. and lowest at 4 a.m. Approximately two-third of asthmatics suffer from nocturnal asthma symptoms and risk of asthma attack greater during night time sleep as compare to day time activity. This is driven by circadian changes in epinephrine (Bronchodilator), cortisol (ant inflammatory substance), Histamine (amedator if bronchoconstriction) melatonin (sleep regulatory hormone) AMP, vagal tone and body temperature. At 4.00 a.m., histamine concentrations peaked at a level that corresponded to the greatest degree of bronchoconstriction.[16,17] Pulsatile-release dosage form can potentially treat the nocturnal asthma by releasing drug after predetermine time delay, provided that most appropriate drugs are administrated. This dose type is given before bedtime, with medication release beginning in the early morning hours, when the risk of an asthmatic attack is highest.

Drug used in pulsatile delivery to treat nocturnal asthma:

Salbutamol:

Salbutamol is a fast-acting, highly selective Beta 2-adrenoceptor agonist with little cardiac adverse effects. This is used to treat asthma through relaxing the smooth muscle of the bronchial tubes causing the bronchi to dilate immediately.[16] The 2-4 mg pill is radially absorbed from the oral route, with a 44 percent absolute bioavailability and a peak plasma concentration of 1-3 hours.

Salbutamol has a short biological half-life (3.8 to 6 hours), a high first-pass metabolism, and a restricted therapeutic window when taken orally. A pulsatile delivery device exposes salbutamol sulphate only when it is needed, potentially preventing undesirable systemic side effects and allowing a lower dose of life-saving medication to be used to treat night-time asthma.[16]

Montelukast:

Montelukast is a drug that can be used to treat asthma and allergic rhinitis. Montelukast belongs to the class of drugs known as leukotriene receptor antagonists. It acts by inhibiting the function of leukotriene D4 in the lungs, resulting in less inflammation and smooth muscle relaxation.[17]

Theophylline:

Theophylline is a methylated xanthine class of medication used to treat respiratory illnesses such as COPD and asthma. It's a bronchodilator and nonselective phosphodiesterase inhibitor that improves respiratory muscle performance and mucociliary clearance. Theophylline's broncho dilating effect makes it effective in the long-term treatment of bronchial asthma.[18]

Peptic Ulcer:

Peptic ulcer is the erosion in lumen of stomach. The human stomach is capable of secreting hydrochloric acid in concentration that create a greater than 2-million-fold gradient in hydrogen ion concentration between the gastric lumen and tissue vascular compartment. Under the fasting condition acid is secreted in relatively low amount to maintain an intragastric pH of approximately 1.5. This slow level rate termed as basal acid secretion. The rate of basal acid secretion is highest between 9pm and midnight.[19]

Meals are often accompanied with an increase in acid secretion and a brief rise in intragastric pH because to the meal's buffering impact. Thus, during a day time. Intragastric pH changes over the day, particularly during

mealtimes. Intragastric pH remains low during the night-time hours when there is no food present. Based on the Circadian rhythm, the secretion of gastric acid is higher in the early morning or late at night, so our goal is to give the medicine late at night and only then receive the right outcome. This condition is well treated by pulsatile delivery that provide a drug after required lag time.[19]

Drugs used in pulsatile delivery to treat peptic ulcer

Lansoprazole:

Lansoprazole is an antiulcer medication that belongs to the proton pump inhibitor class. Lansoprazole is a benzimidazole sulfoxide derivative that inhibits gastric acid output for a long time. Lansoprazole is used for the treatment of duodenal or gastric ulcers, gastroesophageal reflux disease, and zollinger-ellison syndrome. Lansoprazole, which can be targeted to the colon in a pH and time dependent manner, can be used to control medication levels in synchrony with nocturnal acid secretion's circadian rhythm.[20] A pulsatile 'Tablet in Capsule' dosage form containing lansoprazole taken at bedtime with a programmed start of drug release early in the morning hours can prevent a sharp increase in the incidence of high gastric acid secretion, especially during the early morning hours, when the risk of peptic ulcer is highest.

Rabeprazole:

Rabeprazole is medication that decrease stomach acid. It used to treat peptic ulcer, gastroesophageal reflux disease and excess stomach acid secretion. The effectiveness is similar to other proton pump inhibitors. Rabeprazole's bioavailability is approximately 52%, meaning that 52% of orally administered dose is expected to enter systemic circulation (the bloodstream). Rabeprazole is 97 percent protein bound once it reaches the bloodstream. Rabeprazole has a biological half-life of around one hour in humans. After a single orally given dosage, rabeprazole reaches its maximal level in human plasma in around 3.5 hours. Oral absorption is unaffected by the dosage given.[21]

Cardiovascular Disease:

Cardiovascular functions such as heart rate and blood pressure show 24 h variation. Since the majority of these illnesses can result in fatal or severe results, the incidence of cardiovascular disease such as acute myocardial infarction, strokes, and arrhythmia shows obvious diurnal variation. Cardiovascular medicine chrono pharmacology and chronotherapy: Implications for coronary heart disease prevention and treatment[22]

Various Cardiovascular Disease:

1) Blood pressure/Hypertension:

Blood Pressure is well known to exhibit 24 h variation with a peak in the morning. Throughout the day and night, blood pressure varies. The oscillations might last anywhere from seconds to minutes, or they can last a long time from day to night and season to season. The diurnal shifts associated with the sleep-wake cycle are the most easily noticed and important blood pressure variations[22] The pattern of blood pressure measurements acquired from a typical circadian rhythm during the sleep-wake cycle. During awake hours, the pressure rises, then plateaus for several hours until reaching a peak early in the morning.

2) Acute myocardial infraction (AMI):

The AMI Occurs in mostly early morning. A number of physiological functions. Throughout the day and night, blood pressure varies. The oscillations might last anywhere from seconds to several minutes, or they can last a long time from day to night and season to season. The diurnal shifts associated with the sleep-wake cycle are the most easily noticed and important blood pressure variations. The pattern of blood pressure measurements acquired from a typical circadian rhythm during the sleep-wake cycle. Continuous blood pressure monitoring throughout the day and night showed a trend with minimum systolic and diastolic pressure values between midnight and 4 a.m. In during waking time, the pressure rises, then plateaus for several hours before reaching maximum levels early in the morning.

Drug used in pulsatile delivery to treat cardiovascular disease:

Lisinopril:

Lisinopril is a drug of the angiotensin-converting enzyme (ACE) inhibitor class that is primarily used in the treatment of hypertension, congestive heart failure, and heart attacks. It is also used in preventing the renal and retinal complications of diabetes. The drug has a half-life of 12 hrs. This drug belongs to BCS Class III, having good water solubility.[23] Lisinopril is slowly and incompletely absorbed after oral administration with a bioavailability of 25–30%. The objective of the present study was to improve gastric retention, so consequently, the bioavailability of the drug. The distribution is expected to happen in a burst, that is, all at once after a lag period. The goal of developing an acceptable formulation is to deliver the medicine at the proper time, which is early in the morning.

Propranolol:

Propranolol is a competitive antagonist at beta adrenoceptors that is nonselective. It has a high affinity for both beta-1 and beta-2 receptors, but has a lower affinity for the beta-3 subtype. Despite its quick absorption after oral treatment, propranolol has such a limited bioavailability due to considerable first-pass metabolism. Liver metabolism eliminates propranolol, which is strongly bound to plasma proteins.

Metoprolol:
Metoprolol is β1 selective adrenergic receptor blocking agent used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, heart failure, hyperthyroidism and in the prophylactic treatment of migraine.[25] Because the half-life of the medicine is relatively short (about 4-6 hours) and drug administration is necessary every 4-6 hours in the normal course of therapy, sustained release/control release formulations are warranted for prolonging activity and improving patient compliance.

Atenolol:
Atenolol is a β1-receptor antagonist that is widely used in the therapyof diverse CVD such as angina pectoris, cardiac arrhythmia, and systematic HTN. In atenolol, the stereogeniccenter resides at the N-N-dimethyl propoxy side chain, resulting in the existence of enantiomeric pair. S-Atenolol is the S-enantiomer of (±)-atenolol, the eutomer, which alone is responsible for the β-adrenoceptor blockingactivity. The S-enantiomer has been found to lack the reported side effect of a lowered heart rate sometimes encountered with the racemate.

Diabetes mellitus:
In the management of diabetes, the target is to maintain the patient in normoglycemia. Since the time of day, patient activities, and medication timing can all affect the risk of blood glucose peaks and troughs, chrono pharmacological elements are extremely important in the management of diabetes mellitus, Shift workers are known to have greater incidences of diabetes and obesity, poor glucose control, and cardiovascular disease and death. The 24-hour circadian clock is crucial for glucose tolerance.[26] A human study discovered that when eating and sleeping cycles are not in sync with the internal body clock, blood sugar homeostasis and, in particular, glucose tolerance is significantly affected.

Drug used in pulsatiledelivery to treat Diabetes mellitus.
Glipizide:
Glipizide is belonging to class of sulfonylureas. Oral sulfonylurea act through insulin release by inhibiting the K_{ATP}channel of the pancreatic B-cell. Glipizide is an oral rapid and short acting anti-diabetic drug. The biological half-life of Glipizide is 3.4 to 0.7 hrs. to avoid multi dosing of drug pulsatile delivery system helpful to produce therapeutical effect at required time period.[27]

Hypercholesterolemia:
Hypercholesterolemia is a presence of high level of cholesterol in blood. High cholesterol can limit blood flow, increasing the risk of a heart attack or stroke. Cholesterol synthesis is generally high at night time as compare to daylight due to the activity of rate limiting enzyme HMG COA is higher at night. Diurnal cholesterol synthesis can account for up to 30-40% of total daily cholesterol synthesis. To treat this condition of high cholesterol synthesis at night HMG COA refuse inhibitors are used. Studies with HMG COA reductase inhibitor suggest that evening dosing is more effective than morning dosing.[28]

Drug used in pulsatile delivery to treat Hypercholesterolemia
Fluvastatin:
Fluvastatin sodium is an antilipemic agent that competitively inhibits HMG-CoA reductase. It's part of a class of drugs known as statins, and it's used to lower cholesterol levels in the bloodstream and prevent heart attacks and strokes. Its short biological half-life is 3 hrs. and low bioavailability makes it an appropriate candidate for pulsatile delivery system. Fluvastatin pulsatiledelivery is characterized by proportioning medication concentration in the early morning hours when free cholesterol levels are higher. It may be given before night and is capable of releasing drug after a predetermined time delay.[29]

Pravastatin:
Pravastatin is belonging to class of medication know as statin. This are the cholesterol lowering agent used to preventing cardiovascular disease at high risk and treating abnormal lipid conditions. Pravastatin inhibits function of HMG-COA reductase. The half-life of drug 1-3 hrs. It's recommended to use only after other measures, such as diet, exercises and weight reduction have not improved cholesterol level. The use of pravastatin is generally weaker as compare to other statin but for a pulsatiledelivery system they have an ideal approach due to its pharmacokinetics and minimum side effect.[30]

Parkinson's disease:
The loss of voluntary muscle movement is known as akinesia. It's most commonly associated with Parkinson's disease as a symptom (PD). Morning akinesia in Parkinson’s disease Dopamine neurotransmission control is frequently disrupted, which leads to movement problems. Parkinson's disease (PD) is a movement illness caused by progressive dopamine neuron loss and is related with faulty dopamine neurotransmission control. In order to replace the lost dopamine PD motor symptoms is currently treated with oral levodopa (L-DOPA, a precursor of dopamine), which must be emptied from the stomach and absorbed in the proximal small intestine. Levodopa is converted into dopamine in the brain, and stored in the neurons until needed by the body for movement. It remains the single most effective agent in the management of Parkinson's symptoms.[31]

Drug used in pulsatile delivery to treat morning akinesia:
Levodopa and dopa decarboxylase inhibitor:
Pharmaceutical composition that addresses short-

comings of current formulations comprising levodopaand DOPA decarboxylase inhibitors; by providing a composition that enables timed pulsatile release of these compounds. Providing a delayed burst release of a DOPA decarboxylase inhibitor such as carbidopa and a delayed burst release of levodopa after a predetermined lag time, preferably separated in time whereby the DOPA decarboxylase inhibitor is released before levodopa, provides a means for the management of morning akinesia in patients with Parkinson's disease.[31]

With pulsatile delivery, the patient may improve the night time sleeping pattern and be efficiently relieved from a complete disabling state in the morning. Furthermore, such a composition can be taken together with existing marketed immediate and controlled release levodopa products, to provide a full day dose coverage for most patients with Parkinson's disease.

It is an aspect to provide a pulsatile release pharmaceutical composition comprising levodopa and a DOPA decarboxylase inhibitor, and a pulsatile release component providing for a predetermined lag time followed by a pulse release of said levodopa and said DOPA decarboxylase inhibitor.

CONCLUSION:

The development of a pulsatiledelivery system that can effectively treat diseases with non-constant dose therapy has made significant progress. Due to high patient compliance, convenience of administration, and versatility in formulations, oral medication delivery is currently the most preferred route of drug delivery. Hypertension, osteoarthritis, peptic ulcer, asthma, and other circadian disorders necessitate chrono pharmacotherapy. The methods described in this article are based on attempts made over the last decade to achieve Pulsatile release for a variety of diseases.

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REVIEW ARTICLE

A Review on Diverging approaches to Fabricate Polymeric Nanoparticles

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ABSTRACT:
Polymeric nanoparticles (NPs) are tiny particles with a diameter ranging from of 1 to 1000 nm that can be loaded with active compounds or surface-adsorbed onto the polymeric core. It is novel technology by which we improved therapeutic efficacy, bioavailability of API and better patient compliance. Polymeric NPs have shown promising in the delivery of drug to specific sites for the treatment of a range of disease. Polymeric nanoparticles with a size in the nanometer range protect active substance against degradation in vitro and in vivo, release the drug in a controlled manner, and allow for targeted therapy. The current review discusses polymeric nanoparticle manufacturing methods. Using polymeric drug nanoparticles to improve the therapeutic effectiveness of poorly soluble medicines in any mode of delivery is a universal approach.

KEYWORDS: Drug Delivery System, Polymer-Drug, Biodegradable polymers, Nanoparticles.

INTRODUCTION:
Polymeric nanoparticles (NPs) have sparked a lot of attention in recent years owing to its unique features that come from their small size[1-2]. Polymeric NPs as drug carriers have several features, including the capacity to control drug release, shield drug and other biologically active compounds from the environment, and improve bioavailability and therapeutic index[3-4]. The term "nanoparticle" refers to both nanocapsules and nanospheres, which are morphologically distinct[5-6]. Nanocapsules have an oily core in which the medicine is normally dissolved, and a polymeric shell that regulates the drug's release profile from the core[7]. The medication can be maintained inside or adsorbed onto the surface of nanospheres, which are made up of a continuous polymeric network[8-9]. The reservoir framework (nanocapsule) and matrix system (Nanosphere) are two different forms of polymeric NPs[10-11]. Among the most intriguing techniques to obtaining local controlled therapeutic delivery is the use of polymeric nanoparticles.

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Polymeric nanoparticles are colloidal solid particles with a diameter ranging of 10 to 1000nm that can be spherical, branched, or shell structures built from biodegradable and non-biodegradable polymers, wherein substances are embedded into nanoparticles through dissolution, entrapment, adsorption, and attachment, or encapsulation[12]. In order to ensure effectiveness, polymeric nanoparticles are a major advance over traditional oral and intravenous methods. Polymeric nanoparticles could be efficiently utilized in numerous drug delivery operations, such as tissue engineering, as well as therapeutic delivery for non-human animals[13]. Polymeric nanoparticles are attractive candidates for treating cancer, vaccine administration, contraception, and delivery of targeted antibiotics due to their choice of polymer and capacity to adjust drug release via polymeric nanoparticles[14-15].

Advantages of Polymeric Nanoparticle: [17]
- Controlled and sustained release of active during transit and at the point of administration, modifying the drug's organ distribution and subsequent clearance in order to improve therapeutic potential and reduce side effects.
- Lower toxicity and the occurrence of adverse medication responses, as well as improved drug

consumption.

- Targeting ligands could be attached to the surface of the particles or magnetic guiding is used to accomplish site deliberate targeting.
- The system can be used in a diversity of treatment modalities, such as oral, nasal, parenteral, intra ocular, and so on.

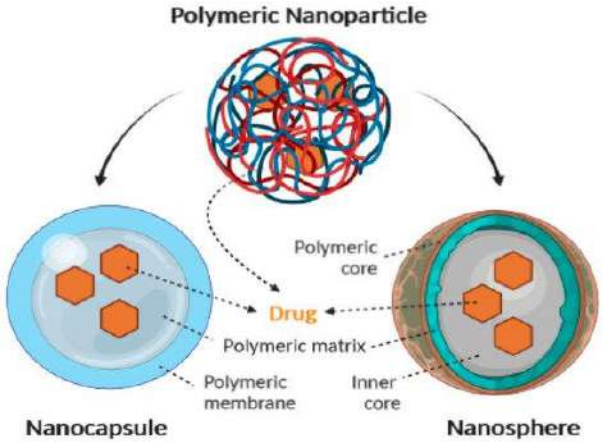

Fig 1: Diagram of Polymeric Nanoparticle[16]

Different Approaches for Synthesis of Polymeric Nanoparticle:

The technological advancement of emulsification equipment has prompted the development of the solvent evaporation technique, which has prompted the development of methods for emulsion preparation with nanoscale droplets over the last decade. Although this procedure is simple and adaptable, it can only be used with liposoluble medicines, it is time consuming, and nanoparticle coalescence during evaporation is a possibility[18].

Emulsification-solvent diffusion:

It involves creating a traditional o/w emulsion from a somewhat water-miscible solvent containing the polymer and medication and an aqueous solution containing a surfactant. To establish the initial thermodynamic equilibrium of both liquids, the polymer solvent and water must be mutually saturated at room temperature for this approach to work. The development of colloidal particles is induced by solvent diffusion from the dispersed droplets into the exterior phase after dilution with a large volume of water [19-20]. Diffusion rather than direct evaporation of the organic solvent from the nanodroplets is a more gentle procedure. Unlike approaches based on solvent evaporation, the droplet size in this technique falls abruptly over a millisecond time scale during solvent diffusion[21-22]. This process is usually used to make nanospheres, but it may also be used to make nanocapsules by simply adding a small amount of oil. Finally, evaporation or filtering can be used to remove the solvent, relying on its boiling point.

Fig 2: Diagram of Emulsification-solvent diffusion[23].

Emulsification–reverse salting-out:

The recently reported emulsification solvent diffusion approach can be thought of as a refinement of the emulsification-reverse salting-out method[24-25]. The key distinction is in the emulsion's composition, which is made up of a water-miscible polymer carrier such as acetone and an aqueous gel comprising the salting-out agent and a colloidal stabilizer. Electrolytes like magnesium chloride, calcium chloride, or magnesium acetate, as well as non-electrolytes like sucrose, are good salting-out agents. The emulsification is accomplished using the Ouzo effect rather than high-shear forces [26-27]. By saturating the aqueous phase, the miscibility of acetone and water is lowered, allowing the production of an o/w emulsion from the otherwise miscible phases. Dilution of the produced o/w emulsion including an excess of water promotes the diffusion of acetone into the aqueous medium, resulting in the precipitation of the polymer dissolved in the emulsified nanodroplets, resulting in a reverse salting-out action. Cross-flow filtration removes the leftover polymer solvent and salting-out agent[28-29]. Adequate miscibility of the organic solvent and water is not required, although it facilitates the execution process. If this is not the case, a higher water/solvent volume proportion will be required throughout nanoparticle production.

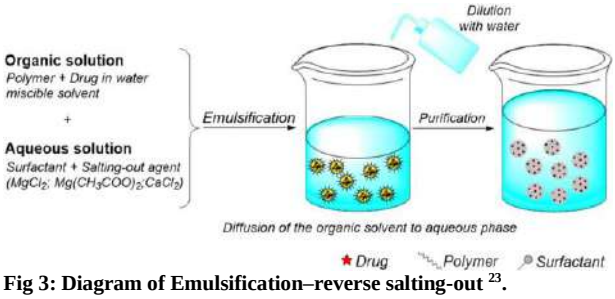

Fig 3: Diagram of Emulsification–reverse salting-out [23].

Nanoprecipitation method:

This technique's core premise is based on the interfacial deposition of a polymer when the organic solvent is displaced from a lipophilic solution to the aqueous environment. The polymer is dissolved in an intermediate polarity water-miscible solvent and then introduced to a stirred aqueous phase in a single shot, sequentially, dropwise, or by controlled addition rate[30]. Nanoparticles form instantly in an order to dodge water

molecules due to the fast spontaneous diffusion of the polymer solution into the aqueous phase. The Marangoni effect appears to govern this phenomenon, in which a decrease in the interfacial conflict between two phases increases the surface area due to fast diffusion, resulting in the creation of tiny droplets of organic solvent[31]. The polymer precipitates in the form of nanocapsules or nanospheres as the solvent diffuses out of the nanodroplets. The organic phase is typically introduced to the aqueous phase; however the procedure might be reversed without impacting nanoparticle production. Acetone is the most commonly used organic solvent since it is miscible with water and quick to evaporate. Nonetheless, ethanol and binary solvent mixes, such as acetone with a tiny amount of water, ethanol, or methanol, can be utilised [32]. As long as the solubility, insolubility, and miscibility requirements are met, either two organic or two aqueous phases can be used. Surfactants are often used in the technique to enhance the integrity of the colloidal suspension, but their existence is not needed for nanoparticle creation. The resultant nanoparticles are typically well-defined in size and have a limited size variation, which is superior to that, obtained using the emulsification solvent evaporation technique [33].

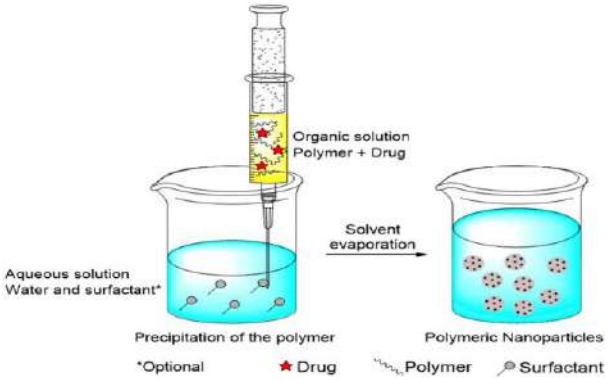

Fig 4: Diagram of Nanoprecipitation Method [23].

Dialysis:

The dialysis approach has been used to make small PNPs with a restricted size distribution successfully [34]. It's regulated by a method similar to that of the nanoprecipitation technique, but with a bit distinct test design. As a physical barrier for the polymer, dialysis tubes or semipermeable membranes with a sufficient molecular MWCO are employed[35]. The polymer is usually emulsified, inserted into the dialysis membrane, and dialyzed against a non-solvent. The inclusion of dilute polymer solutions and the miscibility of the solvents are also essential requirements. As the solvent within the membrane is displaced, the mixture becomes less capable of dissolving the polymer. Furthermore, a rise in interfacial tension causes. Furthermore, an elevation in interfacial tension causes polymer agglomeration and the creation of a colloidal nanoparticle suspension. Whereas dialysis is a straightforward and frequent approach, the enormous volume of counter dialyzing liquid could cause the nanoparticle payload to be released prematurely due to the lengthy process.

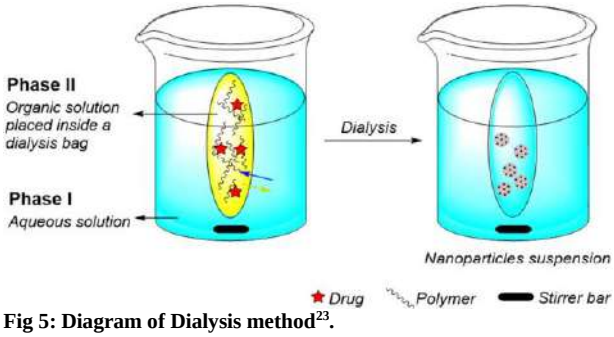

Fig 5: Diagram of Dialysis method[23].

CONCLUSION:

Nanomedicine holds the prospect of developing novel therapeutic platforms that are more effective and have fewer negative effects than traditional formulations. The advantage of delivery systems with nanoscale dimensions is that they have the highest volumeto-size ratio of any dose form. Nanoplatforms have been used in the field of oral medication administration to improve drug solubility, absorption, and bioavailability due to this feature. Nanoformulations also offer the potential to safeguard labile APIs and control drug release, as well as to treat chronic GI illnesses by site-specific and target-oriented delivery.In particular, the development of nanocarriers for oral drug administration has covered three primary sectors of application, in our opinion: increasing the bioavailability of APIs in BCS classes II and IV, treating specific GI regions locally, and delivering biotherapeutics (protein, peptide, and nucleic acids).

To summarise, nanomaterials research for oral medication delivery has recently seen a diversification of material kinds and a rise in the complexity of formulations, as well as the development of new "smart" nanosystems.

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REVIEW ARTICLE

Pharmacosome as a Vesicular Drug Delivery System

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ABSTRACT:
In the area of solubility enhancement, several problems are encountered. Pharmacosomes are a new technology based on lipid drug carriers. Pharmacosomes are colloidal, vesicles, nanometric size micelles or a hexagonal arrangement of colloidal drug dispersions covalently attached to the phospholipid. Because of their unique properties such as active drug loading, small size, high entrapment efficiency, amphiphilicity and stability, they act as a precise drug carrier. They contribute to the controlled release of medicament at the action site, as well as the reduction of therapy cost, increased bioavailability of poorly soluble drugs, decrease toxicity and drug leakage. There has been an advancement in the scope of this delivery system for a number of drugs used for cancer, heart diseases, inflammation and protein delivery besides with a large number of herbal drugs. Pharmacosomes offer new opportunities and challenges for developing a more effective new vesicular drug delivery system.

KEYWORDS: Pharmacosomes, Solubility enhancement, Carrier, vesicular system, phospholipid.

INTRODUCTION:
BCSis a scientific categorization of a drug substance on the basis of its water solubility and intestinal permeability. After oral administration of a drug, it gets dissolved into the gastric (hydrophilic) fluid initially, and then permeated across the biological membranes (lipophilic) and finally reaches in the blood. Many synthetic and natural medications have limited absorption or penetration through the biological membrane, which limits their absorption and total availability to the body system[1].

Poor absorption may be due to their poor water solubility, whereas poor permeation may be due to the poor miscibility with the lipids, thereby severely limiting their capability to pass over the lipid-rich cell membranes of the small intestine.

Therefore, a greater no. of strategies including soluble pro-drug, solid dispersions, cyclodextrin and phospholipids, and vesicular drug delivery systems, etc have been investigated to improve the absorption and penetration of biologically active molecules[2-4].

Vesicular drug delivery systems (VDDS) are important systems used for drug targeting, increasing the bioavailability and stability. These systems have an aqueous core normally adjoining by a lipid bilayer. The system is used as vehicle for the delivery of both water soluble and water insoluble types of drugs. The hydrophilic drugs are entrapped in the inner aqueous core, while hydrophobic drugs are encapsulated in a lipid bilayer. The various advantages offered by VDDS are tissue targeting, high drug entrapment, long retention time, reduced side effects, and increased bioavailability. Furthermore, the system can deliver a drug to the site of action at predetermined rate[5-7].

However, beneficial VDDS have certain limitations. The chief limitations of VDDS are linked to their preparation method, stability, scale-up, loading efficacy, cost-effectiveness, sterilization, burst release, and short half-

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life. Therefore, the pharmacosomes were developed to reduce various defect associated with the conventional vesicular delivery systems. The pharmacosomes are reported as lipophilic prodrug conjugates that self-assemble.

In this review, overview in a very comprehensive manner, the pharmacosomes as an important vesicular delivery system, different components and techniques of preparation and characterization of pharmacosomes, and their applications. In addition, we have analysed the pharmacosomes of different drugs prepared using variety of lipids and their effects on physicochemical properties and pharmacokinetic performance of the drugs. Finally, we conclude by outlining future outcome for the development of pharmacosomes drug delivery.

Pharmacosomes:

Pharmacosomes are colloidal dispersions where the active medicament is covalently bound to the lipid which gives rise to an amphiphilic block. Based on chemical arrangement of drug lipid complex, they reside as fine to an extreme degree of vesicular, micellar, and hexagonal aggregates. The development of vesicular pharmacosomes originates from the surface and bulk interlinking of drugs and lipids. The drug possessing the active functional groups (–COOH, -OH, -NH2) can be covalently linked to lipids with or without spacer chain by esterification or any other suitable conjugation strategy leads to formation of prodrugs. These prodrugs behave like amphiphilic molecules and get self-assembled in one or more layers in contact with the aq. medium. These layers further self-assembled inform of vesicles which resulting in formation of pharmacosomes. In pharmacosomes the drug molecules act as polar head and attached lipids as a non-polar tail. Pharmacosomes avoid problems such as drug leakage, drug incorporation, and reduced shelf life. They can improve bioavailability of drug due to the depletion of interfacial tension, increased area of contact. Pharmacosomes stability is depends on the physical and chemical characteristics of the conjugate system. It possesses several advantages above other vesicular systems such as transferosomes, niosomes, liposomes and hence serves as an alternative to this vesicular systems[8].

Pharmacosomes plays a vital role in the improvement of the drugs dissolution in gastrointestinal fluid and enhancement of their permeation through the lipophilic membrane. Besides, they can enhancedrugs bioavailabilityhaving either low lipid or/and water solubility. The prodrug approach has a high drug trapping efficacy and effectively avoids vesicle leakage and bursts release. As a result, the step of removing the unbound or unentrapped medication from the process of formulation can be skipped, which is a major restriction of liposomes. Pharmacosomes stability is mainly depends on the physiochemical properties of drug-phospholipid complex like solubility, melting point, phase transition temperature, and lipid composition[9-10]. The types of functional groups present in the drug molecule, the length of fatty acid chains in lipids, and the presence or lack of spacer groups all affect the rate of pharmacosome breakdown into active drug molecules. To achieve the desired in vivo pharmacokinetic behaviour, all of these parameters can be tweaked individually. It is reported that a lot of drugs including anti-cancer, cardiovascular drugs, non-steroidal anti-inflammatory drugs (NSAIDS), proteins, and herbal products are delivered through pharmacosomes. Pharmacosomes can be given through different routes like topical, oral and extra vascular routes[11,12].

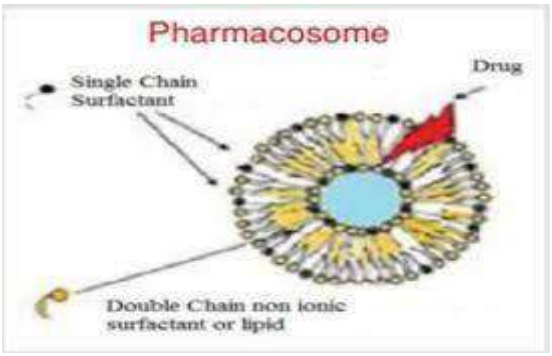

Fig. 1. Structure of pharmacosome

Advantages[13,14]:

1. Drug can be delivered to the active site of infection.
2. Improve bioavailability especially in the case of hydrophobic drug.
3. Reduction in adverse effect and toxicity.
4. Drug carriers like liposomes, nanoparticles, micro emulsions which lead to low physical stability and low drug-loading efficiencysuch as sedimentation, aggregation, and drug leakage during preparation, etc is not present in pharmacosomes.
5. Easily incorporate the drug.
6. Entrapment efficiency is high and predefined since the medication and carrier are covalently bonded and form a stoichiometrically specified unit.

Disadvantage[15]:

1. It requires surface and bulk interactivity of lipids with drugs.
2. To protect the leakage of drugs it requires covalent bonding

Application[16-18]:

1. Pharmacosomes show wider stability profile and longer shelf life.
2. Pharmacosomes has capacity to increase drug absorption and conveyance. Using response surface design, the formulated pharmacosomes were optimized and their attributes examined by

colleagues.

3. Pharmacosomes can increase the permeation rate by improving the fluidity ofmembranes. The approaches have successfully improved therapy, performance, and various drugs such as pindolol diglyceride, amoxicillin, Taxol, cytarabine, dermatansulfate, bupranolol hydrochloride, and so on.
4. Pharmacosomes have a greater degree of selectivity for targeted drug delivery.
5. Pharmacosomes have reach a new level by amplifying therapeutic effects of many drugs like cytarabine, Taxol, dermatan sulphate, pindolol derivative, amoxicillin and so forth.

Component of Pharmacosomes:

The three main components of pharmacosomes are drug, lipid and solvents.

Drug:

A drug having active hydrogen atom (-OH, -NH_2, -COOH,) esterified with a lipid moiety to get amphiphilic block. This amphiphilic block facilitates drug transport via cell membrane, tissue and cell wall. Pharmacosomes of several drugs such as diclofenac, aceclofenac, geniposide, aspirin etc were prepared.

Solvent[19]:

Solvents with high polarity and solubility used for preparation of pharmacosomes. Highly purified and volatile solvent must be used. Generally solvents with intermediate polarity such as acetone, dichloromethane, ethanol, methanol, tetrahydrofuran etc. were preferred for preparation of pharmacosomes.

Lipid[20,21]:

The basic component of a biological membrane is phospholipids. Thephospholipids are of 2 types such as sphingolipidsandphosphoglycerates which are majorly used. Phosphatidylcholine is commonly used lipid for the pharmacosome preparation. It is an amphiphilic block in which hydrophobic acyl hydrocarbon chains pair binds with a water-soluble polar head group of phosphocholine with glycerol bridge. Phosphatidylcholine helps to maintain purity of cell membrane and involved in various biological processes. It acts as source of protein and hepatoprotective agent and utilize in the control of liver disorders. Besides, it prevents fibrosis and also the cirrhosis by enhancing collagen breakdown. Furthermore, it is used to treat different brain conditions such as memory loss, Alzheimer disease, and tardive dyskinesia, and in cancer management. The chemical structure of different lipids used for pharmacosomes preparation.

Table no. 1. Difference between pharmacosomes and liposomes[17]:

Liposomes		Pharmacosomes
Principle	Incorporation of drug in the aq. or lipid phase ofmixture of lipid where the physicochemical properties of the carrier and release of drug will be functions of different lipids used.	The covalent binding of a medication to a lipid results in a molecule that serves as both a carrier and an active agent. The physicochemical properties depend on drug as well as the lipid.
Loss of drug	Through leakage	No leakage, since drug is covalently bound but loss of drug by hydrolysis is possible.
Manufacturing	Cast fill method Extrusion/sonication Injectable method Reverse phase evaporation etc.	Self- dispersion through moderate mixing and sonication.
Separation of free drug	By gel filtration, dialysis, ultrafiltration, ultracentrifugation.	Not necessary since the drug covalently linked.
Volume of inclusion	Decisive in incorporation of drug molecules.	Irrelevant, since the drug is covalently bound
Surface charge	Achieved through lipid combination.	Depends on the physicochemical structure of complex.
Membrane fluidity	Depends on lipid combination and presence of cholesterol fluidity influences the rate of drug release and physical stability of system.	Depends on drug lipid complex phase transition temp. The drug is covalently bound, hence there is zero effect on release rate.
Release of drug	Diffusion through the bilayer, desorption from the surface or release through degradation of liposomes.	Hydrolysis (including enzymatic).
Physical stability	Relatively good Aggregation through double valanced cation.	Depends on physicochemical properties of drug and lipid complex.

Methods of Pharmacosomes Preparation[22-25]:

The pharmacosomes can be prepared by different methods. The methods of pharmacosomes preparation are discussed below.

1. Hand shaking method:

It is one of the simple methods of pharmacosomes preparation where the drug lipid mixture is dissolved in an organic solvent which is volatile in nature in a RBF. Then, the solvent is permit to evaporate utilizing a rotary vacuum evaporator that leads the formation of a thin film in a flask. Finally, the thin film is hydrated using an aqueous medium which offer a vesicular suspension.

2. Ether injection method:

Thecomplex of drug and lipid is dissolved in a definite quantity of ether. This ether solution is then injected in hot buffer or aqueous medium, where vesicles get formed. Vesicles may be in different forms such as round, cylindrical, cubic, or hexagonal type. The shape of vesicles depends on the amphiphilic nature of compound and its concentration.

3. Anhydrous co-solvent lyophilization method:
The drug and phospholipid are dissolved in solution of DMSO and glacial acetic acid. Then, this mixture is blend to form a clear liquid solution and freeze-dried at whole night at condenser temperature. The complex obtained is flushed with nitrogen. Then stored at 4^0C.

4. Solvent evaporation method:
It is a conventional method of pharmacosomes preparation where the drug is firstly acidified to get reactive hydrogen atom which is necessary for complexation. An acid solution of drug extracted with chloroform then recrystallized. The drug lipid complex is dissolved in the organic solvents in an RBF at different ratios. The resultant mixture then refluxed for 1 or 2 hours. Then dried under vacuum evaporator at 40^0C. This dried residue placed in a vacuum desiccator for complete drying. It is time-consuming and involves multistage processing.

5. Supercritical fluid process:
This method is used to overcome shortcomings related with the solvent evaporation technique. The main drawbacks of solvent evaporation technique, time-consuming and involve multistage processing. Besides, the dissolution of pharmacosomes does not improve ideally. Parameters allied to solid morphology, including thecrystal pattern, crystal habit, and particle size, affect the dissolution rate of a compound hence affect bioavailability. The two main techniques used in the supercritical process are gas anti-solvent and solution improve dispersion by supercritical fluid. In this method drug lipid complex is dissolved into supercritical fluid of CO_2 and mixed by using a nozzle mixing chamber. Pharmacosomes formed by fast mixing of dispersion due to the turbulent flow of carbon dioxideandsolvent.

Characterization of Pharmacosomes:
Characterization of drug-lipid complex (prodrug):
1. Chromatography[26]:
The simple chromatographic technique like TLC is primarily used for the confirmation of prodrug. The purity of starting materials and product as well as the progress of drug-phospholipid conjugate synthesis is confirmed by this technique. Nowadays, advanced techniques such as HPTLCand HPLCare widely used over TLC due to higher sensitivity, rapid separation and better resolution.

2. Melting point[27]:
The melting point (MP) is an important parameter that gives information regarding any structural changes in the organic compound. The prodrug formation is characterized by a change in melting point which is normally notably different from that of either pure drug or lipid. The MP of drug will be increased or decreased due to incorporation of lipid moiety. A technique like DSCis widely used to determine the MP of compounds.

3. Ultraviolet spectroscopy:
It is one of the preliminary spectroscopy techniques used to identify the changes in the absorption peaks that occur due to a change in molecular structure. The UV-visible spectrum for pure drug, phospholipid, physical mixture, and prodrug is recorded. The absorption peaks in physical mixtures usually appear at the same wavelength as observed in pure drug and phospholipid. The production of new bonds and recently launched neighbouring groups can be verified by changing peaks, which can be attributable to prodrug synthesis.

4. Fourier transform infrared spectroscopy (FTIR)[28]:
The prodrug formation is confirmed using FTIR by comparing theIRspectrum of prodrug with individual components and physical mixture. The spectrum of a prodrug is commonly different from particular components or physical mixture due to chemical interactions between drug and phospholipid which leads to the generation of new bonds.

5. X-ray diffraction (XRD)[29]:
X-ray diffraction analysis is also performed to confirm the formation of the drug-phospholipid conjugate. In X-ray diffraction pattern the crystalline drugs demonstrate characteristic intense peaks while phospholipid which is amorphous, shows wide peaks.Due to the prevalence of the both free drug as well as phospholipids, the physical mixtures get both sharp as well as wide peaks. The production of a drug-phospholipid conjugate is indicated by the absence or depletion in the intensity of sharp peaks.

6. Solubility studies:
The solubility is again one of the criteria used in the characterization of drug-phospholipid conjugate. The former drug-phospholipid conjugate will affect the solubility profile of the drug. The conjugation of drug with lipophilic moieties decreases solubility and increases the membrane permeability. The solubility studies are performed in water and buffer solutions of different pH values. An excessive amount of sample beyond saturation is added in vials containing different solvents and equilibrated in shaker bath at 37^0C for 24 hrs at controlled rpm. After completion of 24 hrs, a known volume of sample is withdrawn and the amt. of drug solubilized is determined by UV-visible spectroscopy.

Characterization of vesicles[30-34]:
1. Surface morphology:
The shape and size of pharmacosomes are altered by certain parameters such as purity of phospholipids, speed of rotation, method of preparation. Surface morphology can be studied by using SEM and TEM, etc technique.

2. Drug content:

Fordrug content determination an equivalent amount of drug-lipid complex is measured and transferred to volumetric flask which containing solvent. Then flask is sonicated to achieve solubilization for 24 hrs. Finally, the solutions are diluted and drug content is determined using UV-visible spectroscopy or HPLC.

3. *In vitro* drug release study:

The equilibrium reverse dialysis bag technique is used to perform *in vitro* drug release study. Dialysis bag containing donor phase (an emulsion of drug, drug lipid complex) suspended in a vessel comprising of continuous phase outside and stirred. At definite time interval dialysis bag is removed and analysed for drug release. This method has certain advantages as the rise surface area available for donor and receiver phase and increased efficiency due to reduction in the number of steps.

4. *In vivo* characterization:

Specific study models were selected based on the expected pharmacological action of the drug in the pharmacosomes. For evaluating *in vivo* hepatoprotective activity, the effect of test pharmacosomes on animals against alcohol or paracetamol-induced hepatotoxicity can be observed.

5. Stability:

FTIR spectrum of drug lipid complex in solidified form is compared with FTIR spectrum of its micro-dispersion in water later lyophilization at different time intervals. This spectrum data tells about the stability of pharmacosomes.

Table no 2: Pharmacosomes prepared using different drugs, lipids and preparation method with comments[1]:

Drug	Lipid	Preparation method	Comments
Aceclofenac	Soya phosphatidylcholine	Solvent evaporation	Improved bioavailability of Aceclofenac
Diclofenac	Soya phosphatidylcholine	Solvent evaporation	Improved solubility of diclofenac
Aspirin	Soya phosphatidylcholine	Solvent evaporation	Controlled release of Aspirin
Rosuvastatin	Soya lecithin	Hand shaking	improved bioavailability of Rosuvastatin
Acyclovir	Phosphatidylcholine	Tetrahydrofuran injection	Improved solubility of Acyclovir
Ketoprofen	Soya phosphatidylcholine	Solvent evaporation	Increased solubility and dissolution of Ketoprofen
Ornidazole	Soya lecithin	Solvent evaporation	Showed sustained release of Ornidazole
Losartan	Losartan	Solvent evaporation	Improved bioavailability of
Furosemide	Soya phosphatidylcholine	Solvent evaporation	sustained release of
Geniposide	Phospholipid	Solvent evaporation	Improved lipophilicity, absorption and permeation of Geniposide

CONCLUSION:

Pharmacosomes is a stepping stone to improve delivery of drugs containing active hydrogen atom (-OH, -NH_2, -COOH,). In Pharmacosomes drug is bound to the lipid by covalent, van der Waal and hydrogen bonding. The drug-lipid conjugate (prodrug) is amphiphilic in nature and get self-assembled in vesicles in an aqueous medium. In contrast to conventional liposomes, Pharmacosomes are characterized by an unusually high drug loading, amenability to sterilization, higher *in-vitro* stability, and a low burst release. A large variety of drugs formulated as Pharmacosomes using different lipids and preparation techniques have shown improved physicochemical properties and pharmacokinetic performance of the drug.

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Research Article

pH Dependent Mucoadhesive *In-Situ* Gel Formulation Based on *Abelmoschus esculentus* as Sustained Release Carrier for Gastro-retentivity of Famotidine

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ABSTRACT

The major goal of this work was to develop and assess a novel in-situ gel system for sustained drug administration using natural polymers. Okra gum was extracted from the fruits of Abelmoschus esculentus using acetone as a drying agent. The physical and chemical properties of dried okra gum, including pH, solubility, viscosity, moisture content, infrared study using FTIR, and crystallinity study using XRD, were assessed. The in-situ gel was created using the powdered dried okra gum. The pH dependent gelation approach was used to generate an in-situ famotidine gel using varying concentrations of okra gum and tamarind gum. The system makes use of polymers that go through a sol-to-gel phase transition when certain physico-chemical conditions change. Viscosity and in vitro drug release were all considerably affected by the concentration of gelling agents and release retardant polymers. The results showed that the pH ranged from 6.7 to 7.4 and that the drug content ranged from 83.74 to 94.82 %. The viscosity of sol and gel strength was increased with increase in the concentration of polymer, also drug release sustaining. At the end of 8 hours, the in vitro drug release from formulations comprising various amounts of okra gum and tamarind gum was sustained. In all formulations, the drug had a retardant release.

Keywords: Famotidine, Okra gum, Tamarind gum, Gastro retentive drug delivery, in situ gel.

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INTRODUCTION

The creation of regulated and sustainable drug delivery systems has received more attention over the past 30 years. The development of polymeric drug delivery systems has received a lot of research attention. In-situ gel creation has received a lot of interest nowadays. This is mostly due to the in-situ gelling system's significant advantages, which include convenience of administration and decreased frequency of administration and assist to promote patient compliance [1]. The ability to give regulated drug delivery with improved gastro retention within the stomach is provided by gastro retentive in situ gelling systems, also known as stomach-specific systems. In situ gelling systems are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH [2]. Since the gel produced by the in-situ gelling technique is lighter than gastric fluids, it floats above stomach contents or adheres to the gastric mucosa because of the bio adhesive nature of the polymer. This prolongs the time the dosage form spends in the stomach and causes gastric retention, which in turn extends the duration of time the drug is delivered to the gastrointestinal tract. The system makes use of polymers that undergo sol–gel phase transition owing to changes in specific physicochemical parameters. Several polymers are used to form in situ gel, including tamarind gum, xyloglucan, pectin, gellan gum, and sodium alginate [3,4].

Famotidine, histamine is a H2-receptor antagonist that prohibit gastric secretion both locally and systemically, is used to treat gastric ulcers. The dose frequency is twice or three times day and may vary from person to person. Famotidine is rapidly and incompletely absorbed from gastrointestinal tract with the bioavailability of about 45% having an elimination half-life (t1/2) of 3 hours [5]. The use of natural bio-degradable polymer okra gum and tamarind gum was used for this purpose at various combinations in present work. Trisodium citrate, which is a component of the formulation, aids in keeping it liquid until it reaches the stomach. When the formulation enters the stomach, the presence of an acidic environment causes Ca++ to be released, which causes the formulation to gel. The buoyancy of the in-situ gel is maintained to extend period of time due to the release of carbon dioxide in the stomach pH.

Therefore, the goal of the work was to create an in-situ gelling system containing famotidine utilising okra gum and tamarind gum by a pH dependent gelation method, and to assess its physicochemical properties including measurement of pH, viscosity, gelation time, in vitro release characteristics and drug content.

MATERIALS AND METHODS

Famotidine was gift sample from yarrow chem. Pvt. Ltd, Mumbai. Okra gum is extracted in laboratory and

Tamarind gum was obtained from Chhaya industries, Barshi and all other chemicals used were of analytical grade.

Extraction of Okra gum

The fresh *Abelmoschus esculentus* fruits were collected and washed with water. The fruits were crushed and soaked in water for 5–6 hrs, boiled for 30 min and left to stand for 1 hr to allow complete release of the mucilage. The mucilage was precipitated by adding acetone after being separated using a multi-layer muslin cloth (three times the volume of filtrate). The precipitate obtained was collected, dried in an oven at 40°C, and passed through a sieve #80 to obtain discrete powder [6].

Characterization of Extracted Okra Gum [7]

Experiments were carried out in accordance with British Pharmacopeia 2007 and altered based on prior studies.

Solubility Test

Stirring 10 mg of okra powder in 10 mL of water, acetone, chloroform, and ethanol to qualitatively assess the extracted gum's solubility (1 % dispersion). Visual examination of the solute was used to determine solubility.

pH Determination

pH metre was used to determine the pH of the sample's 1 % W/V dispersion in water after it was stirred continuously for 5 minutes.

Viscosity

Viscosity of Okra gum at 1% and 0.5% concentrations was performed using the Brook-field digital viscometer.

Moisture content

Moisture content of okra gum powder was conducted by measuring 100mg of powder using hot air oven with loss on drying at 105˚C.

Fourier Transform Infrared (FTIR)

The Fourier transform-infrared (FTIR) spectrum of the sample was recorded in FTIR Thermo Scientific range between 400–4000 cm^{-1}, in attenuated reflection mode (ATR).

X-Ray Diffraction Analysis

X-Ray diffraction was carried out on Bruker D8 Advance instrument at 250 exposures.

Pre formulation Studies

FTIR Spectroscopy of Famotidine

FTIR spectroscopy was carried out to check the compatibility between drug and polymer. The usual FTIR spectrum of the pure drug was compared to the FTIR spectra of the drug with polymers [8].

UV Spectroscopy

10 mg of famotidine transferred into 100 ml volumetric flask. 0.1 N HCL was used to get the volume assigned to 100 ml (stock-1) by using UV visible spectrophotometer in the scale of 200-400 nm UV spectrum was recorded[9].

Calibration curve of famotidine in 0.1 N HCL

50 mg of famotidine was dissolved in 50 ml of 0.1 N HCL. The solution was then diluted with 0.1 N HCL to obtain 2, 4, 6, 8, 10 and 12 µg/ml solution. It was then measured by UV visible spectrophotometer at 265 nm [10].

Melting Point

Melting point equipment was used to find out the melting point of Famotidine. Drug was placed in a glass capillary with flame-sealed end to determine melting point. Inside the melting point apparatus, which had a magnetic stirring facility, the capillary containing the drug was submerged in liquid paraffin.

Preparation of in situ gelling Sols

The in-situ gel formulations of F1 to F3 was prepared by using okra gum and tamarind gum was 69eionize in F4 to F6 formulation. The polymer solutions (sodium alginate, tamarind gum and okra gum) of various concentrations were prepared by adding to 69eionized water containing 0.17% w/v trisodium citrate and heated to 90°C with continuous stirring. After cooling to below 40°C appropriate amounts of calcium carbonate (0.05% w/v), drug solution of famotidine and preservative (methyl paraben) was added to the polymer solution and volume was adjusted to 20 ml with distilled water. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing (Table 1) [11].

Table 1: Composition of the in-situ gelling formulation

Sr.no	Ingredient	F1	F2	F3	F4	F5	F6
1	Sodium alginate (%W/V)	1	1.5	2	1	1.5	2
2	Okra gum (%W/V)	0.2	0.4	0.6	-	-	-
3	Tamarind gum (%W/V)	-	-	-	0.2	0.4	0.6
4	Trisodium citrate (%W/V)	0.17	0.17	0.17	0.17	0.17	0.17
5	Calcium carbonate (%W/V)	0.05	0.05	0.05	0.05	0.05	0.05
6	Famotidine (mg)	40	40	40	40	40	40
7	Preservative (%W/V)	0.2	0.2	0.2	0.2	0.2	0.2

Characterization of the in-situ gel formulations

Determination of the visual appearance

All the preparations were visually inspected for their appearance, clarity, and consistency.

Measurement of the pH

A calibrated digital pH metre was used to measure the pH of each formulation. For each formulation, the readings were held three times, and the averages of the readings were held into consideration[12].

In vitro gelation study

The gelling capacity was determined by placing 10 ml of solution in 100 ml of stimulated gastric fluid (pH 1.2) freshly prepared and equilibrated at 37 ± 0.5°C. When the formulation came in contact with the gelation medium, it was quickly converted into a gel-like structure. The in vitro gelling capability was analyzed based on the gel's stiffness and how long it maintains its stiffness.

According to the period of time the created gel required and how long it lasts, the in vitro gelling capability was primarily categorised into three groups [10].

• (+): Gels in few second and disperse immediately.

• (++): Immediate gelation does not disperse rapidly.

• (+++): Gelation after few minutes remains for extended periods.

Determination of viscosity

The viscosities of the formulations were measured using fresh samples three times using a Brookfield digital viscometer with an S21 spindle at 50 rpm. The average reading was taken after each measurement.

In vitro buoyancy study

The in-vitro buoyancy study was carried out using stimulated gastric fluid (0.1N HCl, pH 1.2). 37 ±5°C was maintained as the medium temperature. In the dissolution media, 10 ml of the in-situ gel formulation were added. The time taken by the in-situ gel formulation to reach the medium surface (floating lag time) and how long it remained buoyant (the floating duration) was noted [1,13].

Determination of the drug content

80 ml of 0.1N HCl, pH 1.2, was combined with 5 ml of the formulation corresponding to 10 mg of the drug, and the solution was stirred for one hour in a magnetic stirrer. The solution was filtered and diluted with 0.1N HCl, pH 1.2, after 1 hour. The drug concentration was then determined by ultraviolet (UV) visible spectrophotometer at 265 nm against a suitable blank solution [14].

Measurement of density of gel

30 ml of the in-situ formulation was poured into a beaker containing 50 ml of 0.1N HCl. 10 ml of the gel formed was elevated in measuring cylinder and weight of the gel was measured. The density was determined using both the weight and volume of the gel. This method was followed for all the formulations [15].

Measurement of gel strength

30 g of the gel was elevated in a 50 ml beaker and a 50 g weight was placed on the centre of the gel surface and allowed to penetrate through the gel. The time taken by the 50 g weight to penetrate 5 cm down through the gel was noted for all the formulations. The same method was followed for 3 times for each fresh formulation and average time was noted [16].

In vitro drug release studies

A USP dissolution equipment (Type II) with a paddle stirrer operating at 50 rpm was used to assess the drug release from the formulations. This slow speed is necessary to avoid breaking of the gelled formulation. The dissolution medium was 900 ml of the simulated gastric fluid (0.1N HCl, pH 1.2), and the temperature was kept at 37±5°C. In situ gel was formed when 10 ml of the formulation were added to the dissolution vessel without disturbing the dissolving medium. At each time interval, 3 ml of the sample was withdrawn and replenished with fresh medium. The samples were collected, filtered, and suitably diluted before being analysed at 265 nm with a UV spectrophotometer [17].

In vitro mucoadhesive study [18,19]

Using a modified bioadhesion test equipment, the force necessary to separate each formulation from goat tissue was measured in order to estimate the mucoadhesive strength of each formulation.

Modified bioadhesion test apparatus

Modifying the double beam physical balance as shown in figure 1 can create the bio adhesion test apparatus. the two pans of the physical balance were removed. A light-weight plastic glass was used to replace the right-hand pan, and a glass vial was suspended from a strong thread on the left-hand side of the balance, with the height of the vial and it adjusted to allow for a lower vial to be placed beneath it. The two sides of the balance were adjusted so that the right side weighed 5 g more than the left. To determine the bioadhesive strength, a piece of goat mucosa was cut and utilised as a membrane. It was attached to both glass vials using a thread after being properly washed with physiological saline solution so that both mucosal surfaces were exposed on the outsides of the vial surfaces (figure no.1). The buffer pH 5.5 was added to the jacketed glass container, which was kept at a constant 37˚C±1˚C. The vial was then placed inside of it. The membrane was kept at this temperature for 30 minutes to allow for equilibration. the jacketed glass container containing beaker was kept below the right-hand setup of the assembly. The gel was stuck to the lower side of the beaker as a thin layer. The assembly was kept undisturbed for 1 min and the weights were slowly added to the left-hand side till the membrane surface just detached from the gel surface just detached from the gel surface. The excess weight on the left-hand side, i.e., a measurement stress in dyne/cm2 was determined from the

minimal weights that detached the tissues from the surface of each formulation using the following equations: total weight (g) minus blank (weight in gm required to detach mucosal surface without gel layer).

Detachment stress (dyne/cm^2) = m x g/A

Where, m = weight required for detachment for two vials in grams

g = acceleration due to gravity [980cm/s^2]

A= Area of tissue exposed

The goat mucosa was changed for each measurement. For each of the gel formulations, measurements were carried out times.

Stability study

The optimized formulation of in situ gel were placed in an amber colour bottle with aluminium cap as a closure. It was tightly sealed. The stability study was carried out for 1 month. Stability of the in-situ gel formulation was monitored at room temperature (25˚C+2°C). Samples were periodically removed and evaluated for viscosity, drug content, pH and in vitro release [20].

RESULTS AND DISCUSSION

FTIR and compatibility studies

All the characteristic peaks of Famotidine were present in the spectrum of drug and polymer mixture, indicating compatibility between drug and polymers. The spectrum confirmed that there is no significant change in chemical integrity of the drug.

Determination of λ $_{max}$ of Famotidine

λ $_{max}$ of Famotidine was determined by utilised stock solution and analysed spectroscopically at 265nm wavelength.

Calibration curve of Famotidine

The absorbance of the solution was recorded at 265 nm by using UV visible spectrophotometer. 0.1 N HCL was taken as blank. The graph of absorbance vs. concentration was shown to be linear in the famotidine concentration range.

Melting point determination

By using a melting point equipment, the melting point of famotidine was detected to be between 160-162˚C. The reported melting point range for famotidine 163-164˚C.

Characterization of Okra Gum

Solubility test

Okra powder was shown to be sparingly soluble in water and insoluble in acetone, ethanol and chloroform. An increase in solubility was observed when temperature was applied.

pH Determination

The pH of Okra gum is 6.59.

Viscosity

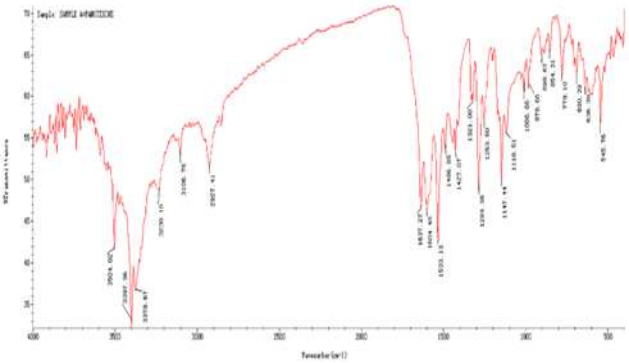
Viscosity of Okra gum 1% solution is higher (228.78cps) compared to the viscosity of Okra gum at a lower concentration (0.5% solution) which is 62.32 cps. This indicates that Okra gum has higher viscosity at a higher concentration.

Moisture content

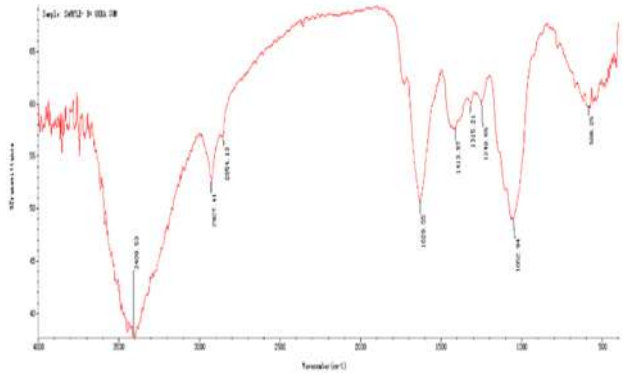
Moisture content of Okra gum is 14.83%, indicating that Okra gum contains bound moisture to the polymer. This is due to the polymer adsorption sites that is able to bind water molecules to the polysaccharide structure via hydrogen bond [9], which leads to a larger permeability of hydrophilic materials.

FTIR and compatibility studies of Okra gum

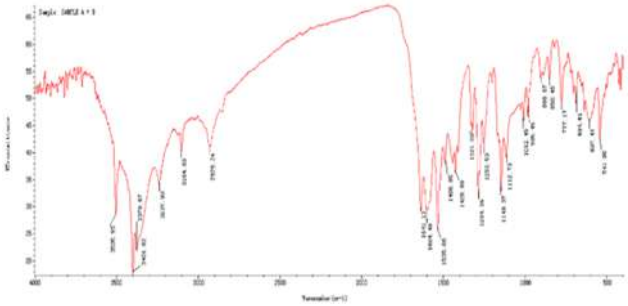
No considerable changes in the IR peaks of the extracted Okra gum as shown in figure 1.



(a)



(b)



(c)

Figure 1: FTIR peak of Famotidine (a), Okra gum (b) and famotidine with okra gum (c)

X-Ray Diffraction Analysis

XRD analysis of Okra as can be seen in Figure 2 showed that it consists of crystalline structure. The sharp peak that could be seen from the X-ray diffraction spectrum indicates the crystalline nature of the polymer.

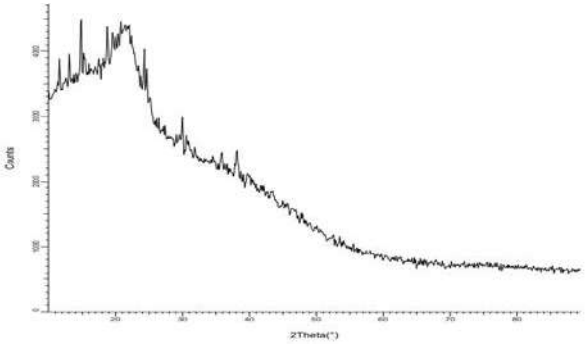


Figure 2: X-ray diffraction analysis of Okra gum

Characterization of the *in-situ* gel formulations

Visual appearance

Visual appearance was evaluated on all of the formulations. The results are shown in Table 2 for the developed formulations of in situ gel containing okra gum, which had a light brownish appearance, and tamarind gum, which had an off-white appearance. The formulations were free running and did not produce any gelation at room temperature.

pH measurements

According to Table 2, the pH of each formulation was determined to be adequate and ranged from 6.7 to 7.4. The pH of each formulation was within the orally acceptable range.

In vitro gelation study

The gelation study was conducted in 0.1N HCL, pH 1.2. On an ordinal scale between + and +++, the gelation properties of the formulations were evaluated as given in table no.2. All the formulation on contact with the gelation medium had undergone sol to gel transition. It was detected that the gel intensity was increased when the concentration of polymers was increased. Table 2 has shown that the formulation F1, F3, F5, F6 were satisfactory to cause gelation.

Table 2: Appearance, pH, Gelling capacity, Pourability

Formulation code	Appearance	pH	Gelling capacity	Pourability
F1	Light brownish	6.7±0.02	+	Easily pourable
F2	Light brownish	7.1±0.06	+++	Easily pourable
F3	Light brownish	7.3±0.03	+++	Easily pourable
F4	Off -white	7.2±0.07	++	Easily pourable
F5	Off -white	6.9±0.01	+++	Easily pourable
F6	Off -white	7.4±0.04	+++	Easily pourable

Table 3: Viscosity, Floating lag time, Floating duration, Percentage drug content

Formulation code	Viscosity (cps)	Floating lag time (s)	Floating duration (hr)	Percentage drug content (%)
F1	69.20±0.02	22	5	88.16 ± 0.34
F2	85.52±0.16	19	7	83.74 ± 0.45
F3	90.60±0.45	16	8	91.46 ± 0.53
F4	82.34±0.49	26	11	84.87 ± 0.23
F5	88.68±0.25	31	12	89.72 ± 0.41
F6	108.52±0.65	33	12	94.82 ± 0.59

In vitro buoyancy study

The floating lag time is the duration of time that the formulation required to appear on the medium's surface and the floating duration is the period of time that the formulation floated constantly. Buoyancy studies results are given in Table 3. A gel barrier forms on the plane of the formulation when it comes into contact with an acidic environment due to the calcium ions' cross-linking and gelation processes. The formulation floats because the carbon dioxide emitted is trapped in the gel matrix. The dispersing of carbon dioxide and drug release are then further constrained by the polymeric network. The floating capability of the formulations mainly depends on concentration of the gelling polymer, carbon dioxide and cation source. The formulation containing okra gum (F1-F3) is less floating lag time but more floating lag time containing tamarind gum (F4-F5).

(a) (b)

Figure 3: *In vitro* buoyancy study of (a) Okra gum and (b) tamarind gum

Viscosity

The viscosity of all the in-situ gelling formulations determined at 50 rpm using Brookfield digital viscometer. The results of viscosity measurement of each formulation are shown in Table 3. The increase in viscosity of the formulations that were observed with the increase in the concentration of polymer can be related to the increasing crosslinking of the polymer. Okra gum-based formulations have greater viscosities, which results in in situ gel and slower drug release. The all formulations of viscosity range between (69.20±0.02 and 108.52±0.65).

Drug content

Drug content is one of the important evaluation parameters for any type of dosage form. The percentage drug content of each formulation was between the range of 83.74-94.82 indicating uniform distribution of drugs in all formulations as per monograph Table 3.

Measurement of density of the gel

Regarding the gastro retentive dosage form's ability to float, density is an important evaluating parameter. The formulation must have a density that is less than or equal to the gastric content's density (1.004 gcm^3) in order to float on them. The density of each formulation given in (Table 4) has density less than the above-specified value. As a result, the floating of the gastro retentive in situ gel is promoted in the stomach.

Measurement of gel strength

All the formulations showed good gel strength in which okra gum as compare to tamarind gum ranges are same from 18.7s to 30.3s. This says the increase in polymer concentration causes an increase in gel strength (Table 4) gives the gel strength of all the formulations.

Table 4: Density and gel strength of the in-situ gel formulation

Formulation code	Density (g/cm^3)	Gel strength (s)	Mucoadhesive strength ($dyne/cm^2$)
F1	0.422±0.36	18.7 ±0.06	1191±33.41
F2	0.501±0.45	24.2±0.12	1275±33.13
F3	0.554±0.42	29.8±0.23	1511±33.41
F4	0.482±0.54	22.5±0.33	785.6±18.02
F5	0.526±0.56	26.9±0.45	915.1±18.02
F6	0.579±0.62	30.3±0.56	1011±21.31

Table 5: Stability studies of in situ gel formulation

Days	pH	Viscosity	Drug content (%)	Drug release (%)
Initial	6.7±0.02	69.20±0.26	88.16 ± 0.34	90.74
After 1 month	6.7±0.06	69.20±0.26	88.16 ± 0.34	90.74
After 2 months	6.6±0.14	67.28±0.35	87.22 ± 0.54	89.35
After 3 months	6.5±0.11	69.28±0.12	88.11 ± 0.13	88.22

***In vitro* drug release study**

The in vitro drug release studies, it was mentioned that the release of the drug from the prepared gastro retentive in situ gel reduces as the concentration of the gelling agent increases. The effect of polymer concentration on in-vitro drug release from in situ gels. The plot of % cumulative drug release v/s time (in hours) was plotted and depicted as shown in Figure no.10. Drug releasing pattern of various formulation contains a different concentration of gelling agent and drug release retardant polymers are given as follows: Okra gum: F1 > F2 > F3 and Tamarind gum: F4 > F5 > F6 as shown in Figure 4. The percentage drug release from formulations containing various concentrations of Okra gum at the end of 8 hrs was observed to be 90.74%, 86.24%, and 82.31%, respectively, for F1, F2, and F3. Similarly, percentage drug release from formulations containing various concentrations of Tamarind gum at the end of 8 hrs was observed to be 91.82%, 88.16%, and 83.74%, respectively, for F4, F5, and F6. The retarded release observes in above formulations.

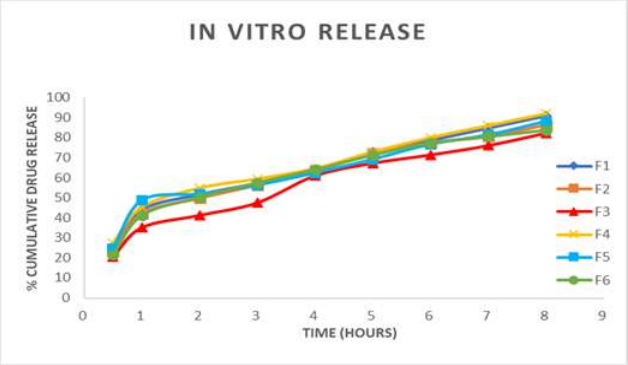


Figure 4: *In vitro* drug release

Mucoadhesive strength

Mucoadhesive strength of the in-situ gel formulation plays major role in giving the idea about gastric residence time of the formulation. Formulation must have enough mucoadhesive property so that it will remain in GIT for longer the gastric absorption of drug. The formulation containing okra gum (F1-F3) is more mucoadhesive strength but tamarind gum containing (F4-F6) less mucoadhesive strength. Result of mucoadhesive strength study is given in Table 4.

Stability studies

The stability study of optimized formulation F3 was carried out for 3 months at room temperature and humidity condition. Stability study's results designated that the there was no significant change in the pH, viscosity, drug content (%) and drug release (%) as shown in Table 5.

CONCLUSION

In the present study in situ gel of famotidine were produced by using different concentration of okra gum and tamarind gum to improve its oral bioavailability and sustained release activity. Okra gum shows less floating lag time and more mucoadhesive strength but tamarind gum shows more floating lag time and less mucoadhesive strength. Both okra gum and tamarind gum show result but okra gum shows significant result as that of tamarind gum. Based upon obtained results it concluded that prepared formulation is satisfactory for clinically use of famotidine and formulation of in situ gel using okra gum was successfully prepared.

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Regulatory Intelligence

Neha Nangare[1], A B. Velhal[2]

Abstract: ***Regulations are a common way for governments to exert control over the activities of individuals, businesses, and communities in order to promote the common good. Regulations can be for any area of society, such as environmental wellness, such as water or air quality, public health, or data privacy for internet safety. They can, however, be produced by various entities with overlapping jurisdictions, resulting in widespread confusion, misunderstanding, and inaction. AI has the ability to play a significant role in assisting various stakeholders in better understanding existing regulations, their gaps and duplications, and recommending methods to strengthen them in order to streamline decision-making. Regulation Intelligence is the term we use to describe the difficulty of enabling improved comprehension of regulations. Pharmaceutical companies all across the world have long struggled with the massive amount of data they must manage. These can be described as the twin issues of manually researching changing regulatory requirements to ensure better compliance and decreasing rework as a result of departmental silos within the business and the lack of readily available historical information. These two difficulties result in lower operational efficiency, as well as more time, effort, and expense. This paper examines how these issues might be addressed comprehensively by adopting a technology-centric approach to developing a smart regulatory compliance solution. This solution will be able to deliver actionable insights and support precise, easily accessible, and contextual information, allowing for on-demand access to literature.***

Keywords: Regulatory intelligence

1. Intelligence on regulatory issues

Regulatory intelligence, in general, refers to the monitoring, collection, and analysis of publicly available and experience-based regulatory information in order to develop strategies for more time and cost-effective drug development.

Regulatory intelligence professionals provide strategic information to the drug development process, act as liaisons with regulatory bodies, and distribute information to the right stakeholders. Kirsten Mesmer and Charity – Anne Schuller, regulator experts, present an overview of applicable delivery methods and general considerations for communicating information via spreadsheets, text documents, slide presentations, strategy reports, and competitive intelligence reports in " Regulatory Intelligence Communication for Business Impact. "

The authors discuss how to get the most out of regulatory information when responding to specific stakeholder requests, as well as communication tips.

Regulatory Intelligence enables regulatory professionals to determine the requirements for global clinical trials, compliance procedures, manufacturing requirements, advise personnel, answer strategic regulatory questions, and develop a global marketing application using data from regulatory intelligence. Going deeper into this blog will give you a better understanding of what regulatory intelligence is and how it operates.

However, three points should be remembered if you want to grasp the essence of RI:

1) Collect information
2) Regulatory strategy
3) Information

1) Collecting information

Regulatory specialists used to limit RI activity to this issue solely at one point. When gaps in the input and output were discovered, it was clear that several critical facets were missing from the shelf. That's when the rest of the puzzle fell into place.

To begin, RI experts conduct extensive study into regulatory requirements for a certain product in a specific geography. There are many sites that RI experts use to consolidate their research material when it comes to obtaining appropriate regulatory information. These are some of the resources available:

a) Regulations on Websites, Blogs, and Social Media Groups
b) Seminars and Training Sessions-Professional Newsletters-Competitor Product Analysis
c) literature
d) Requests for Information (FOIA)
e) E-mails pertaining to regulatory issues – Networking
f) Paperwork
g) Messages

2) Knowledge

Because the initial phase contains a large amount of research material, it is clear that this data must be filtered in order to acquire useful information for the objective. You can think of it as jigsaw pieces, and now we need to make sure that all of them fit together to acquire what we need. An effective regulatory strategy conveys the best answer and fosters adequate planning throughout an organization's numerous disciplines, from manufacturing to marketing.

This task entails keeping track of things like the regulatory industry's current trends and patterns. We've been emphasizing that in order for RI to be effective, it must stay up with the most recent changes in regulations and guidelines. As a result, it becomes clear that this method may undergo several alterations in order to eliminate the required conclusion.

Knowledge of the sector and its history, as well as soft skills, are required of regulatory intelligence specialists. In regulatory affairs and the pharmaceutical and/or medical

device industries, there are no hard-and-fast regulations concerning how many years of experience a new worker should have. However, a reasonable rule of thumb for entering the regulatory intelligence field is that entry-level positions should demand a minimum of 5 years in industry and 3 years in regulatory affairs. The number of years of experience necessary rises in proportion to the position's seniority.

3) Action Plan for Regulation

The main goal of the aforementioned tasks is to develop the most appropriate and realistic regulatory strategy for a company. In different countries, different products have distinct regulatory rules. This is why experts recommend a plan of action that lays out a strategy for implementing regulatory actions in the target distribution markets. However, this strategy never results in a completed work. It keeps moving forward as the regulatory space's mandates change.

Importance of Regulatory Intelligence:

- Provides regulatory professionals with information to:- identify opportunities
- More indications and more precise pre-clinical and clinical development programmes
- Quicken development/improve efficiency

a) Recognize potential pitfalls

- Issues with compliance, as well as changes in the requirements for certain indications

b) Answer specific development questions posed by team RI-Predict review times for product and/or update to product Provide?

- Research for product teams
- Supports execution plan
- Policy comments to shape legislation
- Track legislation
- Track approvals, non-approvals, and withdrawals
- Knowledge management
- Training
- Corporate policy creation

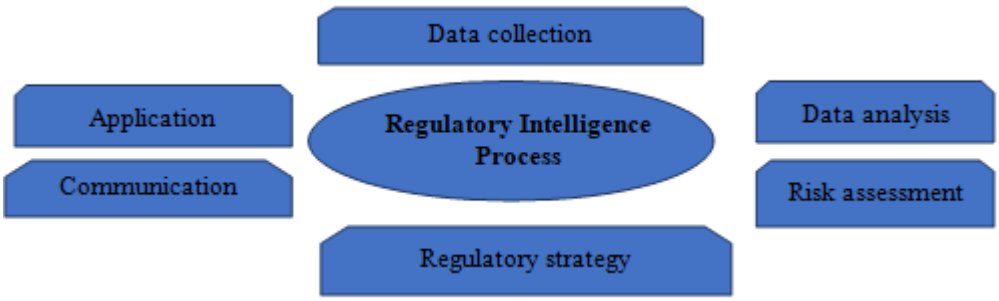
The RI procedure

The practice of delivering strategic information that underpins the making of effective and efficient decisions in relation to the regulatory aspects of the business is known as regulatory intelligence.

The following activities are included in the procedure:

Selection of relevant publicly available data sources;

- Data collection;
- Data analysis;
- Generation of significant information for the definition of the regulatory strategy based on the analysis;
- Communication of the implications of this information for the business;
- Continuous monitoring of the regulatory environment, looking for opportunities to model future regulations, policies, and legislation.



In medication development, what role does regulatory intelligence play?

- Regulatory intelligence professionals provide strategic information to the drug development process; act as liaisons with regulatory bodies, and channel information to the right Ate stakeholders.
- Kirsten Messmer and Charity-Anne Schuller, regulato-Ry experts, present an overview of applicable Delivery methods and general considerations for communicating information via spread-Sheets, text documents, slide presentations, strategy reports, and competitive intelligence Reports in "Regulatory Intelligence Communication for Business Impact. "
- Authors discuss how to get the most out of regulatory information when responding to specific stakeholder requests, as well as communication tips.

Regulations are evolving at a faster rate than ever before –

It's necessary to be on the ball all of the time.

- New technology and goods, such as the world's first 3D-printed medication Approved recently it's possible that it won't fit well in the current regulatory context, necessitating careful adaption.

Harmonization and Expansion

- Australia is constantly implementing new EU legislation – nations may join the EU – Increased transparency equals increased accountability.
- Recent drive for transparency in the EU and the US – for example, trial registrations – More information becomes publicly available – Information overload

In pharmacovigilance, what role does regulatory intelligence play?

QVigilance continuously monitors regulatory information from local, regional and global authorities and organisations for pharmacovigilance related regulatory intelligence to ensure that we and our customers are always up to speed and thereby maintain compliance with the latest regulatory requirements and guidelines;

1) Drug safety
2) That is dependable
3) Scalability should be improved.

The act of acquiring and evaluating publically available regulatory information, communicating the consequences of that information, and monitoring the present regulatory environment is known as Regulatory Intelligence in Pharmacovigilance (PV).

Regulatory intelligence is the process of staying current with new regulatory standards as they are enacted by governments and regulatory agencies. These regulations apply to both pharmaceutical drugs and medical equipment that are in development and have been approved for sale. This means that new or altered PV-relevant regulatory material must be examined and assessed on a regular basis for potential influence on corporate operations and pharmacovigilance strategy. Regulatory Intelligence efforts must be reported to stakeholders, and an effect assessment must be done and documented.

PV Regulatory Intelligence is managed by ProPharma Group for a number of clients. Regulatory Intelligence is also used by our team to keep our own internal knowledge current, such as that of QPPVs (Qualified Persons for Pharmacovigilance), LPPVs (Local Persons for Pharmacovigilance), and others.

Regulatory intelligence sources and communication.

- Regulatory intelligence sources vary by company. Smaller enterprises must rely on public regulatory intelligence sources, but larger, better-resourced companies can obtain rights to paid subscription services like Cortellis or Tarius.
- Regulatory authority websites were cited as the most popular source of regulatory intelligence by survey respondents, which is understandable given that they are the best source of regulatory information.
- It's worth noting that the 2019 poll results showed less use of subscription services than earlier versions of the survey.
- This could indicate that there is more free information on the internet, reducing the need to pay for high-quality regulatory intelligence.

RI in action:

1) Programmed optimization
2) Clinoptimization's Possibility
3) Adjustment of the development plan
4) Questions and answers, as well as a review of regulatory requirements
5) Regulatory overview preparation
6) Contracts for research bidding
7) Internal and external education and training companies Alerts that are specific to your needs, as well as a newsletter
8) Sometimes it's as simple as seeing if a particular medicine is available in other nations.

2. Conclusion

Pharmaceutical companies may function more efficiently and respond quickly to any developing urgent scenario by reorganising outdated procedures and reinventing regulatory information with the help of digital technologies such as artificial intelligence. The integrated Regulatory Intelligence solution offers a more simplified ow of global regulatory requirements by facilitating the reuse of internal data. As a result, the necessity of the hour is to imagine a connected future using digital technologies and RI. Its diverse capabilities and potential can assist pharma companies in overcoming important issues and achieving their goal of becoming a smart firm.

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DRUG DEVELOPMENT PROCESS

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Abstract: Drug discovery is a procedure that tries to find a therapeutically beneficial chemical for curing and treating disease. Identification of candidates, synthesis, characterization, validation, optimization, screening, and tests for therapeutic efficacy are all part of this process. Once a molecule has been proven to be significant in these studies, it will begin the medication development process prior to clinical trials. To create a medicine that is safe, effective, and meets all regulatory standards, the new drug development process must go through numerous stages. One of the main points of our article is that the process is long, complicated, and expensive enough that multiple biological targets must be considered for any new treatment that is eventually approved for clinical use, and new research methods may be required to investigate each new target. It takes a long time and a lot of effort to turn a discovery into a commercial medicine. It takes roughly 12 to 15 years from discovery to licensed drug, and an expenditure of about $1 billion is required. A million molecules are screened on average, but only one is investigated in late-stage clinical trials and eventually made available to patients. This article gives a quick overview of how new drugs are discovered and developed.

Index Terms **- Drug discovery , Development , Validation, Optimization , Screening.**

I. INTRODUCTION

Drug discovery is a multidimensional process that include identifying a drug molecule that is therapeutically useful in the treatment and management of a disease. Typically, researchers discover novel medications by developing new perspectives on a disease process that allow researchers to construct a medicine to counteract or stop the disease's symptoms. The identification of drug candidates, synthesis, characterization, screening, and assays for therapeutic efficacy are all part of the drug development process. Following clinical trials, if a molecule achieves favorable findings in these investigations, it will begin the process of medication development. Due to the high costs of R&D and clinical trials, drug discovery and development is a costly process.[(1)]

A single new medicine molecule takes almost 12-15 years to develop from the moment it is discovered to the time it is accessible on the market for treating patients. For every 5,000-10,000 compounds that join the research, Success necessitates vast resources, including the best scientific and logical brains, cutting-edge labs and equipment, and multidimensional project management. Persistence and good fortune are also required. Drug discovery eventually offers hope, faith, and relief to billions of sufferers[(2)]

II. STAGES OF DRUG DISCOVERY

stage 1: Target identification:

Target identification is the first and key step in the drug discovery channel. A drug target is the specific binding site for drug in vivo through which it exerts action. Usually, drug target refers to a single biomolecule.[(4)]

A drug target can be an established drug for which there is good scientific Know-how which is supported by publications that describe both how the target behaves in normal physiology and how it is involved in human pathology There are many drugs targeting established drug targets. A drug target can also be potential drug targets which are biomolecules whose functions are not fully understood and which lack drugs targeting them.[(5)]

A drug target has any of the following characteristics

1. the drug targets can be a biomolecule(s) such as a protein that could exist in solitary or complex forms.
2. The biomolecules have specific locations or sites that match with the other, the drug
3. The structure of biomolecules may change when it binds to drugs. The changes in structure are usually reversible.
4. Various physiological responses occur when the structure of a biomolecule changes, causing the cell, organ, tissue, or body condition to be regulated.[(5)]

Stage 2: Target Validation:

Target validation is the basis for new drug exploration in the process of drug discovery. Target validation helps to new drug research and development and to provide more insight into the pathogenesis of target-related diseases. (6)

The target validation process includes

1 Discovery of the biomolecule of interest

2. Evaluation of its potential as a target.

3. Designing a bioassay to measure its biological activity.

4. Constructing a high-throughput screening method. [6]

Stage 3: Lead Discovery

Leads can also be obtained by molecular modelling assisted by 3D computer graphics, which allows the development of structures based on new and pre-existing molecules to increase desired features while eliminating undesirable properties to develop highly selective targeted compounds. A combinatorial chemistry wherein unplanned mixing and matching of large numbers of chemical building blocks to produce libraries of all possible combinations can also be attempted to get leads. This technique generates billions of compounds, screened by high-throughput screening (HTS), meaning thousands a day. [3]

Stage 4: Lead optimization:

Lead optimization is a procedure that begins with the identification of a compound that has the potential to have a biological effect and ends with the selection of the best compound. Molecules are chemically modified and described to produce molecules with desirable qualities, which are then converted into drugs. Physiochemical qualities, pharmacokinetic properties, and toxicological elements of leads are optimized in vitro and in vivo for efficacy and potency[7]

Stage 5: Pre-clinical and Clinical Development:

Companies use stylized statistics to illustrate the risks in preclinical research, such as that on average, only one in every five thousand compounds that enters drug discovery to the stage of preclinical development becomes an approved drug[8]

Clinical trials are organized by the National Institutes of Health (NIH) into 5 different types:

1. Treatment trials: This trial tests the experimental treatments or a new combination of drugs.

2. Prevention trials: This trial looks for ways to prevent a disease or prevent it from recuring.

3. Diagnostic trials: This trial finds better tests or procedures for diagnosing a disease.

4. Screening trials: This trial tests methods of detecting disease.

5. Quality of life trial: This study looks into ways to improve the comfort and quality of life for those who have a chronic illness[12]

III. PRE-CLINICAL STUDIES

Preclinical development, also known as nonclinical studies, is a stage of drug development that occurs before clinical trials (human testing) and collects essential feasibility, iterative testing, and drug safety data, generally in laboratory animals.[9]Preclinical studies are used to determine a starting, safe dose for first-in-human studies and to assess the product's potential toxicity, which often includes new medical equipment, prescription medications, and diagnostics.[8]

The pre-clinical development includes developing a method of large-scale synthesis, animal safety studies, carcinogenicity tests, drug delivery, elimination and metabolism studies , drug formulation experiments, a dose-ranging studies in animals. At this stage, wide range dosages of the potential drugs are introduced to the cell line or animal to get preliminary effectiveness and pharmacokinetic information[8]

IV. CLINICAL STUDY

• A clinical study is a research project that uses human volunteers (also known as participants) to further medical knowledge. Clinical trials (also known as interventional studies) and observational studies are the two basic forms of clinical investigations.[10]

• Clinical trials are human research studies that are used to assess a medicinal, surgical, or behavioral intervention. They are the most common technique for researchers to determine whether a novel treatment, such as a new medicine, diet, or medical equipment (such as a pacemaker), is safe and effective in humans. A clinical trial is frequently performed to determine whether a new treatment is more successful than the current treatment and/or has fewer negative side effects.[10]

• Clinical studies are often carried out in stages that build on one other. Each phase is intended to provide answers to specific questions. Knowing the clinical trial's stage is crucial since it might give you a sense of how much is known about the medicine being investigated. [12]

PHASE 0 CLINICAL TRIALS -Despite the fact that phase 0 studies are conducted in humans, they are not the same as the other stages of clinical trials. The goal of this phase is to assist the drug approval process go more quickly and smoothly. Researchers may use phase 0 trials to see if the medications do what they're supposed to do. This could save time and money that would otherwise be spent on later-phase experiments. .Phase 0 studies use only a few small doses of a new drug in a few people known as micro dosing study (11)

PHASE I CLINICAL TRIALS -Phase I studies of a new drug are usually the first that involve people. Phase I studies are done to find the highest dose of the new treatment that can be given safely without causing severe side effects. Study participants ranges from 20-100 healthy volunteers. .It determine safety and dosage.(13)

PHASE II CLINICAL TRIALS: A phase II clinical study is conducted if a new medication is found to be safe in phase I clinical trials and to see if it works in specific forms of cancer. The advantage that doctors seek is determined by the treatment's purpose. It could indicate that the cancer is shrinking or disappearing. Study participants are up to several 100 people with disease and length of study is up to several months to 2 years .Determine efficacy and side effects (14)

PHASE III CLINICAL TRIALS: Before being approved for general use, treatments that have been shown to work in phase II clinical trials must pass a third phase. Phase III clinical studies assess the novel treatment's safety and effectiveness against the current standard of care. Study participants are up to 300-3000 volunteers who have disease and length of study is 1 to 4 years.(14)

PHASE IV CLINICAL TRIALS: In phase IV studies, drugs that have been approved by the FDA are generally monitored for a long time. Even after thousands of patients have been exposed to a new therapy, not all of the treatment's side effects may be known.it is the practice of monitoring safety of drug after it has been released in the market(15)

V. INVESTIGATIONAL NEW DRUG (IND) APPLICATION

The filing of an Investigational New Drug (IND) application is the initial stage in the drug review process. The application to the US Food and Drug Administration (US FDA) for an exemption to send the product to investigators throughout the state has been submitted. To qualify for this exemption, the company must submit the required information via the IND. INDs are divided into two categories:

1. Commercial - For companies looking to have a new medicine approved for marketing.
2. Non-commercial (research) - for companies submitting Investigator IND, Emergency Use, and Treatment INDs. (17)

VI. NEW DRUG APPLICATION (NDA)

The NDA application is the formal proposal to the FDA by drug sponsors, like as biotech and pharmaceutical corporations, to authorize a new pharmaceutical for sale and marketing. Since 1983, every new drug or therapy has required approval of a New Drug Application (NDA) before being commercialized in the United States.(18)

The NDA documentation is expected to detail the drug's entire history, such as what happened during clinical trials, what the medicine's ingredients are, the conclusions of animal research, how the drug acts in the body, and how it is produced, processed, and packed.(17)

Once the FDA has reviewed the NDA, it issues one of the below mentioned three action letters:

- Approval Letter – This letter confirms that the drug has been approved.
- Approvable Letter – This letter shows that the drug will be authorized eventually, but that it will need to be corrected due to a few flaws such as labelling revisions.
- Not Approvable Letter - Indicates that the medicine will not be approved and provides a list of reasons why. (17)

Objectives of NDA

- Whether the medicine's proposed labelling (package insert) is acceptable and what it should include
- Whether the drug is safe and effective in its proposed usage, and whether the drug's advantages exceed the hazards
- Whether the production procedures and quality control measures employed to maintain the drug's identity, strength, quality, and purity are sufficient to maintain the drug's identity, strength, quality, and purity. (16)

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Yashoda Technical Campus
Satara

VII. TIME REQUIRED FOR DEVELOPING NEW DRUG

Drug development takes a long time since a candidate drug is reviewed by regulatory authorities in numerous countries at every step of development before being released on the market. A new drug can take anywhere from 12 to 15 years to create, according to PhRMA (Pharmaceutical Research and Manufacturers of America, a pharma industry trade organization in the Americas). Preclinical testing takes roughly six and a half years. Phase-I trials last around 1.5 years; Phase-2 trials last about 2 years; Phase-3 trials last about 3.5 years; and regulatory body assessment and approval takes about 1.5 years. Once the prospective drug has been approved for use as a medication. It may be subjected to additional Phase-IV trials to gather more safety and effectiveness information.(17)

VIII. DRUG DEVELOPMENT COST ACCORDING TO STUDIES

The cost of medication development has been estimated to be anywhere from $314 million and $2.8 billion, according to the report. Olivier Wouters, an assistant professor of Medicine Policy at the London School of Economics and Political Science, Martin McKee, a professor of European Public Health at the London School of Hygiene & Tropical Medicine, and Jeroen Luyten, an associate professor of the Faculty of Medicine at the Leuben Institute for Healthcare Policy's Department of Public Health and Primary Care, were the authors. (16)

Drug development costs for therapeutic domains with five or more medications ranges from $765.9 million for central nervous system treatments to $2.7716 billion for cancer and immunomodulating drugs, according to the median estimates.(16)

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A Review of the Preparation of Regulatory Dossiers in CTD Format and ECTD Submissions

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ABSTRACT:
The information needed to prepare dossiers for multiple countries is discussed in this article, as well as the CTD format, which is projected to considerably decrease the time and resources needed by the industry to compile global registration applications and reports. The International Conference on Harmonization of the electronic common technical document (eCTD) aims to revolutionize the pharmaceutical submission procedure. In comparison to paper submissions volume, more than three-quarters of individuals with eCTD expertise were able to shorten their total time to approval, and more than 90% of this group was able to demonstrate cost savings.

KEYWORDS: Regulatory,CTD, eCTD, Dossier, ICH.

I. INTRODUCTION:

Regulatory Dossier

Dossier: In English, a dossier is a collection or file of materials about a specific subject, particularly one containing thorough information about a person or a topic. Any formulation is designed for human use, i.e., to alter or investigate physiological processes. [1]

"Pharmaceutical" refers to the use of systems or pathological conditions for the benefit of the recipient. "A product for human consumption." Critiquing and evaluating pharmaceutical dossiers is a process. Administrative, chemistry, preclinical, and clinical data are all included in this product. Information and authorization issued by a country's regulatory agencies to "Marketing approval or Registration" is a term used to describe the process of supporting a product's marketing or approval in a country." Product License" or "Marketing Authorization". [2]

A dossier is a file document that is submitted for drug product approval in several regulatory jurisdictions based on their requirements. CTD is a harmonized format (template) for presenting data in the ICH regions, and it is submitted in many ways such as CTD, and e-CTD.

A dossier is a collection of documents that provide in-depth information about a specific person or subject. (Or) a collection of papers relating to a subject or a person. (Or) A dossier is a file document that contains detailed information about a drug product and is submitted to the regulatory authorities. [3]

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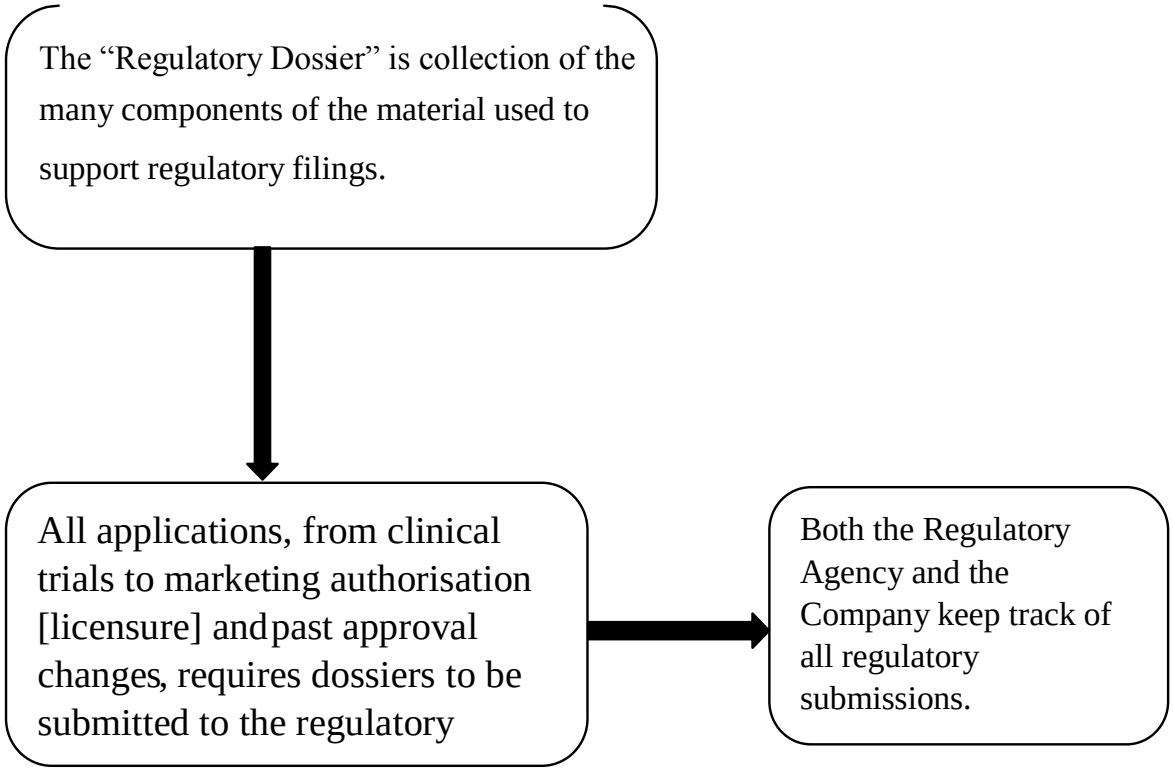

Figure 1: Regulatory Dossier Preparation.

COMMON TECHNICAL DOCUMENT (CTD):

A Common Technical Document (CTD) is a supporting list of leaflets that must be given to the regulatory body with pharmaceutical registration applications to obtain market authorization. CTD mostly describes the data format. It is customary for RA professionals to be aware of the documentation that must be provided when a medication product is approved. CTD, on the other hand, is primarily concerned with the orderly structure of information. CTD documents should be simple, straightforward, and transparent. [4]

CTD is an ICH-defined format that has been agreed upon and accepted by regulatory agencies in Europe, Japan, and the United States. The FDA defines the CTD as an information package containing clinical, non-clinical, manufacturing, and technical data that would be submitted for registration of novel pharmaceuticals in all three ICH regions, namely the United States, the European Union, and Japan. [8] Paper submission of ACTD and CTD format dossiers, as well as electronic submission of CD format dossiers, are used in semi-regulated markets such as ASEAN countries (Circle disk). [6]

(See Fig. 2 for a diagram of the CTD triangle describing the various modules.) As a result, has it five modules. [4]

1. Administrative and prescribing information (Module 1).
2. Common Technical Document (Module 2) Summaries (Quality Overall summary)
3. Module 3: Data of High Quality
4. Non-clinical study reports (Module 4)
5. Clinical Study Reports (Module 5) [13]

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CTD STRUCTURE:

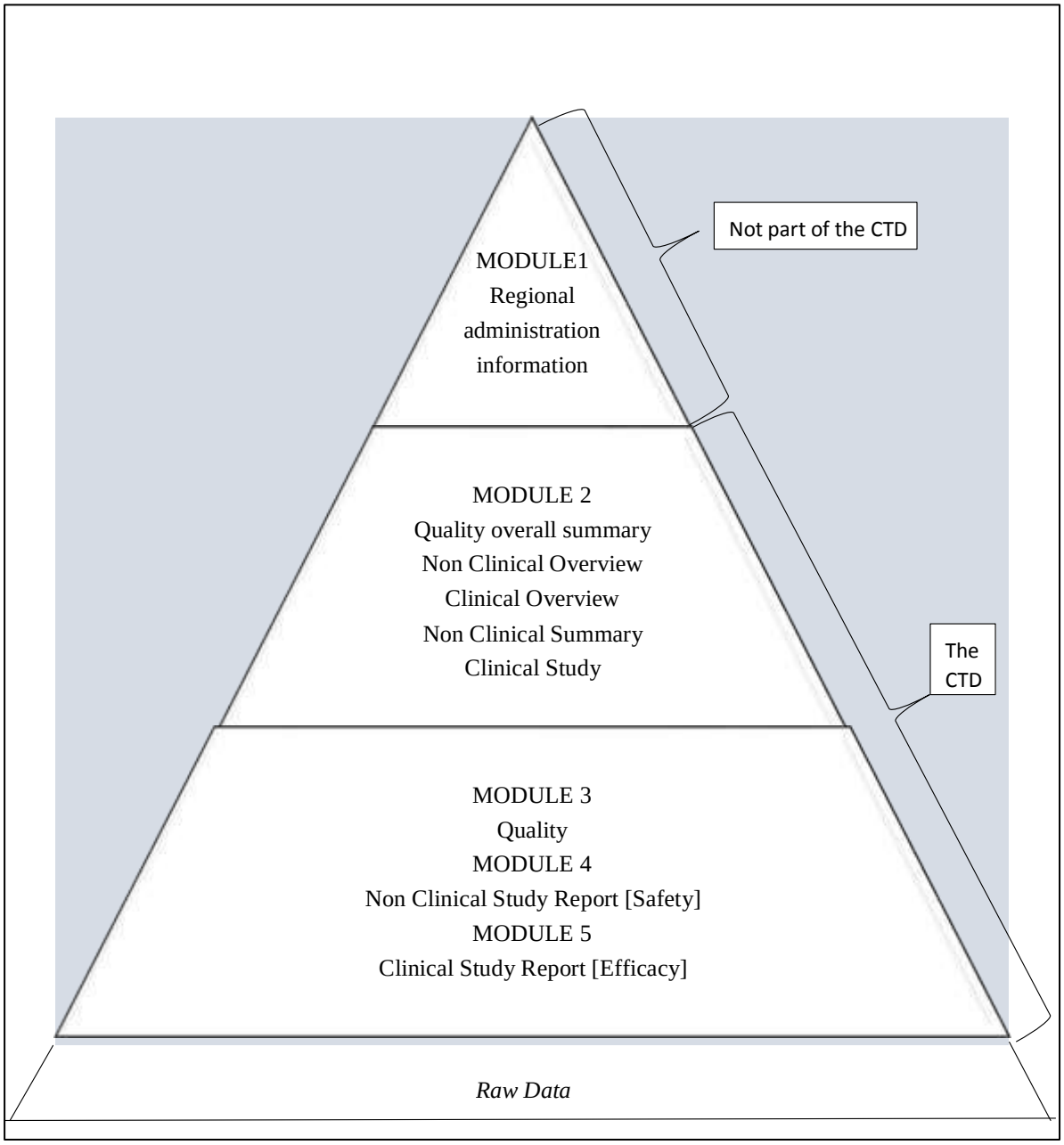

Figure 2: Triagonal Representation of Dossier Preparation

Modules are divided into two categories:
First Regional module
Only the content of the shared modules is defined by the CTD. Each of the ICH regions defines the contents of Regional module1. (USA, Europe, Japan). [14]

ORGANISATION OF CTD:
The Common Technical Document is organized into five modules.

Module 1: Administrative Data.
Administrative information should include papers particular to each region, such as application forms or the proposed regional designation. [5]

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Module 2: Overall Quality Summary
CTD Synopsis Begin with a general overview of the drug (pharmacological class, mechanism of action, and intended clinical usage).
It begins with a general overview of the medicine, including its pharmacological class, mechanism of action, and potential clinical applications. Information (for example, pharmaceutical documentation), as well as the Non-Clinical and Clinical Overviews, NonClinical Written Summaries and Tabulated Summaries, and the Clinical Summary.[7]
Module 2 is divided into seven sections, which should be kept in the following order:
2.1Table of contents. 2.2 The Beginning
2.3 Overall Quality Summary. 2.4 Overview of Non-Clinical Research
2.5 Overview of Clinical Practice. 2.6 Non-clinical Summaries (Written and Tabulated)
2.7 Clinical synopsis
Module 3: Quality Assurance
The M4Q's Quality component establishes a standardized structure and method for delivering CMC (Chemistry, Manufacturing, and Controls) data in a registration dossier. The following are the primary headings in this section (which must not be changed):[5]
3.1 Module 3 Table of Contents
3.2 The data set
Drug Substance 3.2.S
Drug Product 3.2.P
3.3 Module 3 literature references.[11]

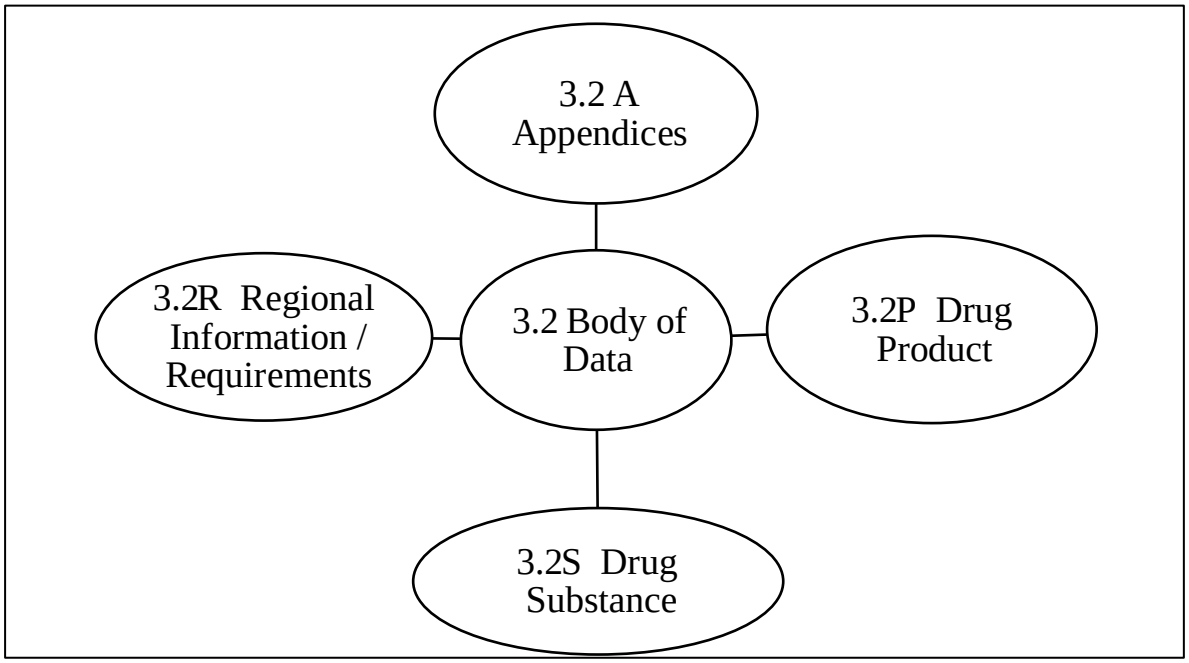

Figure 3: Dossier preparation in the CTD format.

Module 4: Reports on nonclinical and preclinical research
The CTD Safety (M4S) Guideline defines the nonclinical study's structure and format. Module 2 of the Common Technical Document summarises the information in Module 3 of the Common Technical Document and organizes Module 4 of the Nonclinical Study Reports.[16] The Nonclinical Overview should be no more than 30 pages long and should provide an integrated and critical assessment of the pharmaceutical's pharmacologic, pharmacokinetic, and toxicological examination. Nonclinical Written Summaries (100–150 pages) are indicated for more comprehensive summaries and discussions of nonclinical pharmacology,
pharmacokinetics, and toxicological information.[6]
4.1 Module 4 Table of Contents
4.2 Reports on research Pharmacology (section 4.2.1) [11]
4.2.2 Pharmacokinetics is a term that refers to the study of how drug toxicology (section 4.2.3)

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Module 4 uses 4.3 literature references. [5]

Module 5: Clinical Study Reports

The organization and format of clinical data in an application, including summaries and comprehensive study reports, is described by CTD-Efficacy (M4E). The Clinical Overview, a short document that gives a critical review of the clinical data, and the Clinical Summary, a larger document that focuses on data summary and integration, are both included in Module 2 of the CTD. Module 5 contains clinical study reports as well as raw data. [11]

The following are the primary headings in this section (which must not be changed):
5.1 Module 5 Table of Contents
5.2 A list of all clinical studies in a tabular format
5.3 Reports on clinical trials
5.3.1 Biopharmaceutical study reports
5.3.2 Reports on experiments involving human biomaterials and pharmacokinetics.
5.3.3 Human pharmacokinetic (PK) studies reports
5.3.4 Human pharmacodynamics reports (PD) research
5.3.5 Efficacy and safety study reports
5.3.6 Post-marketing experience reports
5.3.7 Individual patient listings and case report forms
5.4 Literature citations. [5]

ADVANTAGES OF CTD:

1. The main goal of implementing a common submission format is to make reviewing each application easier and to avoid other critical data or analysis missions. Omissions of this information can cause approvals to be delayed unnecessarily.
2. A common format for technical documentation will significantly reduce the time and resources required to compile applications for human pharmaceutical registration, as well as make electronic submission preparation easier. [6]
3. A standard document with common elements will be used to facilitate regulatory reviews and communication with the applicant.
4. The implementation of CTD is expected to significantly reduce the amount of time and resources required by the industry to compile global registration applications. [10]

CTD'S SILENT BENEFITS:

1. Global application harmonisation.
2. Establishes guidelines for preparing submission-ready documents during the IND stages.
3. Standardization makes project management and data management easier.
4. Makes life cycle management easier.
5. Aids in the planning of drug development. [10]

ELECTRONIC SUBMISSIONS (eCTD):

The electronic submission equivalent of the CTD is the eCTD. The eCTD serves as a conduit between industry and government agencies for the exchange of regulatory data, facilitating the development, review, lifecycle management, and archiving of electronic submissions. All CTD information is included in the eCTD submissions. The structure of the submission is represented by an XML file (Extensible Mark-up Language) at the heart of eCTD. It contains links to files as well as other metadata such as checksum data. The XML scheme is extremely rigorous. CTD submission, all subsequent submissions for the application should be in eCTD format. The submission's lifecycle management is simplified using eCTD. [7]
The electronic Common Technical Document (eCTD) is a regulatory information transfer link between the pharmaceutical industry and regulatory agencies. The Common Technical Document (CTD) format is used for the main content. The Multidisciplinary Group 2 Expert Working Group (ICH M2 EWG) of the International Conference on Harmonisation (ICH) produced it. [15] Essentially, the electronic Common Technical Document (eCTD) will be a transport format that will allow electronic submissions to be moved into an agency's review environment. The eCTD will act as an interface for the flow of regulatory information from industry to agencies, while also making the production, evaluation, lifecycle management, and archiving of electronic submissions easier. [17]
An eCTD application is a CTD application, but then electronically.

Electronically means for eCTD:

I complete the dossier in electronic format I XML files (XML backbone)
I Specifications followed for the Granularity, folder- & filename convention of the dossier Navigation through the dossier using hyperlinks and bookmarks. [9]

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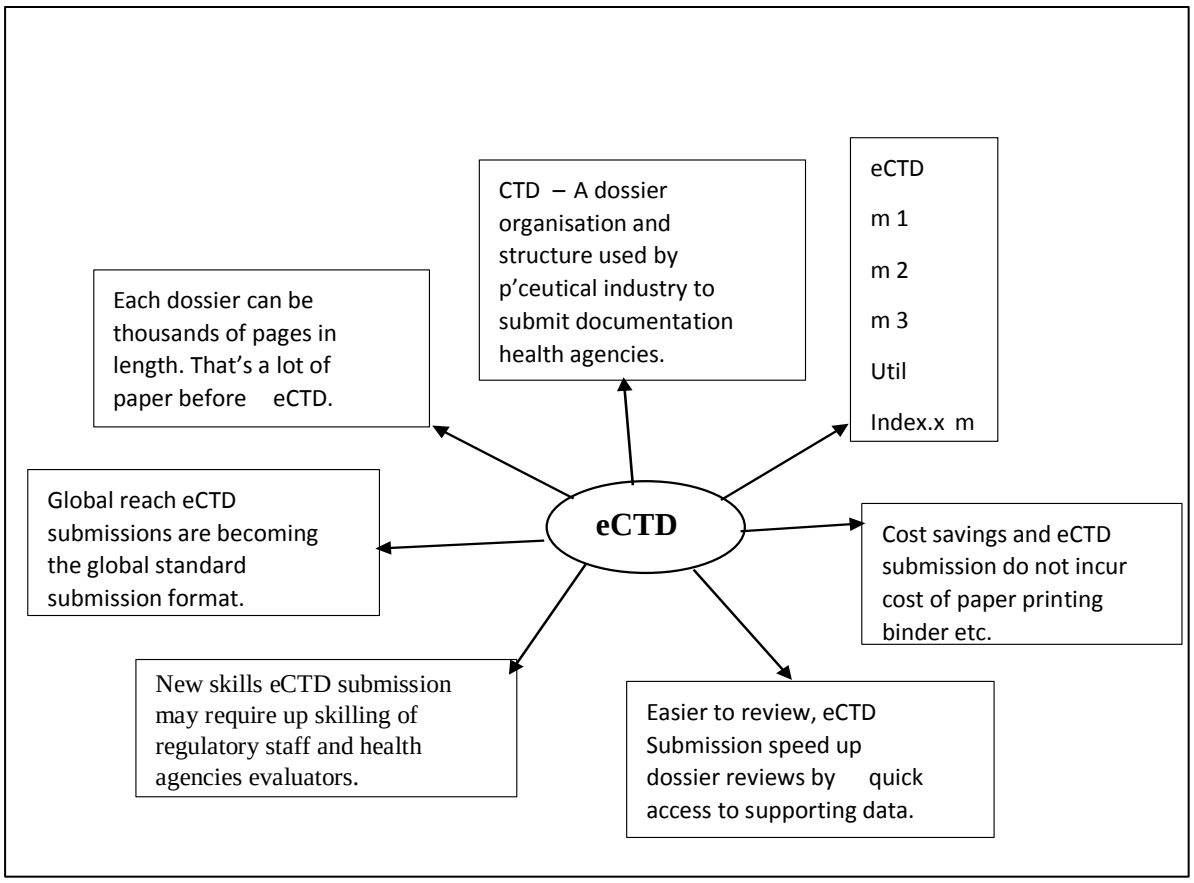

Figure 4: Overview of eCTD Submission.

eCTD Submission Checklist:
eCTD Software
Software training and support from the supplier
Compiling and eCTD

eCTD hyperlinking
QC of eCTD

Submit eCTD on CD/DVD or Use an electronic gateway [2] **eCTD STRUCTURE:**

eCTD is highly recommended by USFDA for NDAS, BLAS, DMFS, and INDs filing. From the year 2010 European Union also make compulsory for electronic CTD submission to all procedures. [2]

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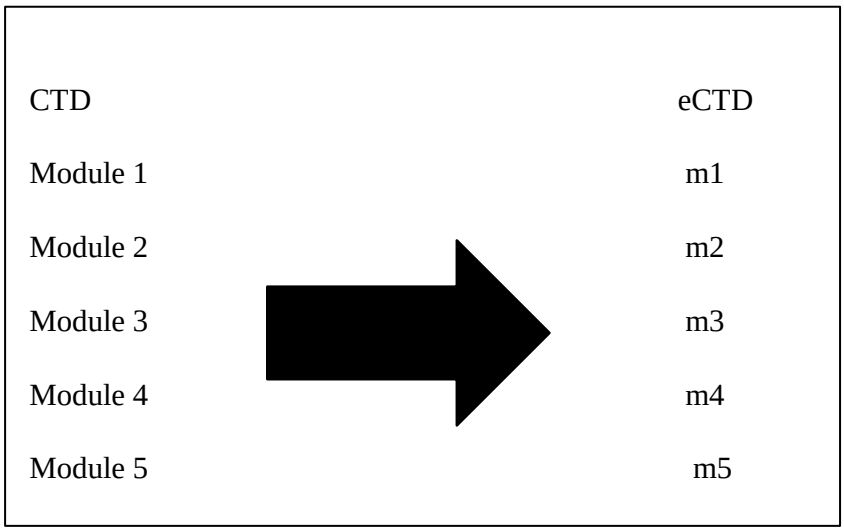

Figure 5: Electronic Submission to all Procedures.

eCTD ADVANTAGES:

The eCTD dossier becomes the single authoritative regulatory archive, thus reducing the use and costs associated with producing and storing paper dossiers. Enhanced ability to organize, prepare, and manage submission content.

Opportunity for streamlined interactions with agency reviewers, decreased response times to agency requests, and ultimately, a faster approval timeline.

Facilitates collaboration between teams of document authors, reviewers, publishers, and external partners. [6]

There are five modules in eCTD as mentioned here:

1. Region-specific information.
2. Summary documents.
3. Information related to quality.
4. Non-clinical study reports.
5. Clinical study reports (CSRs).

Comparison OF CTD and eCTD:

Sr. No.	**Paper CTD [Common Technical Document]**	**eCTD [Electronic Common Technical Document]**
1.	Volumes, tabs, and slip sheets were entered electronically and then printed on paper.	Electronically filed with e-documents in folders.
2.	A4 paper must be used.	A4 or US letter size documents are acceptable.
3.	TOCs and volume are used to navigate the CTD.	XML backbone for eCTD navigation.

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4.	The target CTD section number is included in the cross-reference.	The target is linked to the cross-reference.
5.	TOCs, page numbers, and caption crossreferences are used to navigate the document manually.	TOCs, bookmarks, and hyperlinks are used to navigate electronic documents.
6.	Trucks delivered binders in boxes on pallets.	CD (or DVD) or email portal submissions are accepted.

Table 1.CTD and eCTD Statements in Comparison.

eCTD submissions are accepted for the following applications:

1. Investigational New Drugs (INDs).
2. New Drug Applications (NDAs).
3. Abbreviated New Drug Applications (ANDAs).
4. Biologics License Applications (BLAs).
5. All the applications following submission of the above-stated applications.
6. All the Master Files (MFs) are part of any above-mentioned applications.

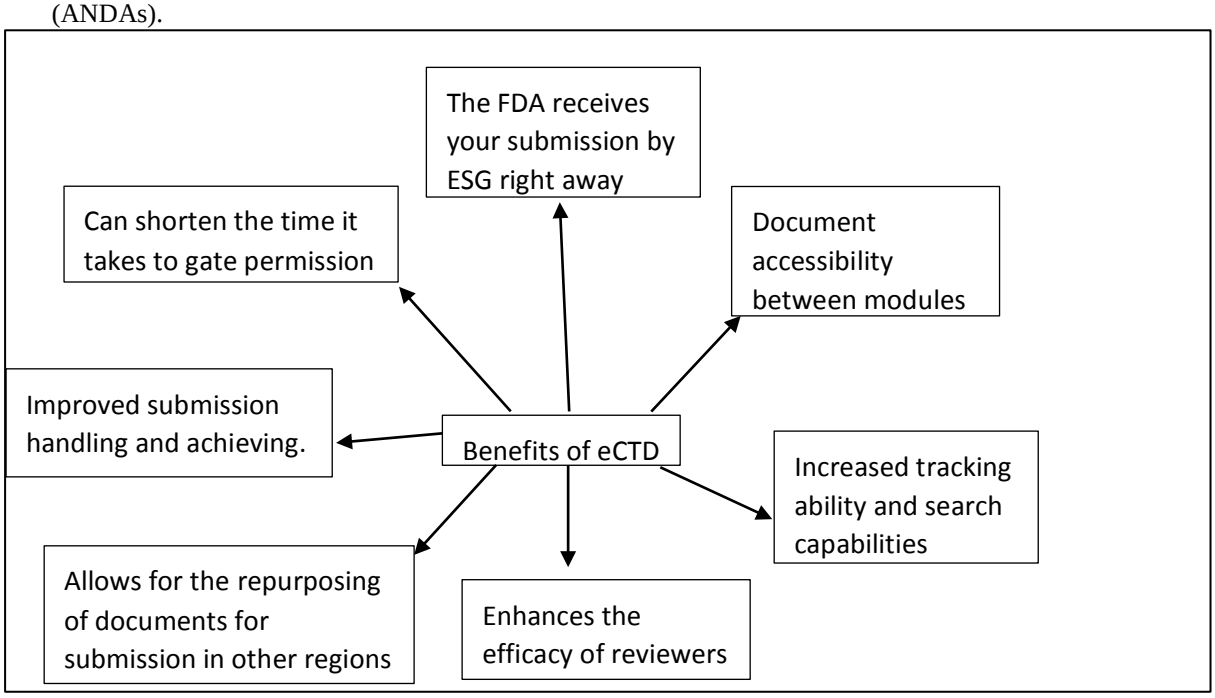

Figure 5: Overview of Benefits of eCTD

Content specification– as defined by ICH specified below-

1. Technical specification- Electronic software
2. CTD -TOC [pdf] [paper]
3. eCTD- XML Backbone

The eCTD is an electronic document similar to the CTD. Is an eCTD backbone describing the Structure of the submission, the XML file (Extensible Mark-up Language) includes links

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to files and other metadata such as checksum information.

1. The schema for the XML is very rigid.
2. Easy to distribute and review.
3. More efficient use of resources with less cost and stress to the organization.
4. Self-validating.

The eCTD Requirements:

You must submit electronic submission using the FDA's current supported version of eCTD.
The current version of eCTD, that is supported is listed in the Data standards Catalog and further explained in the technical specification document below.
Electronic Common Technical Document Specification of the International Council for Harmonisation (ICH). Study tagging files ICH eCTD Backbone file specification. [15] **Software used in eCTD management:**[9]

Sr. No	Software
1.	ECTDXPress-Image solution-http://www.imagesolutions.com.
2.	ECTDXPress-Image solution-http://www.imagesolutions.com.
3.	Data farm, http://www.datafarminc.com.
4.	Take solution: www.PharmaReady.com.
5.	MasterControlsubmissionGateway™-MasterControl, http://www.mastersolution.com.
6.	Lorenz Life Science: www.lorenz.com.

Table 2. Software used in eCTD Submission.

Things to know before using eCTD software
eCTD knowledge
General eCTD tool knowledge
Document format types and editing
Software functions and system requirements
FDA ESG submission requirements
FDA Guidance Submission format delivery

How to send it to Authorities?
Files on a CD or DVD
Files attached to an e-mail
Files submitted via Eudralink
Files submitted via CESP 1 File submitted via a Gateway. [2]

II. CONCLUSION:

Any export market requires a high-quality dossier, which may be created via a methodical Formulation Development process. The right planning and execution of Formulation development will aid in the production of high-quality dossiers and the response to regulatory bodies' questions. It is critical to assemble documentation in a format that is acceptable internationally for both regulated and non-regulated markets when registering pharmaceutical products in any of the exporting countries. Due to significant discrepancies in the requirements for dossier registration for pharmaceutical products, the CTD and eCTD formats were developed. This aids in the compilation of documents in the above-mentioned format as per the registering requirements.
According to the thesis, the way of submitting a Dossier, according to CTD and eCTD format, Module 1- contains Administrative Information, Module 2- contains the Overall summary, and Module 3- contains the Quality Information. In

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summary, Module 4 contains preclinical data, while Module 5 contains clinical data.

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Comprehensive Review On Gmp Of Pharmaceutical Products

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Abstract: The concepts of GMP are not new, it is from ancient times. The concept required for GMP are explained in this report. The main purpose of GMP is preventing mistakes and errors involved in any manufacturing activities. To achieve agreement of guidelines and laws of the manufacturing of medical products for human use there are some public and also private organizations institutes and regulatory authorities who work and cooperate with pharmaceutical industry.

GMP guidelines provide minimum requirements for pharmaceutical or a food product manufacturer must meet to assure that the products are of high quality and do not pose any risk to the consumer or public. Good manufacturing practices (GMP) is a part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

Index Terms **- GMP, Manufacturing activities, Guidelines, Quality assurance, Quality Standards.**

I. INTRODUCTION

The concept of Good Manufacturing Processes was introduced to regulate packaging and manufacturing processes in the pharmaceutical industrial areas.[1]

Manufactures follows various procedures and principles for the therapeutic good which helps in ensures the required quality of products. Good manufacturing practices is the component of quality assurance which helps in ensure the ensure the products are consistently manufactured and controlled to the Quality Standards appropriate to their intended use.

Good manufacturing practices is mainly used to reduce risk involved in production of pharmaceuticals products that cannot be removed through testing of the final products. Good Manufacturing practices covers all views of production from initiating materials, equipment's, premises to personal hygiene and training of employees.

A basic principle of Good Manufacturing Practices is that quality cannot be tested into a batch of product but must be built into each batch of product during all stages of the manufacturing process. It is designed to minimize risk involved in any pharmaceutical production that cannot be eliminated through testing the final product.

Good Manufacturing Practices:

The quality of formulation and bulk drug depends on the quality of those producing it.

Good manufacturing practices is the magic key that opens the door quality

In matter of GMP swim with the current and in matter of quality stand like a rock

Most countries will only accept import and sale of medicine that have been manufactured to internationally recognized GMP

Government Seeking to promote their countries export of pharmaceuticals can do so by making GMP mandatory for all pharmaceutical production and by training their inspectors in GMP requirements. [2,3]

Why GMP is important:

- A poor-quality medicine may contain toxic substance that has been unintentionally added.
- A medicine that contains little or none of the claimed ingredients will not have the intended therapeutic effect. Provides a high-level assurance that medicines are manufactured in a way that ensures their safety efficiency and quality.
- It maintains the consistency in the manufacturing of the medical products.
- To eliminate contamination and to minimise the error GMP is important.

- Good Manufacturing Practices ensures companies execute consistent procedures in safe environments.

- Good Manufacturing Practices helps in ensure the proper design, monitoring and control of manufacturing process and facilities, while securing the identity, strength, quality of their products.

- Good Manufacturing Practices assist cutdown on facility losses and waste and also to protect consumers the manufacturer from harm. [3,4]

How to Comply with Guidelines:

GMP guidelines and regulations address different issues that can influence the safety and quality of a product. Meeting GMP or cGMP standards helps the organization comply with legislative orders, increase the quality of their products, improve customer satisfaction, increase sales, and earn a profitable return of investment. [5,6]

Conducting GMP audits play a big part in assessing the compliance of the organization to manufacturing protocols and guidelines. Performing regular checks can minimize the risk of adulteration and misbrand. A GMP audit helps improve the overall performance of different systems including the following:

- Building and facilities
- Materials management
- Quality control systems
- Manufacturing
- Packaging and identification labelling
- Quality management systems
- Personnel and GMP training
- Purchasing
- Customer service

Good manufacturing practice - the general/current state:

- **Pharmaceutical quality system:**

This guideline describes a comprehensive model for an effectiveness quality system of medicinal products, based on the concepts of ISO quality and its implementation throughout all stages of the lifecycle of the product.

The guideline applies to supporting the development and manufacture of substances of Pharmaceutical Industry, Active Pharmaceutical Ingredient and medicinal products, including biotechnology and biological products throughout the life cycle of the product.

Quality assurance is a broad concept that includes all matters that individually or collectively influence the quality of a product, that is, management of the quality of raw materials, products and other components, services related to production, and management, production and inspection processes. It is applied in pre-production to verify what will be made meets specifications and requirements and also while manufacturing production. [7,8]

Personnel:

According to GMP, the management of an enterprise should determine and provide appropriate resources such as human resources, financial, materials, facilities and equipment to implement and maintain the Quality Management System and improve effectiveness. Effective coordination and management of human resources are key factors in the proper functioning of any enterprise system and improve effectiveness.

For the maintenance of satisfactory system of quality assurance and the correct manufacture and control pharmaceutical products there must be sufficient qualified personnel to carry out all the tasks for which manufacturer is responsible.

Personnel should be aware of principles of GPM that affects them and receive initial and continuing training, including hygiene instructions, relevant to their need. [9,10]

Premises and equipment:

Premises and equipment must meet and comply with all rules, according to the operations to be performed in order to minimize the risk of errors and should allow effective cleaning and maintenance. [11,12]

Some examples are:

a. Walls: Walls in manufacturing areas, packaging areas and corridors should be of plaster finish on high-quality concrete blocks or gypsum board. The finish should be smooth, usually with enamel or epoxy paint. They should be washable and able to resist repeated applications of cleaning and disinfecting agents.

b. Floors: Floor covering should be selected for durability as well as for clean ability and resistance to the chemicals with which it is likely to come into contact. Epoxy flooring provides a durable and readily cleanable surface.

c. Ceilings: Manufacturing areas require a smooth finish, often of seamless plaster or gypsum board. All ceiling fixtures such as light fittings, air outlets and returns should be designed to assure ease of cleaning and to minimize the potential for accumulation of dust.

Raw Material:

All materials used for production should be stored properly according to the appropriate conditions which are set by the manufacturers.

There should be a proper stock management system implemented to ensure that all incoming materials are correct and of high quality. [13]

Documentation:

Good documentation constitutes an essential part of the quality assurance system and it is the key to operate in compliance with GMP requirements.

All types of documents and media used should be fully defined in the manufacturer's Quality Management System.

Given below is a list of the most common types of documents along with a brief description of each [14,15]

Site Master File: A document describing the GMP related activities of the manufacturer.

Quality Manual: A global company document that describes, in paragraph form, the regulations and/or parts of the regulations that the company is required to follow.

Policies: Documents that describe in general terms, and not with step-by-step instructions, how specific GMP aspects (such as security, documentation, health, and responsibilities) will be implemented.

Logbooks: Logbooks are used for documenting the operation, maintenance, and calibration of a piece of equipment.

Logbooks are also used to record critical activities, e.g., monitoring of clean rooms, solution preparation, recording of deviation, change controls and its corrective action assignment.

Test Methods: These documents are typically used and completed by the quality control (QC) department. Test methods provide step-by-step instructions for testing supplies, materials, products, and other production-related tasks and activities, e.g., environmental monitoring of the GMP facility.[16]

Production:

Production operations must clearly follow the procedures. They must comply with the principles of GM Pin order to obtain quality products and be in accordance with the relevant manufacturing.

All handling of materials and products, such as reception and quarantine, sampling, storage, labelling, dispensing, processing, packaging and distribution should be done in accordance with written procedures or instructions and where necessary, recorded.[17]

Quality control:

Quality control is concerned with sampling, specifications and testing as well as the organization, documentation and release procedures which ensure that the required and relevant tests are carried out, and that materials are not released for use, nor products released for sale or supply, until their quality has been judged satisfactory.

QC is not confined to laboratory operations, but may be involved in many decisions concerning the quality of the product.[18]

Quality risk management

Quality risk management is a systematic process of assessing risks that can affect the quality of the product. According to its principles, quality risk management should ensure that:

The evaluation of the risk to quality is based on scientific knowledge, experience with the process and ultimately links to the protection of the patient and users;

The level of effort, formality, and documentation of the quality risk management process is commensurate with the level of risk.

c) The general quality risk management process and integration into the product quality can be referred to in ICHQ9. [19,20]

Validation and qualification

Qualify systems, premises, and equipment if they are fit/ready for their intended use and validate if processes and procedures can repeatedly produce high-quality products. Critical steps in the manufacturing process should be verified to ensure that product quality is consistent and maintained at a high level. According to the WHO (World Health Organization), qualification and validation should establish and provide documentation stating that:

the premises, supporting utilities, equipment, and processes have been designed in accordance with the requirements for GMP (design qualification or DQ)

the premises, supporting utilities, and equipment have been built and installed in compliance with their design specifications (installation qualification or IQ);

the premises, supporting utilities, and equipment operate in accordance with their design specifications (operational qualification or OQ); and a specific process will consistently produce a product meeting its predetermined specifications and quality attributes (process validation or PV, also called performance qualification or PQ) [21,22.23]

Self-inspection:

The objectives of self-inspections are the evaluation and supervision of compliance of the manufacturer with GMP in all aspects of production and quality control. It must be designed to detect any deficiency in the implementation of GMP and to recommend corrective procedures.[24]

Sanitation And Hygiene: -

A high level of sanitation and hygiene should be practiced in every aspect of the manufacture of medicine products. The scope of sanitation and hygiene covers personnel, premises, equipment and apparatus, production materials and containers, products for cleaning and disinfection, and anything that could become a source of contamination to the product. Potential sources of contamination should be eliminated through an integrated comprehensive programmed of sanitation and hygiene

The areas, surfaces, and equipment in and on which products are made must be kept clean. Dirt, and the microbes that it can harbor, must not get into or on products. Disinfectants can be inactivated by dirt. Dirt (particularly oily or greasy films and protein like matter) can also protect microorganisms against the action of disinfectants. So, before disinfection, it is important to first clean surfaces. Where gross amounts of dirt are present, it may be necessary to first remove most of it by scrubbing. Then surfaces may be cleaned by the application of a cleaning agent, followed by rinsing. [24,25,26]

Basic requirements for active substances used as starting materials:

This guideline is intended to provide guidance regarding GMP for the manufacture of active substances under an appropriate system for managing quality. It is also intended to ensure that active substances meet the requirements for quality and purity that they purport or are represented to possess.

These guidelines apply to the manufacture of active substances for medicinal products for human use and to the manufacture of sterile active substances only up to the point immediately prior to the active substance being rendered sterile.[27]

Manufacture of medicinal products-

Manufacture of solid and semi-solid medicinal product:

Since this type of medicinal products is particularly susceptible to microbial contaminants and other contaminants during manufacturing, it is necessary to follow preventive procedures and it should be apriority for the manufacturer MA holder.[28]

Manufacture of herbal medicinal product:

The procedures and techniques used in the manufacture and quality control of herbal medicines are often substantially different from those used for conventional medicinal products. The herbal substance should be of suitable quality. The supporting data should be provided to the manufacturer of the herbal medicinal products. These guidelines apply to all herbal starting materials: Medicinal plants, herbal substances or herbal preparations. These guidelines apply to all herbal starting materials: Medicinal plants, herbal substances or herbal preparations.[29]

Manufacture of biological active substances and medicinal products for human use:

The methods employed in the manufacture of biological active substances and biological medicinal products for human use are critical factors in shaping the appropriate regulatory control, because the manufacture of these involves certain specific, considerations arising from the nature of products and manufacturing processes, being necessary take some special precautions. Unlike conventional medicinal products, which are normally produced and controlled using reproducible chemical and physical techniques, biological products are manufactured through methods that involve biological processes and materials, such as cultivation cells or extraction of material from living organisms.[30]

Manufacture of sterile medicinal product:

The manufacture of sterile products requires special requirements in order to minimize risks of microbiological contamination, and of particulate and pyrogen contamination, being highly dependent on knowledge, training and attitudes of the personnel involved. This type of manufacture must strictly follow methods and preparation processes, carefully established and validated, since the quality assurance, is of particular importance.[31]

sampling of starting and packaging material:

Sampling is an operation where a small fraction of the batch is removed integrating operations to select a portion of a pharmaceutical product for a specific purpose, in accordance with an appropriate procedure. This process should be carried out in accordance with written and approved procedures that are appropriate to the sample and the type of control intended to be applied to the sample and the sample material.[32]

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Drug Regulatory Affairs - Role of Regulatory Affairs in the Pharmaceutical Industry

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ABSTRACT

Pharmaceutical drug regulatory affairs govern the registration parameters of pharmaceutical products. It has an extensive spectrum protecting all factors of documentation and advertising and marketing in legalized form. The pharmaceutical enterprise is a distinctly regulated industry in our country. Regulatory affairs gurus want the current market state of affairs to cater to hyperlink pharmaceutical industries and global regulatory agencies. Regulatory affairs (RA), is an occupation inside synchronized several industries, such as pharmaceuticals, clinical gadgets, and biotechnological industries. Regulatory Affairs additionally has a very precise which means inside the pharmaceutical industries. DRA is a dynamic, moneymaking subject that consists of each scientific and felony element of drug development. Regulatory affairs gurus assist the organization keeps away from issues precipitated using badly stored records, inappropriate scientific wondering, or bad presentation of data. In most product areas the place regulatory necessities are imposed, and restrictions are additionally positioned upon the claims which can be made for the product on labeling or in advertising.

INTRODUCTION

Regulatory Affairs (RA), also called Government affairs is a profession within regulated industries such as pharmaceutical, medical devices, energy, & banking. Regulatory affairs also have a very specific meaning within the healthcare industries (medical devices, pharmaceuticals, biology's functional foods). Most companies, whether or not they are the most important multinational pharmaceutical firms or small, progressive biotechnology organizations have expert departments of Regulatory Affairs professionals [1]. Nowadays the pharmaceutical industry is well organized, systemic, and compliant with international regulatory standards for the manufacturing of chemical and biological drugs for human and veterinary consumption as well as medical devices, traditional cosmetics, and herbal products. Each regulatory system had faced certain circumstances which led to the current well-defined and controlled regulatory framework this has resulted in systemic manufacturing and marketing of safe, efficacious, and qualitative drugs. With the growth of industry, the legislations from each region have become more and more complex and created a need for regulatory professionals. [2]

The regulatory professional's job is to keep track of ever-changing legislation in all the regions in which the company wishes to distribute its products they also advise on the legal and scientific control and requirements. They are responsible for the presentation of registration documents to regulatory agencies and carry out all the subsequent negotiations necessary to obtain and maintain marketing Authorization for the product they give strategic technical advice at the highest level in their companies, right from the beginning of the development of a product, making an important contribution both commercially and scientifically to the success of a development program and the company as a whole. The demand for Regulatory Affairs (RA) professionals is evident across the pharmaceutical industry, consultancy companies, clinical research organizations, and regulatory agencies. [3,4]

History of Regulatory Affairs

Modern medicine regulation started in 19th-century life sciences, especially in pharmacology, chemistry, and physiology. Which laid a foundation for modern drug development and research and started to develop successfully after the Second World War. Unfortunate events have catalyzed the development of medicines regulation more than the evaluation of knowledge base. In 1937 over 100 people died of diethylene glycol poisoning following the use of a sulfonamides elixir in the United States which used the chemical as a solvent without

any safety testing this facilitated introduction of the federal food. Drug and cosmetics act with premarket notification requirement for new drugs in 1938 However, in countries with a poor regulatory environment even recently medicines contaminated with diethylene glycol have killed patients.[5]

Figure No. 1: Thalidomide capsule.

The second incident that influenced the development of medicines regulation more than any event in history was the thalidomide disaster Thalidomide was a sedative and hypnotic that first went on sale in western Germany in 1956 between 1958 and 1960 it was introduced in 46 different countries. Worldwide resulting in an estimated 10,000 babies being born with phocomelia and other deformities. As result, the whole regulatory system was reshaped and substantially increased legislation for drug product quality, safety, and efficacy. This has resulted in stricter norms for Marketing Authorization (MA) and good manufacturing practices (GMPs).[6]

Role of Regulatory Affairs

Regulatory Affairs (RA), is professional within regulated industries Regulatory affairs also have a very specific meaning within the health care industries. The companies are responsible for the discovery, testing, manufacture, and marketing of these products and also want to ensure that they supply products that are safe and make Worth While contributing to public health and welfare. Regulatory Affairs is a professional developed from the desire of governments to protect public health by controlling the safety and efficacy of products in areas including pharmaceuticals, veterinary medicine, medical devices, pesticides, agrochemicals, and cosmetics by the companies. The main need of regulatory affairs is to provide the basis for the assurance of the high quality of food products which can increase Consumer's interest in ensuring efficacy, quality, and safety.[7]

Regulatory Affairs (RA) professionals are employed in industry, government regulatory authorities, and academics. They aim to protect public health in terms of the safety, quality, and efficacy of products like medical devices, pharmaceuticals, veterinary medicines, pesticides, cosmetics, complementary medicine, etc. The wide range of regulatory professionals includes this area. pharmaceuticals, cosmetics, medical devices, biologics and biotechnology, and in-vitro diagnostics. Regulatory Affairs are vital to the proper functioning of society and economies. Regulation protects the rights, safety, and health of citizens and ensures the safe and effective delivery of public goods and services.[8]

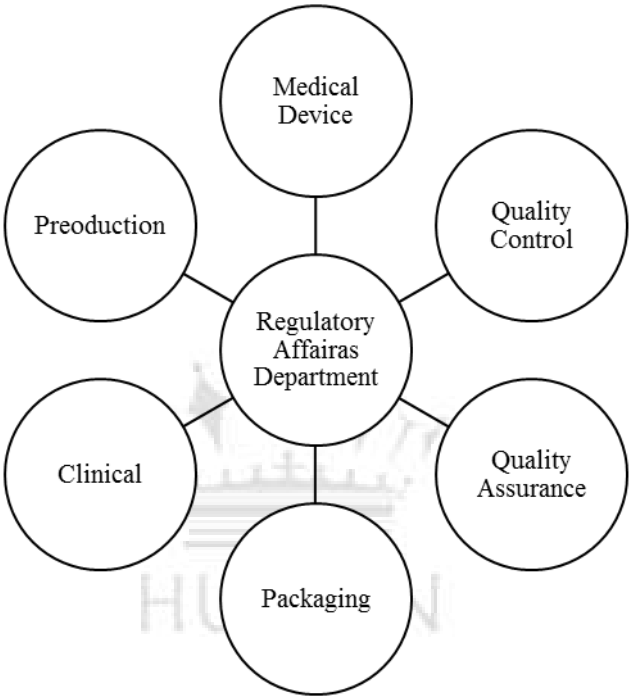


Figure No. 2: Role of Regulatory Affairs

In today's competitive environment the reduction of time taken to reach the market is critical to products and hence the Company's success. The proper conduct of its Regulatory activities is therefore of considerable economic importance for the company. A new drug might also have a value of many tens of millions of Euros or dollars, pounds, to enhance and even a three-month extend in bringing it to the market has sizeable monetary issues and even worse screw-ups to completely record all the on-hand facts or launch of the product being incorrect, labeling might also without problems result in the want for a product recall both incidences may additionally lead to the loss of various hundreds of thousands of units of sales. Not to mention the resulting reduction in confidence of the investors, health professionals, and

patients. The regulatory affairs department is very often the first point of contact between the government authorities and the company. [9,10]

The key role of Regulatory Affairs (RA) professionals is broader than registration products. They advise the company both strategically and technically at the highest level. Their role begins right from the development of the product to make, marketing, and post-marketing strategies. Their advice at all stages both in term of legal and technical requirements help companies save a lot of time and money in developing the product and marketing the same for countries that do not have their regulations the world health organization guidelines on health matters and world trade organization on trade regulations between nations is followed. [11,12]

Regulatory Bodies in the World

Every country has its very own regulatory authority which is accountable to implement the regulations and policies and problem tips for drug development, licensing, registration, manufacturing, advertising and marketing, and labeling of pharmaceutical products. The regulatory bodies are given in table 1. [13,14]

Table No. 1: Regulatory Bodies in the World

Sr no.	Country Name	Regulatory Body
1	USA	Food And Drug Administration (FDA)
2	UK	Medicine and Healthcare Products Regulatory Agency (MHRA)
3	Australia	Therapeutic Goods Administration (TGA)
4	India	Central Drug Standard Control Organization (CDSCO)
5	Canada	Health Canada
6	Europe	European Medicines Agency (EMEA)
7	Japan	Ministry Of Health, Labor Welfare (MHLW)
8	Ukraine	Ministry Of Health
9	China	State Food And Drug Administration
10	Germany	Federal Institute For Drugs and Medical Device

Pharmaceutical drug Regulatory Affairs

This branch is accountable for understanding the regulatory necessities for getting new products approved. They recognize what commitments the organization has made to the regulatory corporations in the place the product has been approved. They additionally post annual reviews and dietary supplements to the agencies. Regulatory Affairs usually communicates with one of the facilities at the FDA headquarters as an alternative to the FDA's nearby district offices. However, they need to recognize and consider adjustments to drug manufacturing and check out things to do to decide if and when the FDA have to be notified the organizations accountable for the discovery, testing, manufacture, and advertising of this merchandise additionally prefer to make certain that they grant merchandise that is protected and make a worthwhile. Contribution to public fitness and welfare. [15,16]

Drug Development Process and Clinical Trial

The innovator company synthesis a New Chemical Entity (NCE) or New Biological Entity (NBE) which can probably be a cure for a disease. The synthesis of NCE/NBE takes place in the preclinical testing period. The innovator company after the synthesis of an NCE/NBE files an Investigational New Drug (IND) application and requests the FDA to grant permission to conduct clinical trials. Clinical trials today have become one of the most important aspects of modern medical research and drug development.[17]

After studying the IND application, FDA grants permission to conduct clinical trials which involve studies in phases like phase1, phase 2, and phase 3. The innovator company files New Drug Application (NDA) and requests the FDA to grant permission to commercialize the product after studying the application, FDA grants permission to launch the new drug in the market the company continues clinical trials of the same molecule in phase 4 called product surveillance studies. Not every compound that is tested in the laboratory is marketed but before is marketed it has undergone several stages of development called drug development. The development of a new drug is a complex and costly process the cost for the development of biopharmaceuticals is higher than those specified earlier. About 10,000 NCE investigated to potentially treat disease, only 250 might make it to the animal testing and these approximately 5-10 would qualify for testing in humans, which means 1-2 of the original 10,000 NCE results in a marketable product. [18,19]

There are five important steps including many phases and stages each of them.

The five steps are:-

1) Step 1: Discovery and Development
2) Step 2: Preclinical Research
3) Step 3: Clinical Development
4) Step 4: FDA Review
5) Step 5: FDA post-market safety monitoring.

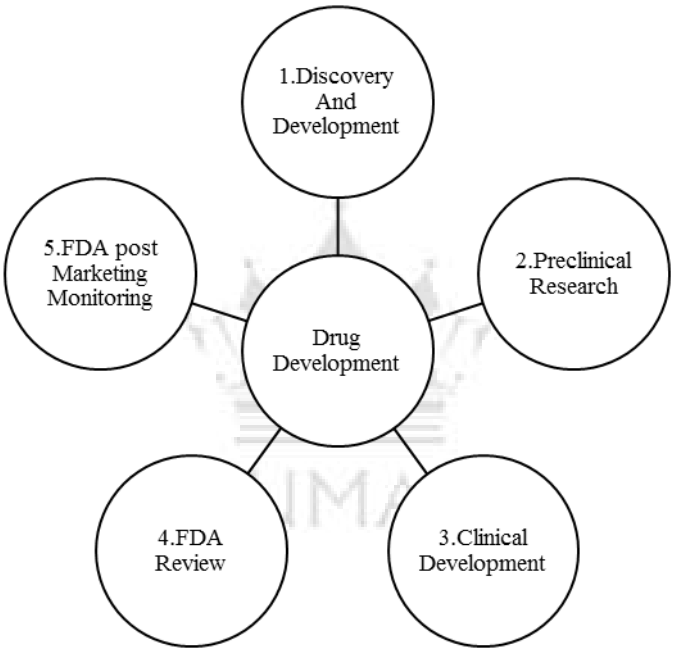


Figure No. 3: Drug Development Process

Step 1: Discovery and Development

Drug discovery efforts address a biological target that has shown to play a role in the development of the disease or starts from a molecule with interesting biological activities. The drug discovery process involves the identification of candidate drugs in their synthesis, characterization, screening, and assay for therapeutic efficacy.

Stages of Drug Discovery

1. Target Identification
2. Target Validation
3. Lead Identification
4. Lead Optimization
5. Pre-Clinical Safety
6. Clinical trials

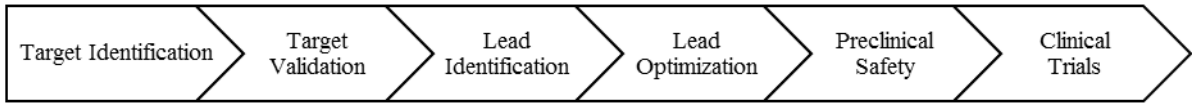


Figure No. 4: Stages of Drug Discovery

Step 2: Preclinical Research

The preclinical research step comprises studies on animals to find out various parameters for a potential drug candidate under the process of development during this stage a sponsor evaluates the drug's toxic and pharmacological effects through in-vitro and in-vivo laboratory animal testing. At the preclinical research step, the US FDA's minimum requirement is that a sponsor should develop a pharmacological profile of the drug; determine its acute toxicity in at least two species of animal and conduct short-term toxicity studies ranging from 2 weeks to 3 months, depending on the proposed duration of use of the candidate drug in the proposed clinical studies.[20]

Step 3: Clinical Development

The clinical development step involves the development of potential drug candidates comprised of pharmaceutical clinical trials which are commonly conducted in 4 phases.

Phase 0: This is an exploratory phase of a clinical trial that expedite the development of a promising drug by establishing early on whether the agent behaves in human subjects as anticipated from preclinical studies.

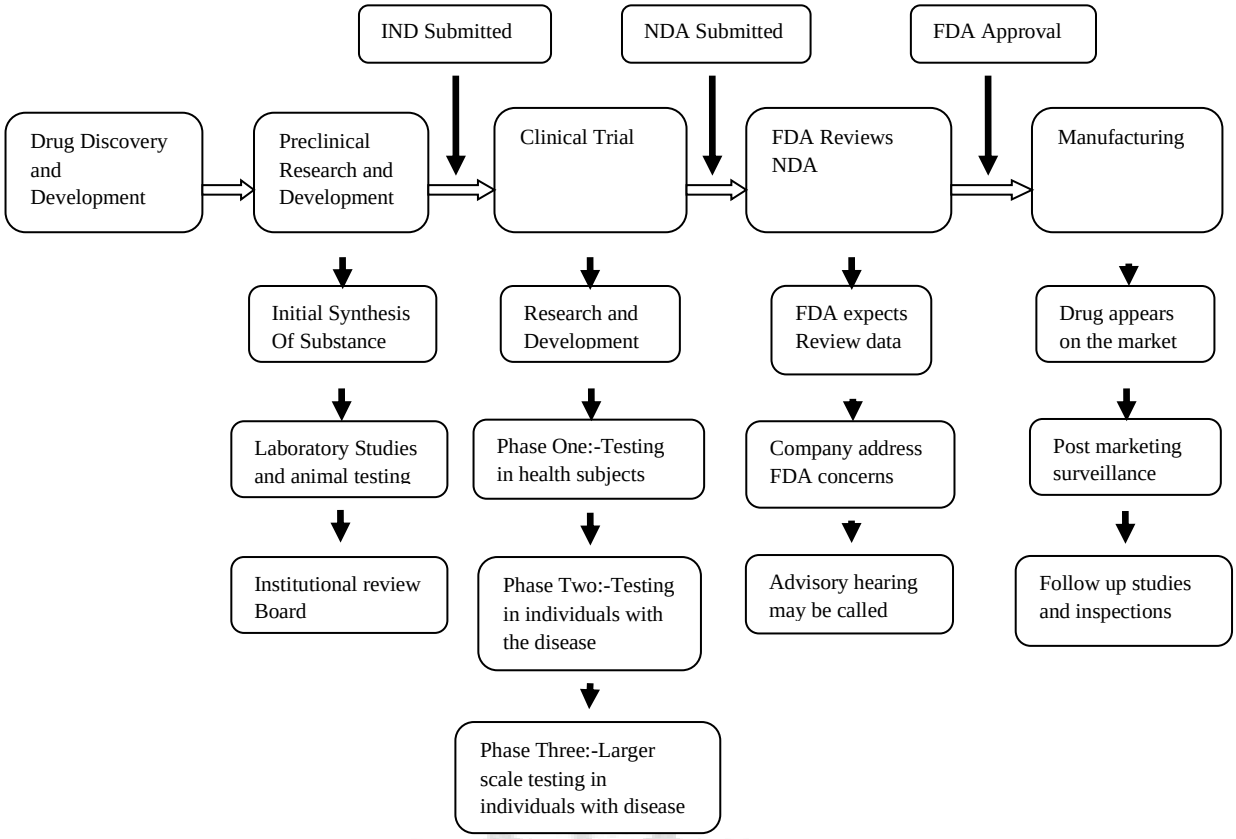
Phase 1: Studies in phase 1 are carried out on a small number of healthy volunteers usually 20 to 100 with the disease or condition and the study requires several months. The purpose of studies in this phase is to identify the metabolic and pharmacological effects of the drug in humans and to determine the side effects associated with increasing doses mainly by determining the safety profile. During phase1sufficient information about the dose of the drug ranging studies so that doses for clinical use can be adjusted approximately 70% of drugs tested in this stage move to the next phase.

Phase 2: Phase 2 includes the early controlled clinical studies conducted to obtain some preliminary data on the effectiveness (efficacy) of the potential drug for a particular indication or indication in patients with the disease or condition testing in this phase help to determine the common short-term side effects and risks associated with the drug under testing these studies are typically well-controlled closely monitored and performed on larger groups of patients usually involving 20-300 the length of study vary from several months to 2 years and approximately 33 % of drugs tested as this phase move to the next phase.

Phase3: Phases3 are expanded, controlled and uncontrolled trials the purpose of study at this phase is to gather additional information about the effectiveness and monitoring of adverse reactions phase 3 includes several hundred to several thousand people usually 300 to 3,000 who have the disease or condition to obtain approval from the US FDA it is typically expected that there must be at least two successful phase-3 clinical trials. The length of study varies from 1-4 years. [21,22]

Step 4: FDA Review

Once the new drug is formulated for its best efficacy and safety, and the results from clinical trials are available, it's advanced for FDA review at this time FDA Review and approves, or does not approve. [23, 24]



Step 5: FDA Post-Market safety monitoring.

This step is also known as Post Marketing Surveillance (PMS) and it is carried out once the candidate drug is approved as a drug and marketed as a medicinal product. This phase aims to find out the drug safety profile in a large patient pool across the world and to establish its Safety profile it is estimated that the success rate of drugs making to market from the laboratory is very less the post-launch safety monitoring helps to detect rare or long term adverse effects of the drugs over a large patient population and time scale than was possible during a clinical trial usually, several thousand volunteers who have the disease or condition are involved in this phase of the trials.[25,26]

Regulatory Affairs in R &D

The Regulatory Affairs personnel work hand in hand with advertising and R & D to develop innovative products that take advantage of new technological and regulatory developments to speed up time to market with new products expected to add great revenues to the company's bottom lines using adaptive clinical trial strategies acquiring quick approval from regulatory authorities and avoiding pitfalls in the method can accelerate the development of new products and help to reduce high-priced errors and time lags.[27,28]

CONCLUSION

Regulatory Affairs (RA) is a profession that acts as interference between the pharmaceutical enterprise and drug regulatory authorities across the world. It is mainly concerned with the registration of drug products in the respective countries before their marketing. The Regulatory Affairs branch is continually evolving and growing and is the one that is least impacted at some stage in the assembly and addition. Regulatory Affairs departments are growing inside companies. The proper implementation of regulatory guidelines and legal guidelines will improve the economic boom of the company and also improves the security of the people. Regulatory Affairs departments are getting larger inside the companies. Due to the altering resource necessary to fulfill the regulatory requirements, some agencies also choose to outsource or out assignment regulatory affairs to external service providers.

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REGULATORY REQUIREMENTS FOR REGISTRATION OF BIOLOGICS IN US

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ABSTRACT

Biological products are used to treat a wide range of diseases, and the number of biological applications submitted for product approval is on the rise. A biosimilars product's development is more difficult and costly than a small molecule generic product. Biosimilars aren't truly generic medications, but they have a lot in common with the reference biological substance. The Biologics Price Competition and Innovation Act of 2009 established a biosimilar pathway in the United States to enhance access to expensive biological therapies. The research included a "Regulatory Prospect for the Registration of Biological Products in the United States" as well as a summary of the biosimilar product development, manufacturing, and approval process. The regulatory framework, [BLA] Biological License Application, is also discussed in this article.

KEYWORDS: Regulatory, Biologics, BLA, Registration, USA.

1. INTRODUCTION

Biologics are items that are made, extracted, or partly synthesized from a biological source and utilized to prevent, cure, or treat diseases and medical problems. The FDA is in charge of regulating them. • These are large, complex molecules generated by biotechnology in a living system like a bacterium, plant cell, or animal cell, and can be made of carbohydrates, proteins, nucleic acids, or complex combinations of these, or they can be living beings. COPS DSU 3 Department of Pharmaceutics

1.1 The Biologics Control Act of 1902 is a milestone in the history of biologics

Hygienic Lab at PHS.

The National Institutes of Health (NIH) has been renamed (1930)

Control of Biologics at the National Institutes of Health (1937)

1937: The National Institutes of Health (NIH) is established, and the division of biologics is given responsibility for biologics control.

The laboratory of biologics control is renamed in 1944.

The Public Health Service (PHS) Act was enacted in 1944.

The National Institutes of Health's National Microbial Institute is established in 1948, and the laboratory of biologics control is merged into it.

Later, the institute of Allergy and Infectious Diseases was established.

In 1955, a polio vaccine was inadvertently inactivated

Biological Standards Division, National Institutes of Health

1.2 OBJECTIVE:

The goal of the dissertation work is to have a basic understanding of the "Regulatory requirements for biologics registration in the United States."

Presentation of application forms, their prerequisites, and instructions for filling out and submitting new biologics applications in the U.S

The CTD requirements for registration of biologics

2. REGISTRATION OF BIOLOGICS IN THE USA

Biologics Registration in the United States

In the United States, "biological products" are regulated differently from "drugs" and have various premarket procedures and intellectual property rights. A biological product, on the other hand, must be approved by a biologics license application (BLA) demonstrating that it is "safe, pure, and effective."

A non-biological drug's sponsor must file a new drug application (NDA) demonstrating the drug's potency. The medication is both safe and effective.

The new biological products will be protected for 12 years, whereas the new medications will be protected for only six months.

Protection for up to 5 years. Different strategies for settling conflicts exist in both biological and drug legislation.

Patent difficulties relating to biosimilars and follow-on products. Before a biologic can be used, it must first be approved by the FDA.

The biologic must go through thorough testing before being licensed and marketed.

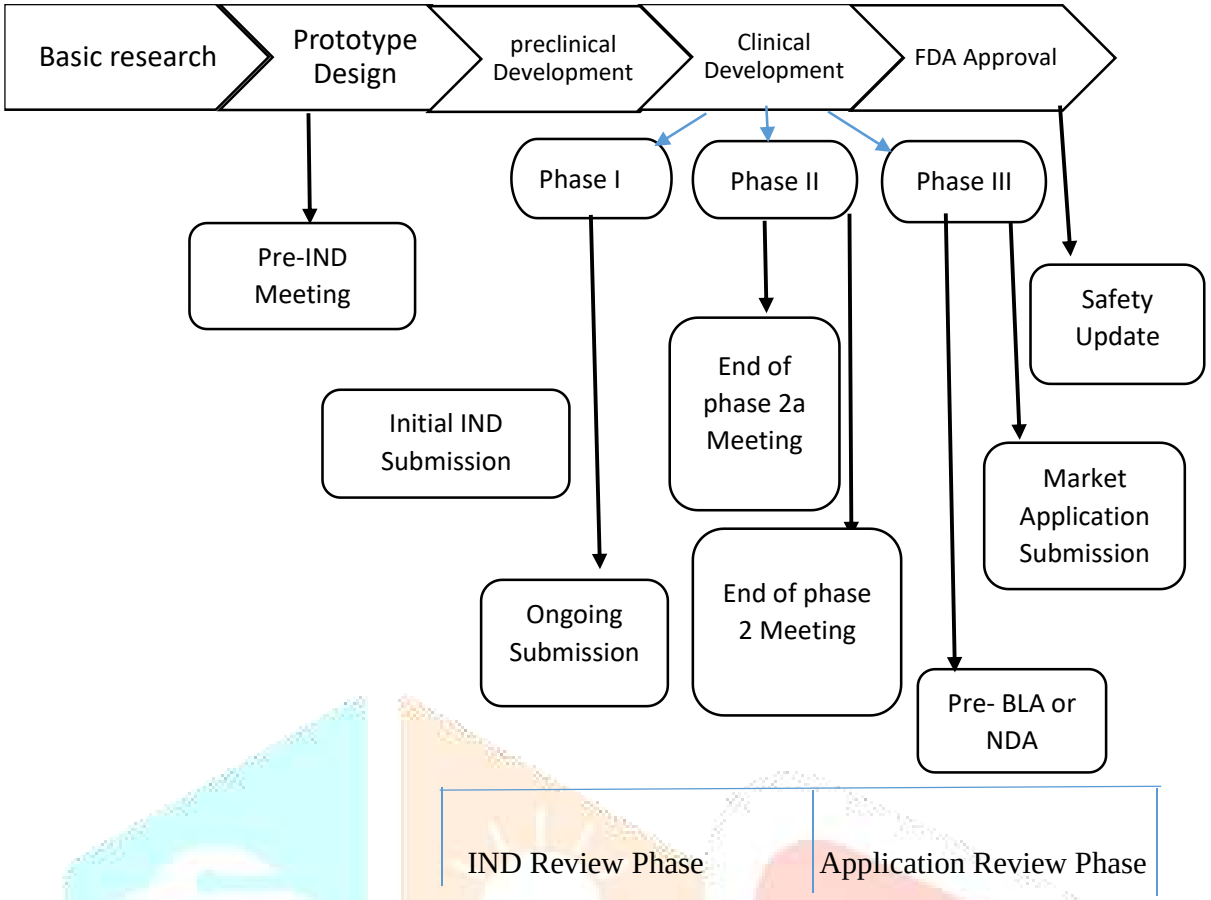

Figure 1 Development of stages of biologics

2.1 BIOLOGICS DEVELOPMENT

BIOLOGICS DEVELOPMENT Living cells or creatures, such as yeasts, viruses, bacteria, or other animal cells, are used to create biologics. A corporation must first demonstrate that it has a viable product to develop before it may create a biological product. This involves a demonstration of the product's ability to be manufactured continuously.

; Figure 1 shows the evolution of biologicals. Preclinical Trial No. 3.1 In vitro and animal experiments are conducted by GLP guidelines. The results of these studies are preclinical data, which are all included in an IND. The FDA reviews the application within 30 days. 3.2

Clinical Research Phase I: A total of 20 to 80 healthy participants were tested for safety and human pharmacology. Phase II: A total of 100 to 200 patients will be tested for basic efficacy and dosing range. Phase III: A multicentre, large-scale investigation involving patients with the target disease

Fundamental research	Discovery	Preclinical Studies	Clinical trials phase I to phase III	FDA Approval

Figure 2: Development of Biologicals

3. APPROVAL PROCESS

(a) General: To receive a biologics license under section 351 of the Public Health Service Act for any reason, according to 21 CFR 601.2.

The manufacturer of a biological product must submit an application to the Director of the Centre for Biologics.

using forms provided by the Centre for Drug Evaluation and Research or the Director, Centre for Drug Evaluation and Research

for such reasons, and shall submit data resulting from nonclinical laboratory and clinical

l investigations for such purposes

demonstrate that the manufactured product complies with all safety, purity, and potency requirements of each nonclinical laboratory study

The applicant, or his or her attorney, agent, or another representative

The application must be signed by an authorized official.

An application for any of the biological goods listed below that are subject to licensure must include the following information:

(1) Therapeutic DNA Plasmid product

(2) Therapeutic synthetic peptides with 40 amino acids or less.

(3) Monoclonal antibody products for in vivo application; and

(4) Therapeutic recombinant DNA-derived products.

(b) [Reserved]

c) (1) To gain marketing authorization for a therapeutic biological product that is subject to licensure.

A monoclonal antibody, DNA plasmid product, a therapeutic synthetic peptide product of 40 or fewer amino acids

An applicant must submit a product that is either an antibody product for in vivo use or a therapeutic recombinant DNA-derived product

By paragraph, submit a biologics license application

If the requirements of this paragraph

2. conflict with other requirements in this section, the requirements of this paragraph

3. take precedence.

(d) The approval or issue of a biologics license application constitutes a biologics license.

The determination that the establishment(s) and product meet all legal requirements to ensure the continuous safety, purity

(e) As of December 20, 1999, any establishment and product license for a biological product issued under section 351 of the Public Health Service Act (42 U.S.C. 201 et seq.) that has not been revoked or suspended constitutes an approved biologics license application in effect under the same terms and conditions.

terms set out in such product license, as well as those elements of the establishment license relating to such product license

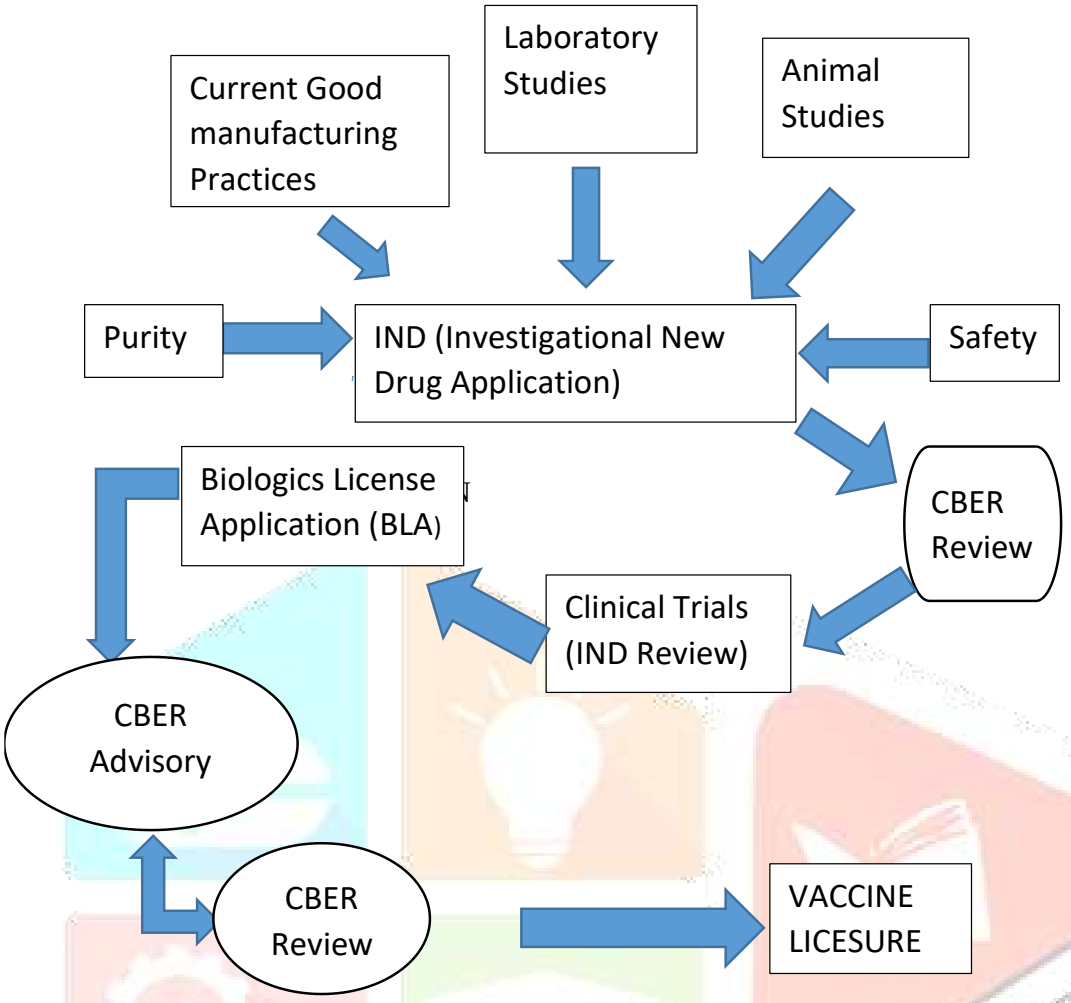

Fig No 3: The process of biological product licensure in the U.S

CONCLUSION:

Biologics are the highly complicated nature medication that is used to treat a variety of diseases and problems. Biotechnology is now being used to create a variety of pharmaceuticals such as antibodies and anticancer medications

Regulatory requirements for registration of new biologics in CTD format according to the FDA include module I which contains administration information, and module II which contains scientific information. module III, in general, contains the quality management system

Moule IV and V contain preclinical and clinical information and information on biologics patent exclusivity the approval process and adjustments made after approval

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An Outline On Improving Solubility And Dissolution Rate In Solid Dispersion Technique.

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Abstract:
To improve dissolution of poorly water-soluble drugs and thus enhancing their bioavailability, the dispersion of one or more active pharmaceutical ingredient in a carrier at solid state is used. This process is known as solid dispersion. It has engrossed significant interest as an efficient means of improving the dissolution rate. Solid dispersions are being employed frequently to improve bioavailability of poorly soluble molecules by enhancing the rate and extent of dissolution in drug product development process. This review discussed the methods for solubility enhancement of poorly soluble drugs and the mechanism by which solubility and dissolution rate enhancement occurs in solid dispersion. The present article also discuss about the manufacturing methods for solubility enhancement, its mechanism and outcome of various low soluble drugs, applications, limitations of the solid dispersions.
***Keywords*: Solid Dispersion, solubility enhancement mechanism, bioavailability.**

INTRODUCTION:

The number of pharmaceutical ingredients (APIs) having low aqueous solubility is currently one of the key issues restricting their biological application. According to estimation, upto 70% of APIs and novel therapeutic entities have poor water solubility, resulting in sluggish absorption and insufficient and unpredictable drug bioavailability [1]. When an active substance is given orally, it must first dissolve in stomach before it can pass through the GI tract's membranes and enter systemic circulation. As a result, a drug with low aqueous solubility will have limited absorption, while a drug with low membrane permeability will have permeation rate limited absorption [2]. As a result, enhancing the bioavailability of active agents by: (i) improving the solubility and dissolution profile of poorly water-soluble medications, and (ii) improving the permeability of drugs. The oral route is the most popular route of administration of the drug due to various reasons like its convenience, good patient acceptance and low medicine production costs [3]. Particle size reduction, salt formation, crystallization, and the use of surfactants and co-solvents are all methods for improving the dissolving capabilities of weakly aqueous-soluble medicines. However, these methods has its own set of constraints, such as the difficulty in forming salts for neutral and weakly acidic/basic medications, and the addition of surfactants/co-solvents leads in liquid formulations with known commercial viability and patient tolerance issues [4]. Furthermore, despite their higher permeability, the majority of potential NCEs are absorbed mostly in the intestine, with absorption dropping substantially after the ileum, showing that absorption is limited [5, 6].

As a result, these medications will have a limited bioavailability if they are not entirely released in this gastrointestinal area. Therefore, improving the water solubility of pharmaceuticals is primary contemporary difficulties facing the pharmaceutical industry [7, 8, 9]. It is feasible to increase bioavailability and prevent side effects by altering the drug release profile of these drugs [10, 11, 12]. Solid dispersions have proven to be most effective ways to enhance the release of low soluble medicines. These are molecular combinations of low aqueous soluble medicines in hydrophilic carriers that have a drug release profile dictated by the polymer characteristics. [13]. Solid dispersion technologies are utilised to improve the solubility properties and, as a result, the bioavailability of low water-soluble compounds. Water insoluble drugs have poor solubility in aqueous gastrointestinal fluids, resulting in insufficient bioavailability. Increased solubility and dissolving rate of the medicine in the gastro-intestinal fluids can improve bioavailability, especially for drugs categorized as Class II by the Biopharmaceutics Classification System. Solid dispersions (SDs) are a well-known approach for improving aqueous solubility and, as a result, oral bioavailability and drug dissolution rate.

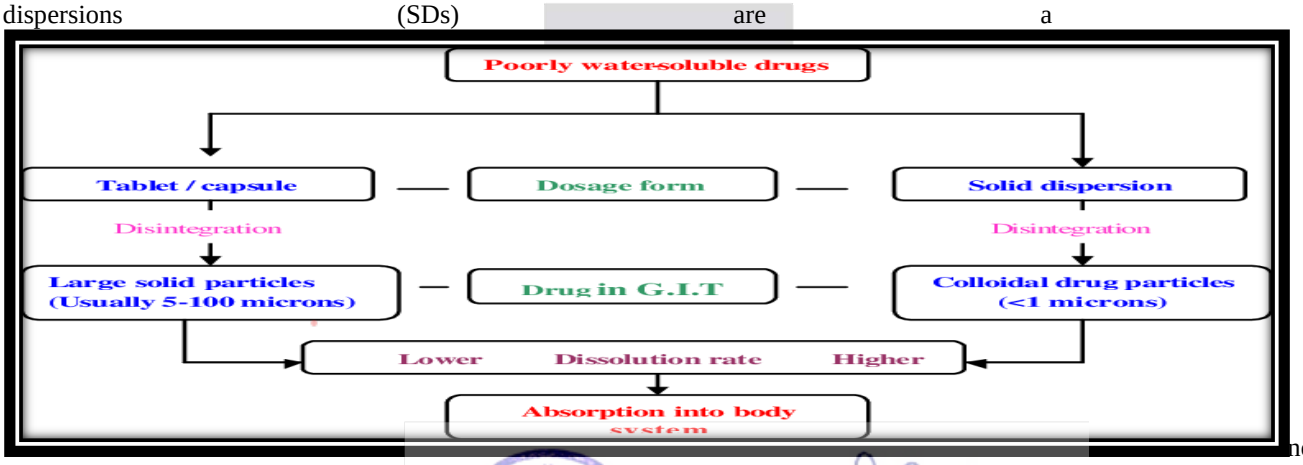

Figure 1: Benefits of a solid dispersion formulation over a conventional tablet or capsule formulation [14].

Applications:

1) The carrier in a solid dispersion plays an important role in enhancing particle wettability. Improved wettability leads to higher solubility, which improves bioavailability [15].
2) Drugs are shown as supersaturated solutions in solid dispersion, which are considered metastable polymorphic forms. Thus, presenting the drug in amorphous form and increases the solubility of the particles [16].
3) Solid dispersions are used for the improvement of the bioavailability of poorly water soluble drugs by enhance the dissolution of the drug [17].
4) Solid dispersions can be formulated as extended release dosage forms.
5) Solid dispersions are better than other particle size reduction methods for improving solubility because other size reduction techniques reduce the size to a limit of about 2-5 microns, which does not result in enough enhancement in drug solubility or drug release in the small intestine, and thus does not improve bioavailability [19].
6) The problems of solid powder such as less size of particles shows poor mechanical properties (include high adhesion and poor flow properties) can be overcome by the use of solid dispersion [19].

Limitations:

1) The polymers employed in solid dispersion can absorb moisture, resulting in phase separation, crystal formation, and the conversion of amorphous to crystalline state [17, 20].
2) Amorphous state of drug undergo crystallization and stability problems.
   Most polymers used in solid dispersions absorb moisture, which can cause phase separation, crystal development, or conversion from amorphous to crystalline state, during storage. As a result, the solubility and rate of dissolution may be reduced [21].
3) In the presence of moisture and high temperatures, solid dispersions may degrade.
4) Difficulty in understanding the physical structure of solid dispersions
5) Difficult to determine the shelf life of Solid dispersion.

Approaches for Solubility Enhancement of Poorly Soluble Drug: The techniques that have commonly been used to overcome drawbacks associated with poorly watersoluble drugs, in general includes [17, 18].

Physical Modifications	*Chemical Modifications*	*Others*
Particle size reduction	Salt Formation	Supercritical fluid method
Modification of the crystal habit.	Co-crystallization	Spray freezing into liquid and lyophillization
Complexation	Co-solvency	Hot melt extrusion
Solubilization by surfactants	Hydrotropic	Electrostatic spinning method

THE MECHANISM BY WHICH SOLUBILITY AND DISSOLUTION RATE ENHANCEMENT OCCURS IN SOLID DISPERSION:

Increased Solubility or Dissolution rate of Drug:

Using a variety of carriers may boost the drug's solubility. As a result, the carrier controls the release of the medication, which is independent of the drug's qualities. Additionally, certain systems exhibit release behaviour that is influenced by drug qualities rather than polymer features. The ability of the matrix carrier to enhance drug's local solubility and wettability is also linked to improved solubility dissolution profile of poorly soluble medicines. In his studies, Goldberg et al. investigated the by melting, fully combining, and hardening the mixture of chloramphenicol and urea for additional solubility and dissolution rate investigations, were able to determine the effect of the hydrophilic carrier urea on the solubility of chloramphenicol [21]. As the urea concentration increased from 0% (w/v) to just over 60% (w/v), the solubility of chloramphenicol in the presence of urea increased by more than seven times [21].

Sr.No	*Drug*	*Method of preparation*	*Conclusion*	*Reference .*
1.	(Diazepam and temazepam)	Solvent evaporation and fusion method	Diazepam and temazepam's solubility increases by around 3.5 and 2.5 times, respectively.	[22].
2.	Chloramphenicol	Melting method.	In the presence of urea, chloramphenicol's solubility increased by more than seven times.	[22].
3.	Glibencamide	Anti-solvent addition method	For the manufacture of drug ASD, the co-spray drying approach was used, which greatly improved solubility and resulted in the formation of Glibenclamide-rich amorphous droplets.	[23].

Reduced Particle size [24]:

Size reduction has been considered to be result of eutectic or solid solution formation. Additionally, it has been proposed that presenting the particles in the dissolution media as physically distinct entities may lessen aggregation. Many of the carriers employed in solid dispersion may have wetting capability, which can prevent agglomeration and enhance surface area by improving wetting. When a weakly soluble medication and a highly soluble carrier present in a eutectic mixture are exposed to water or digestive fluid, the highly soluble carrier dissolves, leaving the drug in a fine crystalline form that is easily dissolved. As can be determined from the Noyes-Whitney equation, greater surface area of insoluble chemical results in increased dissolution rate and thus increased oral absorption. Several solid dispersions were documented employing urea as a high water soluble carrier for poorly water soluble medicines such as sulfathiazole, paracetamol, and chloramphenicol. Compared to conventional formulations of the same medications, these solid dispersions exhibited faster release and improved bioavailability. The small particle sizes of the drug played important role in enhancing bioavailability [9,10]. Similarly, because the drug particle size is decreased to an absolute minimum as it is molecularly disseminated in the carrier in a solid solution, it dissolves faster than a eutectic mixture.

Formation of amorphous structure replacing crystalline structure:

Sr.No	*Drug and SD Method*	*Mechanism*	*Conclusion*	*Reference*
1.	Ball Milling (Curcumin)	Particle Size Reduction	The amorphous nature and self-formed micelles of Curcumin SD resulted in a significant improvement in pharmacokinetic behaviour, as illustrated by a 19-fold increase in oral bioavailability..	[25]
2.	Nobiletin	Hot melt extrusion	Amorphous solid dispersion had a greater drug concentration and a 7.5-fold faster dissolving rate. In accelerated stability circumstances, Nobiletin permeability was primarily increased and shown to be stable for up to 6 months.	[26]
3.	Licofelone	Cogrinding mixtures	Enhanced dissolution rate and decreased particle agglomeration	[27]

In the amorphous state, poorly water-soluble crystalline medicines have a higher solubility. The amorphous solids free energy is greater, has specific entropy, and specific volume when compared to corresponding crystalline materials from a thermodynamic standpoint. Amorphous pharmaceuticals have a higher energy state, have the lowest stability, and can be considered as cooled

liquids. Because the energy necessary to transfer a molecule from a crystal is larger than that required to transfer a molecule from a non-crystalline (amorphous) solid, non-crystalline (amorphous) solids have greater aqueous solubility than crystalline solids. This necessary energy is viewed as an obstacle to medication breakdown. The amorphous state of novobiocin, has ten times the solubility than that of the crystalline form. Chemicals are dissolved or molecularly dispersed in a polymeric carrier in solid molecular dispersions because they lack long-range crystalline structure. The drug is in an amorphous state, which has a higher kinetic solubility and dissolving rate than the crystalline drug (by several orders of magnitude). [7,14,15]. By solvent technique, solid molecular dispersions of diclofenac sodium, naproxen, and piroxicam were generated utilising Poly (2- hydroxyethylmethacrylate) hydrogel as carrier, resulting in the conversion of crystalline drug into amorphous form with enhanced water solubility(16).

Sr. No	***Drug***	***Method of preparation***	***Conclusion***	***Reference***
1.	(Atorvastatin calcium)	solvent evaporation method	The pharmacokinetic study indicated that the Cmax and AUC 0-8h of solid dispersion were improved nearly 2.87-fold and 1.71-fold.Solubility and dissolution rates were enhanced significantly compared with bulk drug	[28]
2.	Vemurafenib	(Micro-precipitated bulk powder technology)	Better dissolution results and a fivefold increase over HPMCAS-L ASD's crystalline form were revealed.	[26]

Complex formation:

In this solid dispersion, in solid state, a drug and an inert soluble carrier form a complex. The solubility and stability constant of the molecule or complex, as well as the drug's absorption rate, determine the drug's availability. It is proposed that the development of a water-soluble compound with a high dissolution constant can increase the dissolution rate and oral absorption. Carbamazepine/PEG 4000 and PEG 6000 solid dispersions, were made using the fusion method, which entails heating a physical mixture of carbamazepine and either PEG 4000 or PEG 6000 to a liquid state. According to dissolving tests, complex formation between carbamazepine and PEG 6000 may be to account for the improvement in solid dispersion dissolution. (27). One of the most frequently used complex carriers are within the class of Cyclodextrins. Cyclodextrins (CD) are cyclic oligomers typically composed of 6–8 glucose units. CDs are a type of solubilizing agent that, by inclusion, produce non-covalent, dynamic complexes with lipophilic molecules.

Sr.No	***Drug and SD Method***	***Mechanism***	***Conclusion***	***Reference No.***
1.	phenacetin (solvent evaporation)	Mechanical Particle size reduction.	The water-soluble hydroxypropylcellulose swells in water and is trapped in the water-insoluble ethylcellulose so that the release of the drug is slowed.This study shows that it is feasible to control PHE release from MC-CP solid dispersions by controlling the complex formation between MC and CP	[26]
2.	Carvedilol	Complexati on and kneading technique	The complexation constant of the medication and the carriers confirmed the formation of stable complexes. The carvedilol had been transformed to an amorphous state, according to solid state data.	[29]

Swelling and capillary action of carrier [24]:

Superdisintegrants like crospovidone, crosslinked polyvinylpyrrolidone etc can considerably used to improve dissolution rate of poorly water soluble drugs. swells 7- to 12-fold in less than 30 sec. Croscarmellose swells 4- to 8-fold in less than 10 seconds in two dimensions, retaining fibre length equal.This indicates that rate, force, and extent of swelling have an important role in disintegrants that work by swelling.

By improving porosity solid dispersion [24]:

The porosity of particles in solid dispersions has been discovered to be greater. Porosity increases with carrier qualities; eg Solid dispersions with linear polymers produce larger, more porous particles than those with reticular polymers, resulting in a faster dissolving rate.

Interactions of the drug with Carrier functional groups:

In addition to improving the drug's local solubility and wettability, carrier matrix also helps to improve the medication's aqueous solubility and dissolving rate through specific interactions with the drug. [19,20]

a) The intermolecular hydrogen bonding:
b) By elevating the Tg(transition temperature) of the solid dispersion mixtures:
c) Inhibited drug precipitation from supersaturated solution:
d) By formation of Metastable drug polymorphous with improved solubility and dissolution rate:

Sr.No	Drug and SD Method	Mechanism	Conclusion	Reference No.
1.	AZD0837	Hot melt extrusion	The molecule remained amorphous throughout the dissolving process and was kept in a super saturated and stable condition, according to the findings.	[30]
2.	Indomethacin (IND),	Hot melt extrusion	BCS Class II medication that benefits from the addition of a porous carrier to a ternary mixture and exhibits better dissolving capabilities than the drug-polymer binary mixture alone.	[26]

Sr.No	Drug and SD Method	Mechanism	Conclusion	Reference
1.	**Rivaroxaban**	Melt quenching approach	Physical stability of prepared ASDs was aided by intermolecular interactions with moisture.	[31]
2.	**Griseofulvin**	Freeze drying	Because of its high degree of supersaturation and high crystallisation propensity, there was a significant improvement in dissolving and oral absorption.	[32]
3.	**Itraconazole**	Solvent evaporation	In comparison to PVPVA, HPMCAS demonstrated excellent storage stability at RH levels greater than 60%, which can be attributed to its greater glass transition temperature and lower hydrophobicity.	[26]

4.	**Etoposide**	Solvent evaporation	According to experimental studies, the solubility of etoposide above the Critical micellar concentration (CMC) grew linearly, and the ASD permitted super saturation. By boosting P-gp saturation, a high level of super saturation via ASD increased the drug's in-vivo permeability.	[26]
5.	**Curcumin**	Solvent evaporation	Because of the hydrogen bond interaction between the curcumin and the polymer, HPMC E5 has a substantial impact on crystallisation inhibition and enhanced the permeability of the amorphous drug..	[32]

Conclusion:

Solid dispersion systems have been realized as extremely useful tool in improving the dissolution properties of poorly water-soluble drugs. In recent years, a great deal of knowledge has been accumulated about solid dispersion technology, but their commercial application is limited. Various methods have been tried recently to overcome the limitation and make the preparation practically feasible. The problems involved in incorporating into formulation of dosage forms have been gradually resolved with the advent of alternative strategies.

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EVALUATION OF ANTICATALEPTIC ACTIVITY OF BACLOFEN ON HALOPERIDOL & PILOCARPINE INDUCED CATALEPSY

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ABSTRACT

Aim: Evaluation of Anticataleptic activity of baclofen on Haloperidol & Pilocarpine induced catalepsy. **Materials and methods:** A cataleptic behaviour was measured with a high bar test method. Catalepsy score was measured each hour for 4 h after haloperidol and pilocarpine administration, by gently placing both the forepaws of the rat over a metal bar (diameter 2–5 mm) situated 6 cm above the tabletop. The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cut-off time of 180s. **Result:** Haloperidol 1mg/kg p.o and Pilocarpine 100mg/kg p.o caused a time dependent increase in catalepsy, with maximum score occurring at 4 hours after administration. Animal treated by Baclofen (10mg/kg) p.o could significantly reduce catalepctic score as compared to toxicant control animals. **Conclusion:** It is concluded that Baclofen at the dose of 10mg/kg p.o can possess anticatalepctic effect.

1. INTRODUCTION

Catalepsy is a condition characterized by inactivity, decreased responsiveness to stimuli and a tendency to maintain an immobile posture. It may be associated with the nervous system drug toxicity, psychotic disorders and other conditions. Catalepsy is the neurodegenerative disease of unknown etiology and characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. Catalepsy is characterized by an abnormal basal ganglia activity. Nonmotor comorbidities, such as cognitive impairments (the comorbidity of anxiety and depression like Parkinson's disease) are likely the result of an intricate interplay of multi-system degenerations and neurotransmitter deficiencies extending beyond the loss of dopaminergic nigral neurons (costall,B and Naylor 1974).

Haloperidol is an antipsychotic drug, which is used in the treatment of schizophrenia and other psychotic disorders. Antipsychotics are often associated with distressing extrapyramidal side effects. Haloperidol-induced catalepsy occurs due to the blockade of dopamine (D2) receptors and reduced dopaminergic transmission. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g. haloperidol) is a robust behavioural model to study nigrostriatal function and its modulation by cholinergic, serotonergic, nitrergic and other neurotransmitter systems.

Baclofen (P-p-chlorophenyl GABA) is used clinically in the treatment of spasm of voluntary muscles. Its mode of action has been attributed to the facilitation of GABA-mediated transmission in the central nervous system. Baclofen would therefore be expected to inhibit the activity of nigro-striatal dopaminergic neurones and in the process potentiate the cataleptogenic effect of haloperidol. Gianutsos & Moore (1977) reported that baclofen produced a dose-dependent increase in the concentration of brain dopamine in mice without affecting noradrenaline levels. They suggested that this effect was achieved by a reduction in impulse flow in dopaminergic neurones in a similar way to that produced by y-butyrolactone (GBL), (Roth, Walters, Murrin &Morgenroth, 1975). This study examines the effect of Baclofen on the cataleptogenic effect of haloperidol.

2. MATERIALS AND METHODS

2.1 Animals and housing condition: The experiment was conducted with Wistar male rats of 150-200 g and 2–3 months old. Female rats are excluded from the present study since estrogen has been reported to possess neuroprotective property and this might mask development of Catalepsy. These animals were procured from registered breeder and were acquainted in the quarantine area for one week. After acquaintance, animals were transferred to the standard of22±2°C temperature,50±15%relative humidity, 12hr.dark/12hr. light)cycle and the animals had free access to pellet diet & water was provided *ad libitum*. The study protocol was presented to the IAEC for approval.

2.2 Study design

Haloperidol induced catalepsy

The 36 male albino wistar rats were divided into 6 groups: group I received vehicle, group II received Haloperidol (1mg/kg), group III received Haloperidol (1mg/kg) and Levodopa(100mg/kg), group IV received Haloperidol(1mg/kg) and Baclofen(5mg/kg), group

V received Haloperidol(1mg/kg) and Baclofen(10mg/kg), group VI received Haloperidol(1mg/kg) and Baclofen(15mg/kg).

Pilocarpine induced catalepsy

The 36 male albino wistar rats were divided into 6 groups: group I received vehicle, group II received Pilocarpine(100mg/kg), group III received Pilocarpine(100mg/kg) and Levodopa(100mg/kg), group IV received Pilocarpine (1mg/kg) and Baclofen(5mg/kg), group V received Pilocarpine (100mg/kg) and Baclofen(10mg/kg), group VI received Pilocarpine (100mg/kg) and Baclofen(15mg/kg).

2.3 Experimental procedure: A cataleptic behaviour was measured with a high bar test method. Catalepsy score was measured each hour for 4 h after haloperidol and Pilocarpine administration, by gently placing both the forepaws of the rat over a metal bar (diameter 2–5 mm) situated 6 cm above the tabletop. The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cut-off time of 180 s. Finally, scores at different time points (0, 30, 60, 120, 180 and 240 min after haloperidol and Pilocarpine administration) was added and expressed as a cumulative catalepsy score for comparison purpose.

2.4 Scoring of catalepsy

Cataleptic animal maintaining this position for a period of time dependent upon the degree of catalepsy. If the animal maintained the imposed posture for at least 20s it was said to be cataleptic and given one point. Scoring is modified from that used by Costall and Naylor (1974). Animals maintaining the cataleptic posture from 0 s to 10 s scored 0; 10 s to 30 s = 1; 30 s to l min=2; l min to 2 min=3; 2 min to 3 min=4; 3 min to ∞ =5. Animals were tested for catalepsy 0.5, 1.0, 2.0,3.0 and 4.0h after haloperidol and Pilocarpine administration (Costall& Naylor, 1974).

2.5 Statistical Analysis: The data obtained by the various parameters was statistically evaluated by one way analysis (ANOVA) followed by Dunnett's multiple comparison test by graph pad prism software (Graph pad software Inc.…5.0.0). The mean values±SEM were calculated for each parameter. Level of significance was kept at $p<0.05$.

3. RESULTS

3.1 Effect of Baclofen on cataleptic score using High bar Test

Table 1: Haloperidol induced catalepsy.

Groups	Dose	30 min	60 min	120 min	180 min	240 min
Normal control(vehicle)	---	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Negative control (Haloperidol)	1mg/kg p.o	0.0±0.0	0.0±0.0	3.16±0.28	4.66±0.43	4.83±0.47
Positive control (Haloperidol+ Levodopa)	1mg/kg p.o +100mg/kg p.o	0.0±0.0	0.0±0.0	0.83±0.08	0.66±0.04	0.00±0.00
Low dose (Haloperidol+ Baclofen)	1mg/kg p.o +5mg/kg p.o	0.0±0.0	0.0±0.0	1.83±0.15	1.66±0.12	1.66±0.15
Intermediate dose (Haloperidol+ Baclofen)	1mg/kg p.o +10mg/kg p.o	0.0±0.0	0.0±0.0	0.66±0.05	0.66±0.03	0.66±0.04
High dose (Haloperidol+ Baclofen)	1mg/kg p.o +15mg/kg p.o	0.0±0.0	0.0±0.0	4.66±0.41	4.63±0.41	4.63±0.40

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Haloperidol 1mg/kg showed significant increase in number of catalepsy as compared to control group.
2. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Levodopa 100 mg/kg showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
3. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (5mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
4. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (10mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
5. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (15mg/kg) did not reduce catalepsy score as compared to negative group (Haloperidol 1mg/kg).

Table 2: Pilocarpine induced catalepsy.

Groups	Dose	30 min	60 min	120 min	180 min	240 min
Normal control(vehicle)	---	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Negative control (Pilocarpine)	100mg/kg p.o	0.0±0.0	1.55±0.13	2.33±0.20	3.16±0.28	3.66±0.32
Positive control (Pilocarpine + Levodopa)	100mg/kg p.o+100mg/kg p.o	0.0±0.0	0.64±0.06	0.57±0.05	0.55±0.06	0.55±0.04
Low dose (Pilocarpine + Baclofen)	100mg/kg p.o+5mg/kg p.o	0.0±0.0	1.12±0.10	1.06±0.08	1.03±0.12	1.03±0.11
Intermediate dose(Pilocarpine + Baclofen)	100mg/kg p.o+10mg/kg p.o	0.0±0.0	0.64±0.04	0.55±0.05	0.55±0.06	0.53±0.04
High dose (Pilocarpine + Baclofen)	100mg/kg p.o+15mg/kg p.o	0.0±0.0	3.55±0.30	3.83±0.38	4.66±0.40	4.83±0.45

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Pilocarpine 100mg/kg showed significant increase in number of catalepsy as compared to control group.
2. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Levodopa 100 mg/kg showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
3. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (5mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
4. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (10mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
5. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (15mg/kg) did not reduce catalepsy score as compared to negative group (Pilocarpine 100mg/kg).

3.2 Effect of baclofen on locomotor activity using actophotometer

Table 3: Haloperidol induced catalepsy.

Groups	Treatment(dose)	Locomotor activity counts(10 min)
Group I(Normal control)	Vehicle	482.0±13.91
Group II(negative control)	Haloperidol(1mg/kg)	267.0±11.87
Group III(positive control)	Haloperidol(1mg/kg)+Levodopa(100mg/kg)	422.5±11.78
Group IV(Test I)	Haloperidol(1mg/kg)+Baclofen(5mg/kg)	311.5±12.11
Group V (Test II)	Haloperidol(1mg/kg)+Baclofen(10mg/kg)	350.8±12.40
Group VI (Test III)	Haloperidol(1mg/kg)+Baclofen(15mg/kg)	232.0±13.82

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. Negative group (Haloperidol 1mg/kg) showed significant decrease in locomotor activity as compared to normal control group.
2. Animals treated with Levodopa 100mg/kg(positive control) showed significant increase in locomotor activity as compared to negative group.
3. Animals treated with Baclofen (5mg/kg) showed significant increase locomotor activity as compared to negative group (Haloperidol 1mg/kg).
4. Animals treated with Baclofen (10mg/kg) showed significant increase locomotor activity as compared to negative group (Haloperidol 1mg/kg).
5. Animals treated with Baclofen (15mg/kg) did not show significant locomotor activity as compared to negative group (Haloperidol 1mg/kg).

Table 4: Pilocarpine induced catalepsy.

Groups	Treatment(dose)	Locomotor activity counts(10min)
Group I (Normal control)	Vehicle	468.8±10.07
Group II (Negative control)	Pilocarpine(100mg/kg)	143.7±12.48
Group III (Positive control)	Pilocarpine(100mg/kg)+Levodopa(100mg/kg)	442±13.84
Group IV (Test I)	Pilocarpine(100mg/kg)+Baclofen(5mg/kg)	335.6±12.35
Group V (Test II)	Pilocarpine(100mg/kg)+Baclofen(10mg/kg)	360.7±12.95
Group VI (Test III)	Pilocarpine(100mg/kg)+Baclofen(15mg/kg)	242.3±14.72

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. Negative group (Pilocarpine 100mg/kg showed significant decrease in locomotor activity as compared to normal control group.
2. Animals treated with positive control Levodopa 100mg/kg(positive control) showed significant increase in locomotor activity as compared to negative group.
3. Animals treated with Baclofen (5mg/kg) showed significant increase locomotor activity as compared to negative group (Pilocarpine 100mg/kg).
4. Animals treated with Baclofen (10mg/kg) showed significant increase locomotor activity as compared to negative group (Pilocarpine 100mg/kg).
5. Animals treated with Baclofen (15mg/kg) did not show significant locomotor activity as compared to negative group (Pilocarpine 100mg/kg).

4. DISCUSSION

Catalepsy can be produced by blocking dopaminergic striatal pathways or by stimulating the cholinergic pathways (Liisa Ahtee). At striatal dopaminergic receptors, catalepsy has been linked to a functional absence of dopamine(Van Rossum 1966).By inhibiting post-synaptic dopamine receptors, neuroleptics like haloperidol produce this effect. Additionally, it's probable that haloperidol functions as a "feedback" blocker of presynaptic or autoreceptors that control dopamine production (Fuxe, Hokfelt, Ljung-dahl, Agnati, 1975). Dopamine turnover is enhanced overall, and dopamine receptor stimulation is inhibited. Pilocarpine increased the striatal homovanillic acid (HVA) content up to three times the original concentration. According to reports, baclofen inhibits the rise in dopamine turnover caused by neuroleptics, most likely by slowing down the firing rate of dopaminergic neurons and making them less sensitive to neuronal "feedback" mechanisms. It has been proposed that baclofen increases the concentration of brain dopamine in a dose-dependent manner, whereby the reduction in impulse flow in dopaminergic neurones results in an increased activation of tyrosine hydroxylase. (Gianutsos and moore 1977). Since a GABA-ergic system has been hypothesised that inhibits the nigro-striatal dopaminergic system, it would be expected that increasing endogenous brain GABA levels or potentiating GABA-ergic transmission would increase the cataleptogenic action of neuroleptics.

In this study, two animal models were used actophotometer and high bar test. All the models are widely accepted behaviour models for assessing pharmacological anticataleptic activity. The results described in this paper agree with the above findings in that there is a potentiation of catalepsy up to 60 minutes. However, we found that after 60 min the cataleptogenic effect

of haloperidol and pilocarpine was attenuated and remained significantly so when compared to control animals for up to 4 hours. It would appear that the facilitation of GABA-ergic inhibition on the nigro-striatal dopaminergic neurons produced by baclofen diminishes after 60 min. We propose that the reversal of catalepsy by baclofen is a result of the reininstatement of impulse flow in the nigro-striatal dopaminergic neuronal system (J.A Davies and J.Williams).

5. CONCLUSION

It is concluded that Baclofen at the dose of 5mg/kg p.o can possess anticatalepctic effect. Baclofen at the dose of 15 mg/kg did not reduce cataleptic score as compare to toxicant control animals. The effect was more prominent when animals treated with Baclofen (10mg/kg).

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Evaluation of protective role of a Ferulic acid on Letrozole induced polycystic ovarian syndrome in female rats

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ABSTRACT: Background: *Ferulic (*hydroxycinnamic) *acid* is an antioxidant of phenolic phytochemical group used for the skin care product. Polycystic Ovarian Syndrome (PCOS) is a state of hormonal disorder causing an enlarged ovary with small cysts at the outer edges. Aim: The study was designed to investigate the protective effect of ferulic acid (3-methoxy-4-hydroxycinnamic acid) in letrozole induced polycystic ovarian syndrome in rats (PCOS). Methods: All the experimental animals except control group were orally administered with Letrozole (1mg/kg) dissolved in 0.5 % w/v Carboxymethyl cellulose (CMC) solution per oral route for 21 days to induce PCOS. Followed by a dose of ferulic acid (10, 20, and 40 mg/kg p.o.) for 15 days using water as vehicle. Results: The PCOS was confirmed in the letrozole induced rats with increased concentration of androgen, abnormal lipid levels, glucose, glycosylated haemoglobin and also depletion of antioxidants. The administrated of letrozole cause to abnormalities in serum hormone profile, lipid profile, blood glucose levels and increases body weight and ovary weight. Ferulic acid successfully exerted its protective effect by restoring all the parameters to normalize and improving or disappearance of ovarian cysts. Histopathological observations showed a remarkable recovery of the ovarian tissue and the presence of normalized structure of antral follicle. Conclusion: Ferulic acid showed protective effects in letrozole induced PCOS in rats. Biological effects of ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS conditions.

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INTRODUCTION:

Polycystic ovary syndrome (PCOS) is a common and complex female endocrine disorder in women of reproductive age [1,2] with an estimated prevalence of 6 to 10 % [3]. Clinical manifestation of PCOS amenorrhea, abdominal obesity, hirsutism, and androgen excess (Hyperandrogenism), infertility, and expanded ovaries with multiple cysts. Women with PCOS are at increased risk for diabetes, dyslipidemia, atherosclerosis,

bleeding, hypertension, cardiovascular disease as well as endometrial carcinoma [4]. It is also related with psychological impairments like depression and related mood disorders.

Lipid imbalance, insulin resistance, oxidative stress, and genetics are some of the contributing factors of PCOS [5]. Currently, many therapies are available to induce ovulation and manage PCOS, but it is associated with mild to severe side effects, like; arthritis, hot flushes, muscle or joint pain and psychological side effects like, mood swings, depression, irritability, and bloating. Therefore now-a-days focus is being laid on natural source herbal medicinal plants that have been utilized for the treatment of the various disorders related to the reproductive system due to the lesser or no side effects [3].

Ferulic acid(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) is water soluble, phenolic compound found in active chemical constituent in Chinese medicine herbs such as female ginseng ,and many staple foods, like; fruits, cereals, vegetables and coffee [6,7]. Ferulic acid has been reported to possess a wide variety of biological effects like Antioxidant, anti-inflammatory, hypoglycaemic, and Hyperlipidemic activities [8]. In this study we evaluated that Ferulic acid (3-methoxy-4-hydroxycinnamic acid) may be beneficial in management of PCOS induced by Letrozole due to the reported activity.

MATERIALS:

Drugs and reagents:

Letrozole and Clomiphene citrate were purchased from retail Shop Satara, India. Ferulic acid was obtained from Dolphin Pharmacy Instruments, Pvt., Ltd. Mumbai.

METHODS:

In this study the experimental models used is Letrozole induced PCOS models. The model was widely used accepted for assessing PCOS activity. All animals were selected and divided into six groups and housed eight female rats per cage. All animals in five groups except control group were orally administered with Letrozole for 21 days.

Two animals from each group were scarified by using CO_2 chamber. Ovaries was removed and observed for presence of cysts. On 22nd day, Test group I, II, and III was administered with Ferulic acid for 15 days, whereas standard group was dosed with Clomiphene citrate for 15 days per oral route [9-11].

Animals:

This prospective comparative study was conducted at Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, and Maharashtra, India. Healthy, Virgin, cyclic and adult female wistar rats (150 to 200 g) were used in the present study. These animals were procured from registered breeder and acquainted in the quarantine area for one week.

Housing of animals:

The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions of 22 ± 2°C temperatures, 50 ± 15 % of relative humidity, 12 h dark/ 12 h light cycle with free access to pellet diet and water provided *ad libitum*. The study protocol was approved form institutional animal ethic committee. The experiments were performed as per as guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Governments of India. The Institutional Animal Ethics Committee approved the study protocol YSPM/YTC/PHARMA-IAEC/48/2020.

PCOS induction:

All the experimental animals except control group were orally administered with letrozole (1 mg/kg) dissolved in 0.5 % w/v CMC solution per oral route for 21 days to induce PCOS. Vaginal smear checked or examined daily and the animals in regular estrous phase were selected for study. Vaginal smears were collected and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS. Two animals from each group were scarified by using CO_2 chamber. Ovaries were removed and observed for presence of cysts [11,12]. In female rats, the estrous cycle characterized by proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrusII) in normal animals. During estrus cyclic differences in vaginal cytology occurs in response to the morphological changes and continuous changes in cell types (leukocytes, nucleated epithelial and cornified epithelial) occurs in PCOS induced animals [8,9].

Treatment groups:

Animals were randomly assigned into six group (Table 1) and adequate supply food and drinking water.

Study design:

The study consisted of 48 female Albino Wistar rats equally divided into 6 groups as group 1 (control

group), group 2 (PCOS induced group), group 3 (Standard group), group 4, 5, and 6 as treatment groups. Following Letrozole administration, standard group was administered with Clomiphene citrate at a dose of 1mg/kg in 0.5 % CMC per oral and treatment group 4, 5, and 6 were administered Ferulic acid with the dose of 10, 20, and 40 mg/kg of body weight respectively in water per oral for 15 days. After 21 days, PCOS control group and after 36 days, animals from other groups were fasted overnight and blood was collected by retro orbital puncture then serum was separated and was used for estimation of hormones, lipid parameters and glucose. Body weight was measured at the end of study (On day 36th) animals were then sacrificed and ovaries were excised, cleaned of fat and weighed [11].

Table 1. Treatment Groups.

Group 1: Control	Healthy rats were administered vehicle (10 ml/kg)
Group 2: Negative control	Animals were administered with Letrozole (1 mg/kg)
Group 3: Positive control	Animals were administered with Letrozole (1 mg/kg) + Clomiphene citrate (1 mg/kg)
Group 4: Test group with low dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (10 mg/kg)
Group 5: Test group with intermediate dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (20 mg/kg)
Group 6: Test group with high dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (40 mg/kg)

Biochemical estimation:

***Measurement of fasting blood glucose*:**

Blood glucose level was measured by using Accu-cheak active glucometer (Roche Diabetes care GmbH Sandhofer Strasse11668305 Mannheim, Germany).

***Hormonal assay*:**

Blood samples were collected by retro-orbital puncture; serum was used for hormonal estimation (FSH, LH and Testosterone). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone was measured via Enzyme Linked Immunosorbent Assay (ELISA) with the help of commercial kits (ELISA kit).

***Lipid profile*:**

The lipid profile (LDL, HDL, Total cholesterol, Triglycerides) was estimated at the end of the study. Lipid profile (LDL, HDL, Total cholesterol, Triglycerides) were quantified by using enzymatic kits procured from Aspen Laboratories pvt, Ltd

***Histopathology*:**

The excised ovaries were fixed in 10 % v/v formalin solution. According to histological procedure, they were subjected to tissue processing by washing with water which was followed by dehydration through ascending grades of alcohol then cleared through xylene. Then paraffin embedding method was used. The blocks were sectioned by using microtome and were placed on slides. These sections were stained with hematoxylene-eosin (HE), dehydrate, cleared and mounted on DPX mount under glass cover slips. The light microscope was used for observation which was connected to a camera to capture image.

Statistical analysis:

The statistical analysis was done by using Graph pad software version 5.0 and results were compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. The results were analysed by Two-way analysis of variance followed by Bonferroni posttests. A p value <0.05 was considered as statistically significant.

RESULTS:

Examination of oestrus cycle:

Fig 1. showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research, Fig 2 demonstrated not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

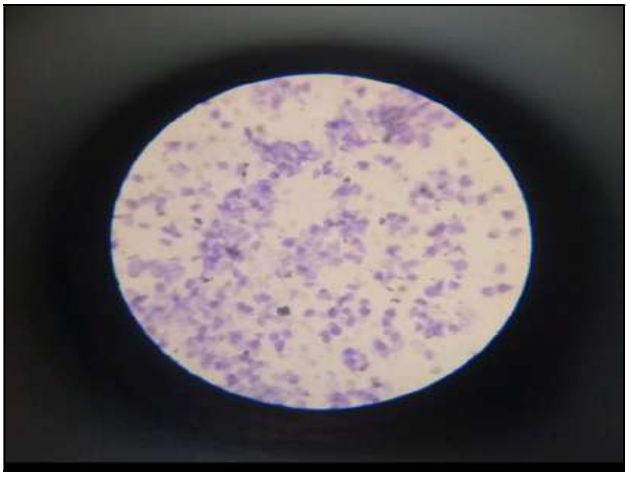

Fig 1. Smear with cornified squamous epithelial cells (Normal animals).

Showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research.

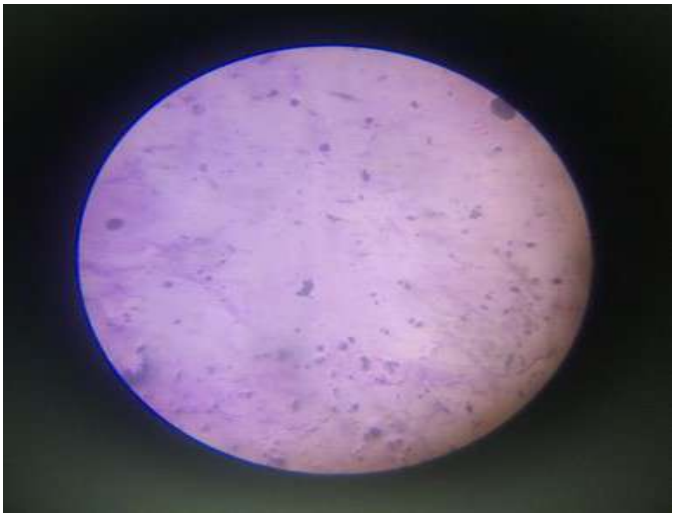

Fig 2. Examination of oestrus cycle (PCOS induced animals).
Not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

Morphology of ovary:

Fig 3 shows Normal ovary structure, where as Fig 4 shows Fluid filled cysts in PCOS induced group.

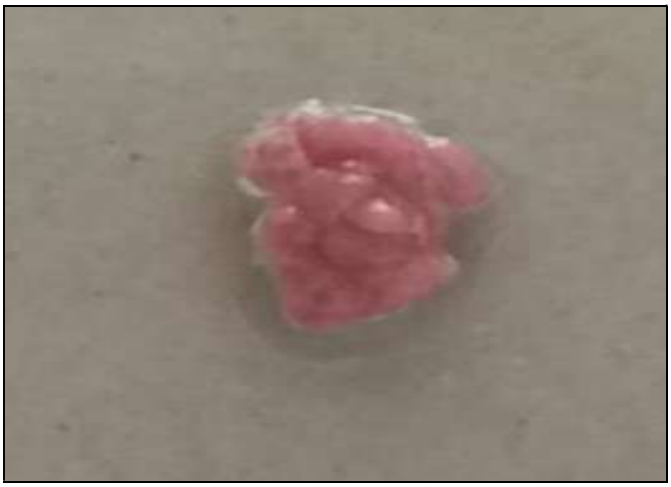

Fig 3. Morphology of ovary (Normal ovary).

Fig 4. Morphology of ovary (Fluid filled cysts in PCOS induced group).

Body weight:

The effect of Ferulic acid on body weight was represented in Fig 5. Letrozole treatment to a significantly increase in body weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.001$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the body weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decreased ($P<0.001$) body weight when compared to Negative control rats.

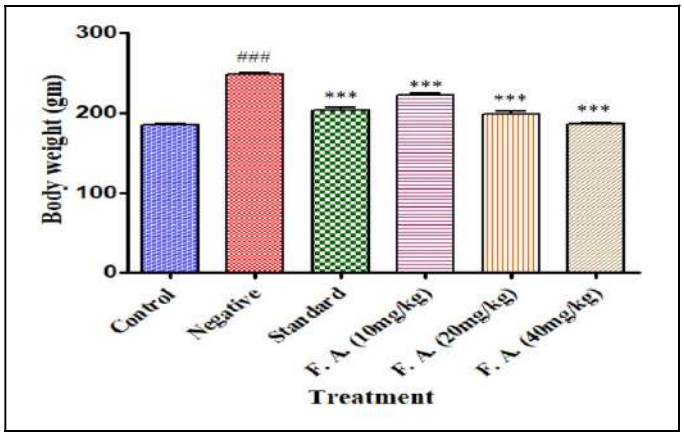

Fig 5. The effect of Ferulic acid on body weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Organ weight:

Letrozole treatment to a significantly increase in ovarian weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.01$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the ovary weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decrease ($P<0.001$) ovary weight when compared to Negative control rats as given in Fig 6.

Serum hormonal profile:

The serum levels of Testosterone and luteinizing hormone (LH) were increased in PCOS induced group ($p<0.001$, $p<0.001$; respectively) while follicle stimulating hormone significantly decreased ($p<0.001$) in comparison to the control group. A significant fall ($p<0.001$) in testosterone levels was observed in standard, low dose, intermediate dose and high dose groups. Treatment with at dose of Ferulic acid 10, 20, 40 mg/kg and standard ($P<0.01$, $p<0.01$, $p<0.001$, and

Table 2. The effect of Ferulic acid on serum hormonal level.

Groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control	0.092 ± 0.003	12.17 ± 0.70	25.67 ± 2.72
Negative	0.140 ± 0.003$^{###}$	19.33 ± 1.25$^{###}$	10.50 ± 0.99$^{###}$
Standard	0.112 ± 0.001***	11.17 ± 0.60***	21.67 ± 0.80***
F. A. (10 mg/kg)	0.119 ± 0.002***	15.0 ± 0.68**	15.33 ± 1.11
F. A. (20 mg/kg)	0.092 ± 0.002***	14.50 ± 0.76**	17.50 ± 0.99*
F. A. (40 mg/kg)	0.083 ± 0.002***	11.17± 0.60***	20.17 ± 0.60***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control. LH and FSH are luteinizing and follicular stimulating hormone.

Table 3. The effect of Ferulic acid on lipid profile.

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
Control	61 ± 1.65	26 ± 1.18	22.17 ± 1.30	82.50 ± 1.97
Negative	102 ± 2.58$^{###}$	14.67 ± 0.66$^{###}$	51.17 ± 2.10$^{###}$	132.80 ± 2.82$^{###}$
Standard	76.67 ± 1.74***	22.67 ± 0.88***	38.67 ± 0.88***	90.83 ± 2.57***
F. A. (10mg/kg)	90.67 ± 1.97**	19.17 ± 1.07*	41.50 ± 0.76**	109.70 ± 2.48***
F. A. (20mg/kg)	71.17 ± 1.35***	21.50 ± 0.76***	37.17 ± 1.32***	90.67 ± 1.97***
F. A. (40mg/kg)	62.50 ± 1.89***	27.67 ± 0.88***	26.67 ± 2.33***	75.67 ± 2.96***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control.

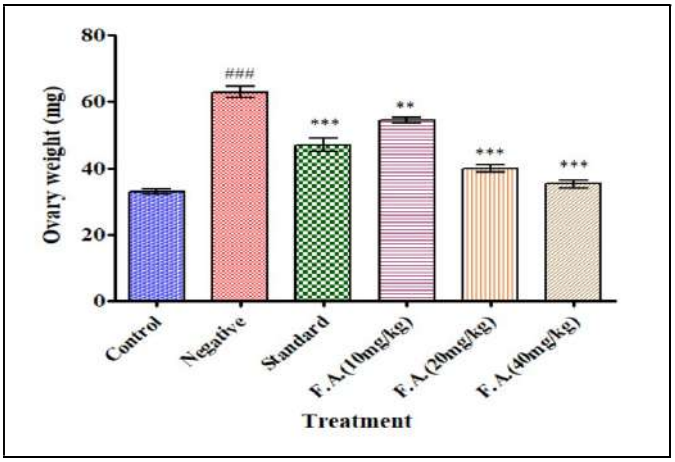

Fig 6. The effect of Ferulic acid on ovarian weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{###}$p<0.001; when compared with normal control. **p<0.01, ***p<0.001; when compared with negative control.

p<0.001; respectively) produced a significant decreased in Luteinizing hormone levels when compared with Negative group. Animals treated with at dose of Ferulic acid 20, 40 mg/kg and standard produced a significant increase (p<0.05, p<0.05, and P<0.001; respectively) in FSH levels when compared with Negative group (Table 2).

Ferulic acid reduces blood glucose level:

The effect of Ferulic acid on blood glucose levels was represented in Fig 7. Letrozole treatment to a significantly increase in blood glucose levels (p<0.001) as compared to control group. Oral treatment with at dose of Ferulic acid 10, 20, 40 mg/kg, for 2 weeks (P<0.001, P<0.001 and P<0.001; respectively) significantly decreased the blood glucose levels in experimental animals while treatment with Clomiphene citrate (1mg/kg) significantly decrease (P<0.001) blood glucose levels when compared to Negative control rats.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed

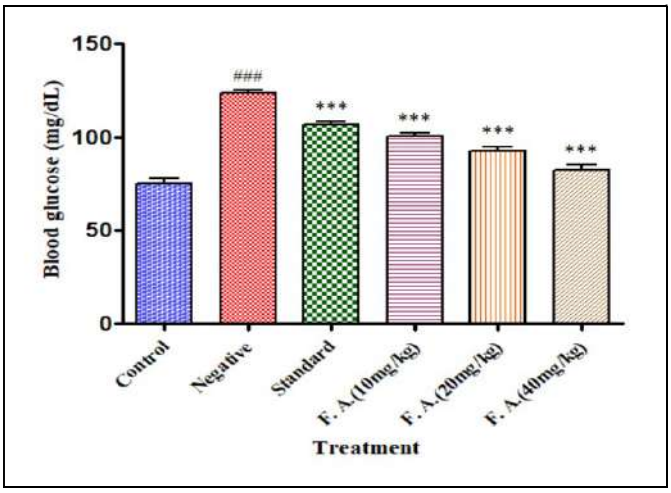

Fig 7. Ferulic acid reduces blood glucose level.
Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed significant changes in serum lipid as compared to control. Cholesterol, LDL and triglyceride were greatly increased as p<0.001, p<0.001 and p<0.001 respectively while HDL levels were decreased (p<0.001) in PCOS induced group (Negative group). Clomiphene treatment significantly decreased Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001) levels when compared to PCOS induced group. While HDL levels significantly increased (p<0.001) when compared to PCOS induced group. Low dose of Ferulic acid (10 mg/kg) decreased the levels of Cholesterol (p<0.01), LDL (p<0.01) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.05) in comparison to negative group. Intermediate dose of Ferulic acid (20 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group. High dose of Ferulic acid (40 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group.

Histomorphological changes

Histopathological examination of stained sections of ovary showed ovarian changes and ovarian follicular cysts (Fig 8). Yellow coloured arrow showing numbers of ovarian follicular cysts. Negative group showing multiple numbers of ovarian follicular cysts compared to normal control group. Oral administration of Clomiphene citrate (1 mg/kg), low dose of Ferulic acid (10 mg/kg), Intermediate dose of Ferulic acid (20 mg/kg), and high dose of Ferulic acid (40 mg/kg) significantly improved or disappearance the number of ovarian follicular cysts compared to negative group.

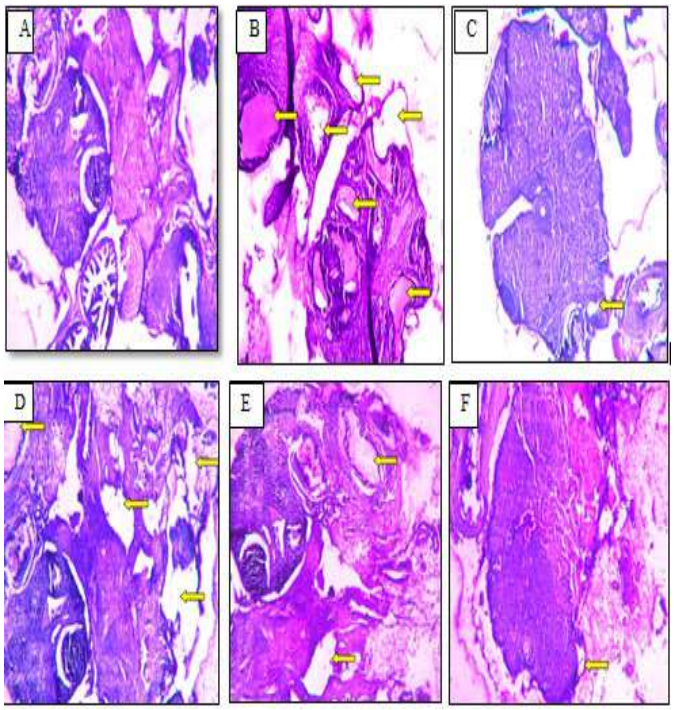

Fig 8. Effect of Ferulic acid in HE-stained ovary tissue (40X).
A. Normal control: showing normal histology of ovary. B. PCOS control: showing large numbers of ovarian follicular cysts. Yellow arrow indicates cysts. C. Letrozole + Clomiphene citrate showing less numbers of cysts. Yellow arrow indicates cysts. D. Letrozole + Ferulic acid (10 mg/kg) showing fewer moderate numbers of cysts. Yellow arrow indicates cysts. E. Letrozole + Ferulic acid (20 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts. F. Letrozole + Ferulic acid (40 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts.

DISCUSSION:

Polycystic ovarian syndrome (PCOS) is major female health problem. It is a chronic metabolic disorder characterized by hyperglycaemia, obesity, excess androgen level, hyperlipidaemia, and decrease FSH level. The World Health Organization estimates that it affects 116 million women worldwide as of 2012 [13]. Various experimental models for PCOS have been developed in rats like administration of testosterone propionate (TP), dehydroepiandrosterone (DHEA), and 5a-dihydrotestoterone (DHT) and Estradiol valerate (EV). It is models fully convincing and identify with the

condition of human PCOS completely [14]. Letrozole is a non-steroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting increased testosterone and decreased E2 production and stimulate PCOS like condition by causing circulating hyperandrogenism, hormonal imbalance, and intra ovarian androgen excess leading to appearance of polycystic ovary. Letrozole induced PCOS was causehyperglycaemic condition which may contribute to insulin resistance, hyperlipidaemia leading to metabolic syndrome [10-15]. Letrozole induce animal model causes polycystic ovarian syndrome in our research study. It is PCOS rat model characterized by an increase in androgen biosynthesis. P450 aromatase enzyme is responsible converting testosterone and androstenedione to estradiol and estrone. This enzyme inhibits activity led to enhance ovarian androgen production or concentration and resulted in PCOS disorder. Due to inhibit of aromatase enzyme activity increases ovarian androgen secretion and resulted into increase level or concentration of testosterone, LH, and FSH, Letrozole treatment showed some metabolic feature, like increased body fat, triglycerides, cholesterol and body weight [10,14]. Ferulic acid showed marked significantly decreased body weight and ovary weight in PCOS rats that may be responsible for reduced the fatty formation, decreasing follicular cysts (follicular fluid). The body weight was considerably reduced by treatment with Ferulic acid (20 and 40 mg/kg). The weight of ovaries in the negative control group was greater than that of normal control group rats. Ferulic acid (20 and 40 mg/kg) treatment significantly decreased ovaries weights which matched to those in control group animals. Type-2 diabetic mellitus and insulin resistant hyperglycaemia are inter-linked with PCOS. Altered insulin levels which can directly stimulate ovarian androgen production in PCOS Insulin stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotrophic hormone (ACTH) and increase pituitary LH release. Increase androgen level cause ovarian cyst. FA improves altered insulin levels, impaired glucose homeostasis and insulin sensitivity [15]. PCOS induced rats showed marked rise in blood glucose level relative to control group. Oral administration of Ferulic acid significantly reduced the increased blood sugar levels, and indicating the beneficial impact of Ferulic acid on insulin resistance and diabetic condition. Women with PCOS are hyperandrogenemic which is associated with alteration in circulating lipoprotein and lipid level resulting in dyslipidemia. Regulation of carbohydrate metabolism, insulin plays important role in the metabolism of lipids. Insulin is inhibitor of lipolysis, since it inhibits the activity of the hormone-sensitive lipases in adipose tissue and increased FFA concentration into the circulation. Increased FFA concentration also raises β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol. FA decreased the levels of FFA, TG, Cholesterol and phospholipids in plasma [16-19]. Characteristically PCOS patient have increased cholesterol level. The women with PCOS tend to be obese probably due to high cholesterol and lipid content. The same effect was seen in current research work after PCOS induction. In comparison with the normal control group, the negative control group reported significantly enhanced LDL, Cholesterol, triglycerides concentration and lowered HDL concentration. Ferulic acid (10, 20, and 40 mg/kg) decreased significantly LDL, cholesterol, triglycerides levels and enhanced HDL level. Ferulic acid displayed beneficial outcome against hyperlipidaemia. In this research, non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This lead in testosterone and LH level increased while FSH level decreased. This imbalanced hormonal level leads to inconsistent oestrus cycle [20,21]. The similar condition has been noted in our research. Letrozole induced rats showed considerably increased levels of testosterone, LH and decreased FSH levels compared to control. Standard drug Clomiphene citrate (1 mg/kg), and Ferulic acid (20 and 40 mg/kg) treated rats showed significantly decreased testosterone, LH level and FSH level increased. The Histopathological report of Letrozole induced rats indicated the existence of polycysts in the ovary. Negative group showed large numbers of ovarian follicular cysts. After treatment with Ferulic acid (20 and 40 mg/kg), decreased or improved numbers of ovarian follicular cysts. All the biochemical and Histopathological parameters in our results advocate the Ferulic acid is most constructive treatment against PCOS.

CONCLUSION:

Treating the various parameters in PCOS induced rats, the impact of Ferulic acid treatment with intermediate (20 mg/kg) and high (40 mg/kg) dose was observed to be similar with standard treatment (Clomiphene citrate). In Letrozole induced PCOS animals, Ferulic acid restored the lipid profile, hormone and glycemic status

as well as ovarian morphology. Ferulic acid might be beneficial in managing PCOS condition due to multiple pharmacological actions like hypoglycemic effects, antihyperlipidemic, anti-inflammatory, protective action against obesity, phytoestrogenic and antioxidant activity. Biological effects of Ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS condition.

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RESEARCH ARTICLE

Role of Aminated Derivatives of Natural Gum in Release Modulating Matrix System of Losartan Potassium

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ABSTRACT

Objective: The investigation aimed to synthesize amino derivatives of various natural gums like Xanthan gum and Tamarind gum for using them as a release modulating polymer in the formulation of the hydrophilic matrix system of losartan potassium and to find the best amongst them. Developing oral sustained release matrix tablets for a drug with a constant release rate has always been a challenge to the pharmaceutical technologist.

Materials and Methods: Release modulating hydrophilic matrix tablets of losartan potassium were prepared by wet granulation method. A total number of 6 formulations of release modulating hydrophilic matrix tablets of losartan potassium were prepared using different polymeric ratios of Carbopol 934, aminated Tamarind gum and aminated Xanthan gum based on preliminary trial bathes. The formulated tablets were evaluated for both pre-compression and post-compression evaluation studies.

Results: Based on in vitro drug release study the effective formulations AXG 3 are shows a maximum similar release profile to other remains formulations with a theoretical drug release profile of losartan potassium for sustained release. Finally optimized formulation AXG 3 containing carbopol 934 (60 mg), aminated xanthan gum (40 mg), MCC (190 mg) and magnesium stearate (10 mg) showed 100±0.024 % drug release in 12 hr which is acceptable with theoretical drug release of losartan potassium for sustain release dose. Conclusion: Aminated derivatives of xanthan gum and Tamarind gum extend the drug release for 12 hr. Based on in vitro drug release studies of formulations, we concluded that the alteration in the concentration of carbopol 934 with an aminated derivative of xanthan gum in sustain release formulation development was more effective and economical.

Keywords

Release modulating matrix tablets; Amination of natural polymers; Aminated xanthan gum; Aminated Tamarind gum; Losartan potassium; Theoretical drug release profile

ABBREVIATIONS: *LSP: losartan potassium; IRD; Immediate release dose; CDR: Cumulative drug release; TDRP: theoretical drug release profile*

Introduction

Polymers are stable on chemical modification(Deore & Khadabadi, 2008). Several chemical modification methods of natural gums have been reported, including amination of Tamarind gum and amination of Xanthan gum reaction, both utilizing the reactivity of hydroxyl groups on the natural polymers(Goswami & Naik, 2014). In the present investigational study, a novel two-step derivatisation procedure was taken on to make aminated tamarind and Xanthan gum. Newly derivatized aminated natural gum contains a huge portion of 1^0 and 2^0 amine groups. The modified natural polymer's derivatives have many potential applications(Chivate et al., 2008). Both modified natural gums derivatives are reactive species in preparation for the release modulation matrix system of the BCS II class drug(Dioscorus et al., 2014; Shukla et al., 2018). Aminated derivatisation of natural polymers increases bioadhesive and drug-release properties(Bassi & Kaur, 2015). In recent times chemical modifications of natural polysaccharides were used to improve the functional properties of natural gums(Ahmad et al., 2019). Reports in the literature show that derivatives of polysaccharides like amine can be used to increase swelling & bioadhesion properties and also drug release (Jain et al., 2008; Mankala et al., 2011; Yeole et al., 2006).

Tamarind gum and Xanthan is neutral, nontoxic and very stable to heat, pH, and shear changes and has near Newtonian flow behaviour (Dey et al., 2011). A Chemical or synthetic transformation procedure of amination is applied to ameliorate the performance of these uncontrollable but reasonable polysaccharides. Cationic polysaccharides have capacious usefulness in drug and gene delivery, and new areas like biologics and fluorescent labelling applications. Hence to meliorate the effectiveness of the Tamarind and Xanthan gum, a cationic structural transformation for complete amination was implemented for synthesis and some of its properties are described in this article. Aminated Tamarind and aminted Xanthan have been developed to enhance the biological characteristics of the native Tamarind gum and Xanthan gum including biocompatibility, hydrophilicity, bioadhesive property, and antibacterial activity. The authors have developed release-modulating matrix systems by using aminated Tamarind and aminted Xanthan combined as efficient carriers for the delivery of anti-hypertensive (losartan potassium) drugs.

Losartan potassium (LSP) is a potent and highly specific angiotensin II type I receptor agonist having antihypertensive activity. LSP was having about 33% of oral bioavailability and plasma elimination half-life in the 1.5 to 2.5 hrs in range(Chopra et al., 2007). Upon administration of LSP in the form of controlled release drug delivery combined with two types of release characteristics which firstly burst release and then extended release over 8hrs(Debnath et al., 2011). These characteristics would be more desirable because they would allow the rapid onset of action and subsequently gives a prolonged antihypertensive effect with the help of maintaining plasma drug concentration above therapeutic concentration(Kalbhare et al., 2020; R. Kumar et al., 2009)

Derivatisation of polymers by the introduction of functional groups leads to an increase in the bioadhesive strength of the derivatized polymer as compared to the inhabitant polymer(Dey et al., 2011). For example, thiolated trimethyl chitosan nanoparticles exhibited 2.1 to 4.7 fold greater mucoadhesion than trimethyl chitosan nanoparticles. These have also been utilized as viscosity enhancers, stabilizers, disintegrates, solubilises, emulsifiers suspending agents, gelling agents and bioadhesive and binders in the above-mentioned dosage form. A sustained drug delivery system of BCS II class drugs has significantly improved the therapeutic efficacy of drugs by using aminted derivatized natural gum(Dumortier et al., 2006). The modified amino derivatives show better antimicrobial activity in comparison to chitosan. Aminated Tamarind and Aminted Xanthan gum are many explanations for the effectiveness of medication dosage forms to improve the bioavailability of the designed dosage form and decrease the frequency of administration to prolong the period of successful blood levels. Modified aminated derivatives of Tamarind gum and Xanthan gum also decrease peak variability by concentration and side effects, and probably improve the effective delivery of the Losartan potassium (LSP).

Materials and Methods

Materials

Losartan potassium (LSP) was provided as a gift sample by Viraj Pharmaceutical. (Mumbai, India). Carbopol 934, magnesium stearate and microcrystalline cellulose were supplied by Thermosil Fine Chem. industry. (Charhol. Tamarind gum and xanthan gum were purchased from Phyto Life sciences Pvt Ltd. (Gandhinagar, Gujarat). Starch, isopropyl alcohol and ethylene diamine are supplied by SD. Fine Chemicals limited. (Mumbai, India). Sodium borohydride was purchased from Karan enterprise. (Mumbai, India). All other reagents and solvents used were of analytical grade and used as received.

Methods

Amination of natural polymers

In 3000 ml water add 60 gm of natural gum. To this solution add aminating agent ethylene diamine (25 ml) with continuous stirring at constant temperature (20-60°C) for (6 hr). Then slowly add the reducing agent sodium borohydride (NaBH4) for 2 hr until the formation of a thick gel. Wash this gel several times with ethyl alcohol and collect the precipitate of aminated derivative. The synthesized aminated polymer was studied under further parameters for the determination of flow

properties, chemical stability and thermal properties(Liu et al., 2008).

Calculation of theoretical drug release profile and fixation of dose

The theoretical sustained release drug profile was calculated based on the pharmacokinetic parameters of the drug. The immediate release dose (IRD) of the drug (drug release at 1st hr) was calculated using the following formula (1).

$$IRD = \frac{C_{ss} \times V_d}{F} \quad \ldots\ldots(1)$$

Where Vd-Volume of distribution; F-Bioavability; C_{ss}-Steady state concentration

Study of release behaviour of aminated derivatives

To assure the consistency of the premix blend, the drug, Carbopol 934 as a binder, and the MCC as a diluent were physically sifted through #40 and well blended. The chosen quantity of derivatized polymers (Aminated TG and Aminated XG), previously passed through #40, were then combined with several drug-diluent premixes for 5 minutes. With the aid of isopropyl alcohol, the premix mixture was wetly granulated. The granules were then sized through #18 and dried at 45 °C for 15 min. Starch and magnesium stearate was used to lubricate the dried LSP granules. The tablets were compressed using the cold compression technique on a calibrated hydraulic press (KBR press) with 12.0 mm, round, flat punches at compressional pressure of 5 tones with a dwell duration of 15 seconds. The average compression weight of the compressed tablets was 400 mg.

Formulation development

Different formulations of Losartan potassium (LSP) 100 mg release modulating matrix tablets were prepared using the following excipients: Aminated TG (30–40 mg), Aminated XG (30–40 mg), Carbopol 934 (60-70 mg), starch (10.25 mg), magnesium stearate (10 mg) and MCC (q.s. to 190 mg). The amounts of ATG and AXG used to prepare each of the 3 in different concentrations, formulations are given in Table no 2.

To maintain the consistency of the premix blend, the drug, Carbopol 934 (as a binder), and the MCC (as a diluent) were physically sifted through #40 and properly blended. The chosen ratio and combinations of hydrophilic polymers (Aminated TG and Aminated XG), which had been previously sorted through #40, were then blended with several drug premixes for 5 minutes. With the aid of isopropyl alcohol, the premix mixture was wetly granulated. The granules were then sized through #18 and dried at 45 °C for 15 min. Starch and magnesium stearate was used to lubricate the dried LSP granules. The tablets were compressed using a cold compression method on a dialed hydraulic press (KBR press) with punches that were 12.0 mm in diameter and flat with a compressional pressure of 5 tones and a dwell duration of 15 seconds(Chopra et al., 2007; Larsen et al., 1973; Mandal et al., 2007).

Table 1. Formulation table for trial batches

Ingredient	Batches				
	T1	T2	T3	T4	T5
LSP* (mg)	100	100	100	100	100
Carbopol 934(mg)	100	75	75	50	50
ATG** (mg)	-	25	-	50	-
AXG*** (mg)	-	-	25	-	50
Microcrystalline cellulose (mg)	190	190	190	190	190
Magnesium Stearate (mg)	10	10	10	10	10
Total weight tablet (mg)	**400**	**400**	**400**	**400**	**400**

Losartan potassium; **Aminated Tamarind gum; * Aminated Xanthan gum*

Table 2. Formulation table of developed formulations

Ingredient	Batches					
	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
LSP	100	100	100	100	100	100
Carbopol 934	70	65	60	70	65	60
ATG	30	35	40	-	-	-
AXG	-	-	-	30	35	40
Magnesium Stearate	10	10	10	10	10	10
Microcrystalline cellulose	190	190	190	190	190	190
The total weight (mg)	**400**	**400**	**400**	**400**	**400**	**400**

**All values expressed in milligram (mg).*

Both trial formulation and developed formulations were studied under further parameters for determination of flow properties, post compression parameters and in vitro drug release study. In vitro drug release profile was compared with therapeutic drug release profile of LSP for sustain release dose. The formulated batch shows maximum similarity with the therapeutic drug release profile which the batch considered for optimization.

Uniformity of drug content

Five tablets of different formulations were weighed separately and powdered. The powder equivalent to the average weight of tablets was weighed and the drug was extracted in methanol, the drug content was determined by measuring the absorbance at 217.0 nm after suitable dilution using a UV Visible Spectrophotometer (UV-1800)(Basu et al., 2011; Yeole et al., 2006).

In vitro drug release studies

Dissolution studies were performed in triplicate, trying to maintain the sink conditions for all the preparations. A 5 ml equal volume of sample was withdrawn at appropriate intervals, filtered and analyzed spectrophotometrically at 205.3 nm. For the formulations, the total per cent drug release was quantified and the drug release data were curve-fitted using MS-excel to evaluate the possible drug release mechanism from swollen hydrophilic matrices(Y. G. Kumar et al., 2013; Mandal et al., 2007).

Swelling and erosion studies

The tablets were slashed, and gently started cleaning using tissue paper to remove surface water after 2 h in 0.1 N HCl and 6 h in phosphate buffer pH6.8, and a Scanning Electron Microscopy (SEM) analysis of the hydrated swelling tablets was performed(Hu et al., 2007). Water uptake and mass loss were determined gravimetrically according to the following equations,

$$\text{Degree of swelling (water uptake)} = \frac{\text{Wet weight} - \text{Original dry weight}}{\text{Original dry weight}} \quad \ldots..(2)$$

$$\text{Erosion (\% mass loss)} = \frac{\text{Original weight} - \text{Remaining dry weight}}{\text{Original weight}} \quad \ldots..(3)$$

Results

Flow properties and physicochemical evaluation of aminated polymers

The bulk density, tapped density and angle of repose of synthesized polymers were increased as compared to natural polymers due to the reason of chemical modification was done in the natural polymer. The compressibility index and Hausner's ratio describe the flow properties of natural polymers and derivatized polymers. Observations as per compressibility index the aminated Tamarind gum shows poor flow properties as compared to natural Tamarind gum. In the case of aminated xanthan gum compressibility index is partially increased and shows good flow properties. The values of Hausner's ratio are < 1.25, which shows good flow. Here all derivatized polymers show good flow properties except aminated Tamarind gum. Evaluation parameters like bulk density, tapped density, angle of repose, compressibility and Hausner's ratio were carried out for the natural polymers and derivatized polymers and were found to be within the limit as given in Table no 3.

Compatibility study of ATG and AXG with Drug

The synthesized modified aminted derivative of Tamarind and Xanthan is characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by the ATR-FTIR study shown in figure 1. The ATR- FTIR study of aminated Tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to the NH_2 group. ATG shows an endothermic peak at 82.40°C and 394.50°C with the heat of flow of 1.002 w/g and 9.231 w/g respectively shown in figure 2 (I). The AXG shows an endothermic peak at 71.74°C and 541.66°C with the heat of flow of 1.644 w/g and 9.424 w/g. All amine derivatives of natural polymers do not show an exothermic peak in DSC thermograms due to the loss of water content in the polymer. The diffraction curve of ATG and AXG was typical of amorphous material with no sharp peaks shown in figure 2 (II).

Calculation of theoretical drug release profile and fixation of sustain release dose

Immediate release dose of drug represents predicted fraction of drug release in 1h was calculated using the formula (1)

$$\text{IRD} = \frac{C_{ss} \times V_d}{F}$$

Where C_{ss} represents steady-state concentration which is calculated by the following formula

$$C_{ss} = \frac{F \times D}{CL \times \tau}$$

Where CL= clearance (liter/kg); D = conventional single dose (50 mg); τ = dosing interval (100 mg bid = 2 h)

Table 3. Flow properties of aminated derivatives of natural gum

Polymers	Bulk Density	Tapped Density	The angle of repose (°)	Compressibility index (%)	Hausner's ratio	pH (1% w/v)
Tamarind gum	0.37	0.41	15.4	9.75	1.1	6.4
Aminated Tamarind gum	0.41	0.55	18.2	25.45	1.34	7
Xanthan gum	0.38	0.45	16.7	15.55	1.18	7.6
Aminated xanthan gum	0.43	0.52	19.1	17.3	1.2	8.2

**All value is mean of triplicate*

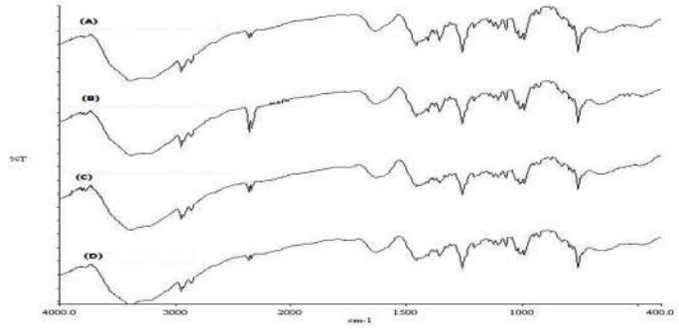

Fig. 1. ATR-FTIR of drug and prepared aminated derivatives **(a) LSP, (b) LSP + ATG, (c) LSP + AXG, (D) Formulation (AXG 3)*

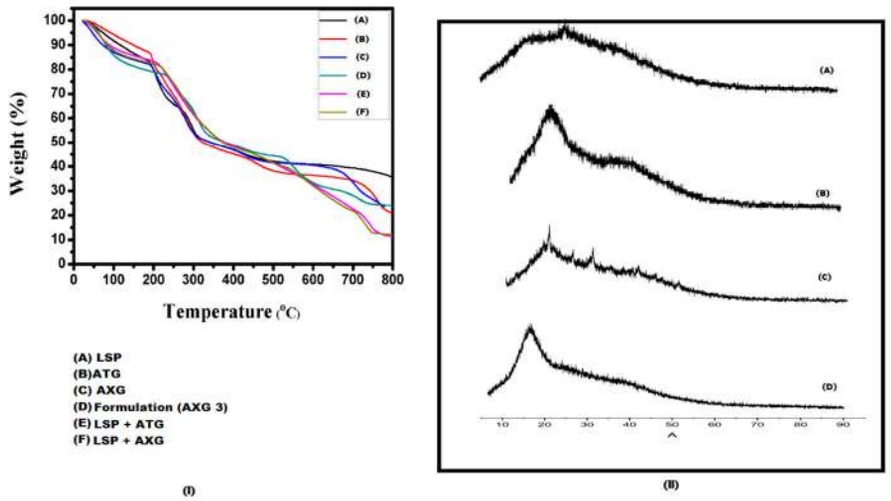

Fig. 2. DSC (I) and XRD (II) of drug and prepared aminated derivatives, *(A) LSP, (B) ATG, (C) AXG, (D) LSP+ ATG, (E) LSP+ ATG, (F)

$$C_{ss} = \frac{33 \times 50}{42 \times 10} = 7.85\text{mg/lit}$$

Now,

$$\text{IRD} = \frac{\text{Vd} \times \text{Css}}{\text{F}} = \frac{34 \times 07.85}{33} = 8.08\text{mg} \sim 50\text{mg}$$

The total dose required to achieve sustained drug delivery from formulation throughout 12 h can be calculated using the following formula,

$$Total\ dose \div MD = 8.08\,[1 + (0.693 \times 2)] = 19.57 \cong 100mg$$

Where $t_{1/2}$= half-life (2 h); t = time up to which sustained action is needed (12 h). Therefore, from a total of 100 mg which was fixed as a sustained release dose of formulation, 19.57 mg (19.57 %) which was considered an immediate release fraction of the drug should release within 1 h to get the therapeutic effect and the remaining 80.83 mg of drug fraction for another 11h at a rate of 19.57 % at each hour. Thus, the theoretical drug release profile can be predicted using the above considerations as shown in figure 3 and is used to compare with the release profile of experimental batches.

CR- Cumulative drug release

Release behaviour of synthesized aminated derivative of natural gum

A matrix tablet of Losartan potassium was prepared by the wet granulation method. Five batches were prepared, consists T1 as a without aminated derivative of natural gum containing carbopol 934 (100 mg). Simultaneously T2, T3, T4 and T5 consist of ATG (30-40 mg), and AXG (30-40 mg) in various concentrations. Powder blends were prepared and subjected to further evaluation. Finally, tablets were compressed using a round flat-faced 12 mm die and punch. The compression force of 5 tones was kept constant for all formulations up to 15 s dwell time.

Evaluation of granules

All formulation shows a compressibility index between 7.7±1.4 to 9.8±1.8. As per the compressibility index, all formulation batches show excellent flow (Table no. 4). Hausner's ratio of granules of trial batches was found to e less than 1.5. All granules of trial batches show excellent flow properties as per Hausner's ratio. The angle of repose of granules in trial batches shows good flow property.

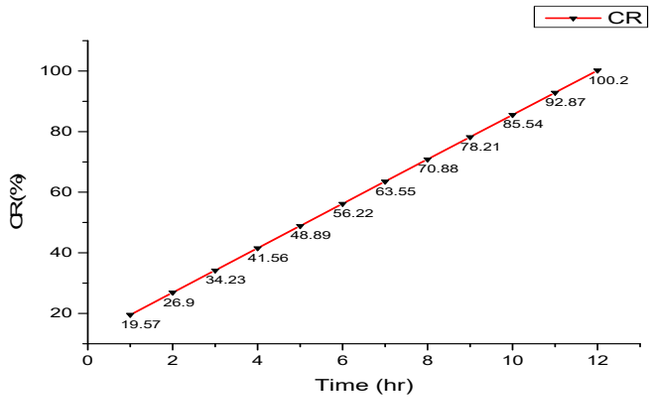

Fig. 3. Theoretical drug release profile of LSP for sustain release dose **CR- Cumulative drug release*

Table 4. Pre-compression parameter study of trial batches

Batch[a]	Bulk density	Tapped density	Compressibility index	Hausner's ratio	Angle of repose
T1	15±0.12	16±0.16	8.1±0.55	1.1±0.0065	28±0.65
T2	15±0.13	16±0.11	7.7±1.4	1.1±0.016	29±0.38
T3	15±0.17	17±0.19	9.8±1.8	1.1±0.022	28±0.37
T4	15±0.13	16±0.19	8±1.8	1.1±0.022	29±0.38
T5	15±0.13	16±0.19	8±1.8	1.1±0.022	29±0.38

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant; [a] preliminary trial batches*

Evaluation of matrix tablet

The thickness and diameter of the tablet were measured by a Vernier calliper. The thickness and diameter of the tablet were found to be in an acceptable range. The hardness of the tablet was measured using a Monsanto hardness tester. The hardness of the tablet was found to be in the range of 4.1±0.058 to 4.8±0.1 (Table no. 5).

In vitro drug release study

In vitro drug release study of release modulating hydrophilic matrix system of losartan potassium was described in table no.6.

Batch T1 contain carbopol 934 (100 mg) and show 71% drug release in 12 hr, this is not acceptable for sustained release delivery of losartan potassium. To overcome the concentration of carbopol 934 in the matrix system, aminated polymers are taken in different ratios. The concentration of carbopol 934 decreases with the concentration of aminated polymers increases. Carbopol 934: ATG / AXG (ratio 3:1) shows 80 % and 86 % drug release in 12 hr respectively, the release profile of T2 and T3 does obey the release profile of LSP for sustain release dose shown in figure 4.

Carbopol 934: ATG/ AXG (ratio 1:1) show 99 % and 100 % drug release respectively. The release profile of T4 and T5 was very different from TDRP. Based on in vitro drug release study of trial batches we concluded that the required drug release profile of formulation is achieved by decreasing concentrations of carbopol 934 i.e. 60 to 70 mg. Concentrations of ATG and AXG extend the release of LSP for 12 hr shown in figure 5.

**T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1; CDR- Cumulative drug release in percentage*

**T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1.*

Preparation of Losartan potassium release modulating matrix tablet

A release modulating matrix tablet of LSP was prepared by the wet granulation method. The concentration of ATG and AXG was selected based on preliminary trial bathes. As per the experimental design, powder blends were prepared and subjected to further evaluation. Finally, tablets were compressed using a round flat-faced 12 mm die and punch. The compression force of 5 tones was kept constant for all formulations up to 15 s dwell time.

Evaluation of granules

Evaluation of granules is used to determine of flow property of granules. In the development of tablet formulation flow property of granules also affect the post-compression parameter of the tablet. The flow property of the tablet is described with various evaluation parameters shown in table no. 8. E.g. Bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index.

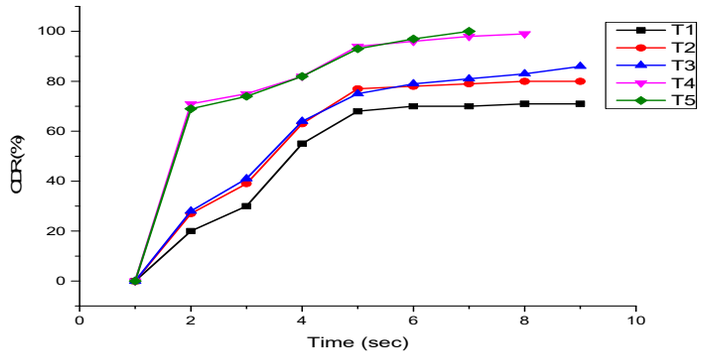

Fig. 4 *In vitro* drug release profile of preliminary trial batches. Drug release extending is directly propositional to the concentration of aminated derivative of natural polymers. **T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1; CDR- Cumulative drug release in percentage*

Table 5 . Post-compression parameter study of trial batches

Batch[a]	Thickness (mm)	diameter (mm)	Hardness (kg/cm2)	Friability (%)	Weight variation	Uniformity of drug content
T1	3.1±0.058	12	4.8±0.1	0.54±0.095	398.95	99±0.36
T2	3.1±0.0	12	4.3±0.15	0.66±0.21	398.6	99±0.36
T3	3.20±0.058	12	4.3±0.058	0.71±0.068	398.95	99±0.45
T4	3.1±0.058	12	4.1±0.058	0.6±0.057	396.15	99±0.47
T5	3.1±0.058	12	4.1±0.058	0.6±0.059	395.65	99±0.21

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant; [a] preliminary trial batches*

Table 6. *In vitro* drug study of trial batches

Time	T1	T2	T3	T4	T5
0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
30	20±0.26	27±0.27	28±1.4	71±2.1	69±2.1
60	30±0.43	39±0.95	41±0.96	75±1.5	74±1.4
120	55±1	63±1	64±0.41	82±0.23	82±0.25
240	68±0.67	77±0.99	75±0.41	94±0.23	93±0.29
360	70±0.66	78±0.98	79±0.41	96±0.23	97±0.25
480	70±0.67	79±0.79	81±0.43	98±0.23	100±0.24
600	71±0.57	80±0.91	83±0.41	99±0.21	-
720	71±0.58	80±0.84	86±0.41	-	-

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

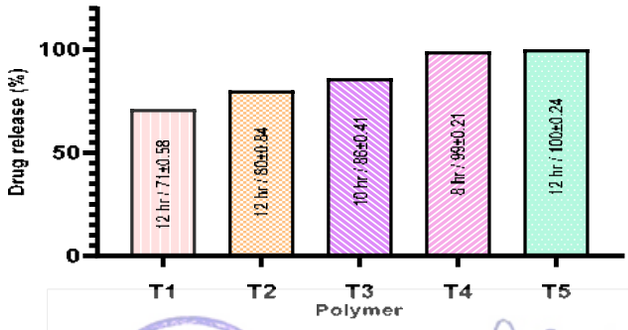

Fig. 5. Comparative study of polymer concentration on drug release of preliminary trial bathes *T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1.

Table 7. Formulation table of development of LSP matrix tablet

Ingredient*	Batches					
	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
LSP	100	100	100	100	100	100
Carbopol 934	70	65	60	70	65	60
ATG	30	35	40	-	-	-
AXG	-	-	-	30	35	40
Magnesium Stearate	10	10	10	10	10	10
Microcrystalline cellulose	190	190	190	190	190	190
The total weight (mg)	400	400	400	400	400	400

**All values expressed in milligram (mg).*

Bulk density and tapped density of prepared granules were found to be 15±0.075 - 15±0.18 and 17±0.12 - 17±0.2 respectively. The per cent compressibility index of granules was found in the excellent range. Hausner's ratio of granules of all batches was found to be less than 1.25, thus all granules show excellent flow properties. Granules of all batches show good flow properties as per angle of repose between 28±0.38 - 29±1.4.

Evaluation of release modulating Losartan potassium matrix tablet

The thickness and diameter of prepared formulations were measured by Vernier calliper. The thickness of the prepared formulation was found to be in the range of 3.1 to 3.2. All formulation show uniform diameters i.e. 12 mm. Hardness of all formulations achieved about the same. Friability of prepared release modulating hydrophilic matrix tablet shows in the range between 0.35±0.11 to 0.36±0.19. The weight variation of formulated tablets was found to be between 396.6 to 399.85, and it is acceptable (Table no. 9).

In vitro drug release study

In vitro drug release study of release modulating hydrophilic matrix tablet was described in table no. 10. In vitro drug release profile of release modulating hydrophilic matrix system was studied in 0.1N HCl for 2 hr and in pH 6.8 PBS for next 10 hr. Batch AXG 3 maximum similarity with predicted TDRP of LSP for sustain release dose. The lowest concentration of carbopol 934(60 mg) and the highest concentration of AXG (40 mg) show extended drug release i.e. about 100 % in 12 hr which is acceptable for sustained delivery of LSP. If the concentration of carbopol 934 was decreased, the rate of drug release was increased for an extended period.

*Cdr- Cumulative Drug Release

Swelling studies

The swelling and erosion functioning of prepared matrix tablet in 0.1N HCl and Phosphate Buffer Solution, pH 6.8, as a function of time, is shown in figure 7. It can be noticed that the hydrophilic matrix tablets undergo both swelling and erosion at the same time. Batch AXG 3 are subjected to swelling and erosion study. Because AXG 3 show a similar dissolution profile with TDRP of LSP for sustained release. Batch AXG 3 show less swelling after up to 2 hr and shows more swelling after 4 hr in both media. Batch AXG 3 contains 40 mg aminated xanthan gum which shows good swelling properties in both mediums. It helps in the development of sustained release formulation of losartan potassium.

After 1 hour, Batch AXG 3 swelled to its full extent, which may be attributed to LSP's first burst release. According to the matrix tablet' simultaneous swelling and erosion, such hydrophilic systems can produce constant release.

Optimization

Optimization of formulations was carried out by comparing the drug release profile of formulation with TDRP of LSP for sustained release dose. The theoretical drug release profile was calculated for a 100 mg dose of LSP for one day. TDRP show 19.57 % drug release at 1 hr was to be significant for the initial burst release of LSP. After the initial burst release, drug release was increased by 7.33 % / hr up to the 12th hr.

AXG 3 shows about similar drug release profile concerning predicted TDRP of LSP for sustained release. ATG 3 shows about 100±0.024 % drug release up to 12th hr, but as per predicted TDRP there are about 100.2 % drug release was required at 12th hr. AXG 3 exhibits greater similarity with TDRP shown in figure 8. So, thus AXG 3 was selected as the best formulation for oral sustain drug delivery of LSP.

Discussion

Reductive amination of native Tamarind and Xanthan was synthesized successfully by using ethylene diamine and sodium borohydride as an aminating agent and reducing agent respectively. The amination reaction was successfully implemented after some of the hydroxyl groups on natural gums were deoxidized. The resulting products showed a great quantity of primary amine group and secondary amine group which can be used as swelling agents. Aminated Tamarind gum shows poor flow properties as compared to aminated Xanthan gum and its native form in the performed investigation. But both derivatized moieties are compatible with a model drug used in the formulation of sustain release matrix tablet. Amination of natural polymers affects the

Table 8. Pre-compression parameter study of release modulating matrix of LP

Batch	Bulk density	Tapped density	Compressibility index	Hausner's ratio	Angle of repose
ATG1	15±0.14	17±0.2	12±0.44	1.1±0.0056	28±0.38
ATG2	15±0.075	17±0.16	11±0.42	1.1±0.0053	29±0.65
ATG3	15±0.15	17±0.19	11±0.62	1.1±0.0079	28±0.38
AXG1	15±0.12	17±0.15	12±1.3	1.1±0.017	29±1.4
AXG2	15±0.18	17±0.12	11±0.89	1.1±0.011	29±0.38
AXG3	15±0.12	17±0.14	12±1.0	1.1±0.013	29±1.4

** All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Table 9. Post-compression parameter study of release modulating matrix of LP

Batch	Thickness (mm)	diameter (mm)	Hardness (kg/cm2)	Friability (%)	Weight variation	Uniformity of drug content
ATG 1	3.2±0.0	12	4.2±0.1	0.36±0.19	396.6	99±0.36
ATG 2	3.1±0.058	12	4.2±0.1	0.35±0.11	398.55	99±0.37
ATG 3	3.1±0.058	12	4.3±0.15	0.31±0.17	399	98±0.55
AXG 1	3.2±0.058	12	4.3±0.15	0.31±0.17	399.85	98±0.36
AXG 2	3.1±0.058	12	4.3±0.058	0.34±0.15	399.8	99±0.27
AXG 3	3.1±0.058	12	4.2±0.058	0.33±0.14	398.55	99±0.45

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Table 10. *In vitro* drug release study of release modulating matrix of LP

Time	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
0 min	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
30 min	29±0.72	31±0.012	15±0.046	29±0.14	20±0.016	15±0.32
1 hr	42±0.13	42±0.51	19±0.095	42±0.56	36±0.64	22±0.38
2 hr	65±0.83	67±0.014	44±0.045	70±0.17	75±0.36	35±0.24
4 hr	80±0.62	85±0.23	58±0.076	81±0.35	86±0.045	51±0.31
6 hr	82±0.77	86±0.025	71±3.3	85±0.56	90±0.084	64±0.38
8 hr	84±0.47	88±0.045	87±0.4	88±0.42	92±0.74	79±0.24
10 hr	86±0.81	89±0.0124	92±0.95	89±0.71	94±0.086	91±0.24
12 hr	86±0.83	91±0.23	97±0.45	92±0.76	96±0.076	100±0.24

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Formulation (AXG 3)

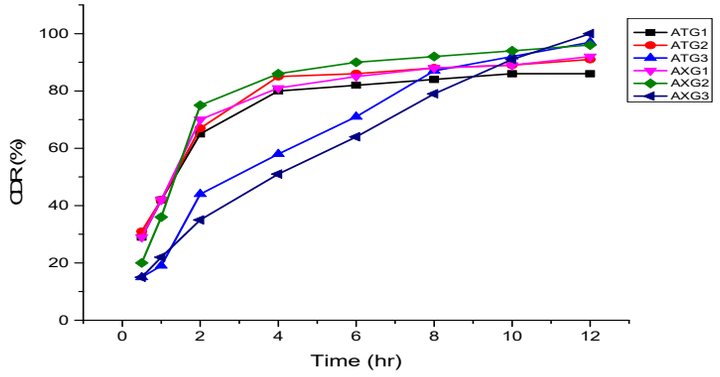

Fig. 6. Dissolution Profile prepared formulation up to 12th hours. AXG3 exhibit minimum burst release up to 2nd hours with a high concentration of AXG. Increasing the concentrations of aminated derivatives of natural polymer are extend the drug release up to 12th hours. **CDR- Cumulative drug release*

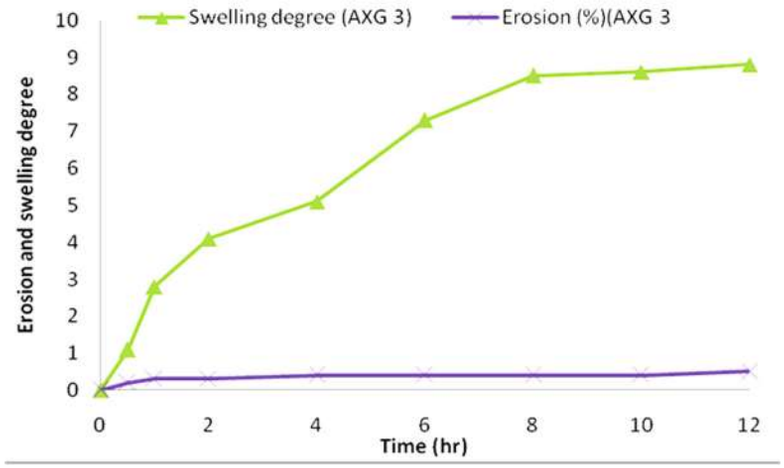

Fig. 7. Erosion and swelling behaviour of optimized formulation i.e. AXG 3 shows good swelling compared with other formulations.

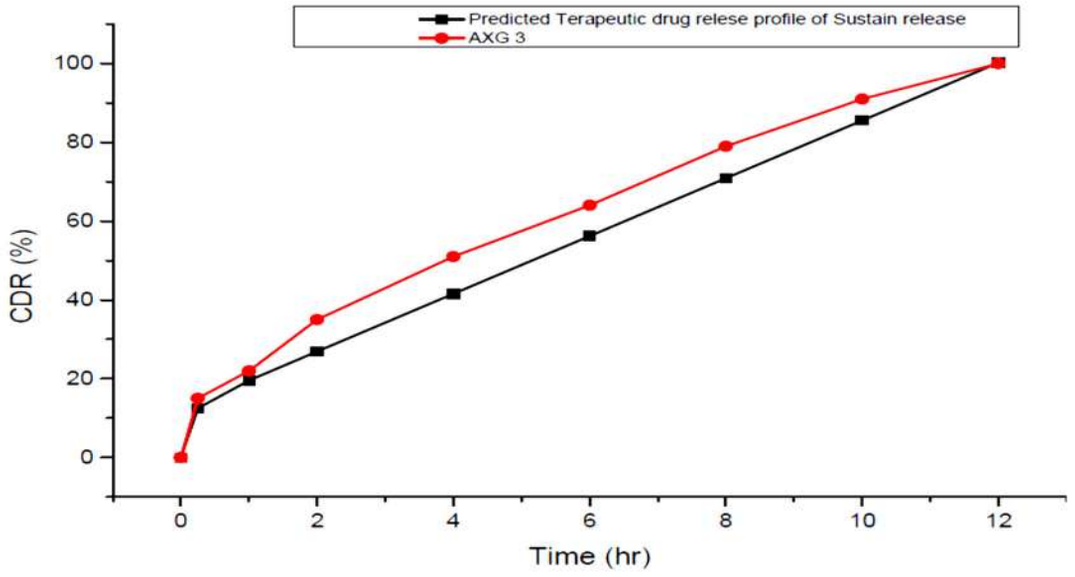

Fig. 8. Dissolution profile of optimized batch AXG 3 compared with predicted TDRP of LSP for sustain release dose, AXG 3 shows maximum similarity with predicted TDRP. **CDR- Cumulative drug release in percentage*

crystallinity of their native form. The amino derivative of natural gum shows amorphous behaviour more than its original form. Physical form-changing properties of native gum after amination exhibit good binding and long-lasting bio-adhesion strength. In the present investigation sustain release matrix tablet formulation was developed using derivatized ATG and AXG as a swelling agent and both derivatized polymers show good adhesive properties. In prepared formulation batches concentration of Carbopol 934 decreased as the concentration of aminated polymers was increased. Hydrophilic matrix tablets of LSP with ATG and AXG were prepared and optimized using a theoretical drug release profile of LSP for sustained release dose. The dissolution profile of optimized formulation AXG 3 was compared with the calculated TDRP of LSP for sustained release dose. On basis of the dissolution profile of optimized formulation, we observed that batch AXG 3 shows maximum similarity with TDRP of LSP for sustain release.

Overall, the study indicates that aminated tamarind gum and aminated Xanthan gum can be promising candidates for designing sustain-release bioadhesive drug delivery systems. Because developed matrix tablets absorb more amount of water and form a sponge-like structure. In this case, the spongy nature of modified gum with the aqueous medium can be applicable for long-lasting bioadhesive formulation. Modified amino derivatives of natural polymers can be promoted excellent mechanical strength to bioadhesive drug delivery for sustained release to obtain constant release from formulation. The formulated smart crosslinked ATG and AXG demonstrated acceptable biodegradability

with no cellular toxicity, suggesting their applicability as pH-sensitive oral drug carriers.

Conclusion

Tamarind gum and Xanthan gum have been functionalized with the amino group. ATG and AXG at very high concentrations (i.e. 40 mg) in prepared matrix form extend the release rate and maintain the constant therapeutic concentration of the drug in the bloodstream. The bonding of the amino group to the ATG and AXG polymers was confirmed by FTIR spectra. The modified amino derivatives of Tamarind and xanthan have the same solubility as native forms. The native form of gum showed a broad melting point around 75-78◦C whereas modified amino derivatives were at 105-115◦C. In addition, modified amino derivatives exhibited good thermal properties. This modified derivative has potential applications in the medical and bio-tronics field because it possesses biocompatibility and strong binding behaviour with a very useful application.

Trial batches of LSP matrix tablets give an idea about formulation development. A high concentration of carbopol 934 (100 mg) shows unnecessary drug release behaviour as compared to TDRP of LSP for sustained release dose. Thus, synthesized aminated derivatives of TG and XG are subjected to formulation to minimize the concentration of carbopol 934. Carbopol 934: ATG / AXG is incorporated in the formulation in different ratios i.e. 3:1 and 1:1. Here low concentration of aminated derivatives in matrix tablet of LP show about 70-72 % drug release for 12 hr and high concentration of aminated derivatives show about 100 % drug release in 8-10 hr. Based on trial batches we concluded that, for obtaining similar TDRP of prepared formulation, the concentration of carbopol 934 (70-60 mg), ATG (30-40 mg) and AXG (30-40 mg) was selected.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

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Creation and Development of Promethazine (PT) Fast Dissolving Tablet Using Quality by Design Methodology

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ABSTRACT

Patients continue to choose the oral route for medicine delivery over other available dosage forms. According to the research, over half of patients will eventually favor oral disintegrating tablets (ODTs) over alternative solid oral dose forms. The oral route is the most favored for administering medication since it has several advantages over other routes. Patients get a variety of pharmacological therapeutic substances used in ODTs to spread their effects throughout the body. Because of the extremely high risk of aspiration and difficulty swallowing, ODTs are recommended for asthma patients. In addition to the restrictions listed above, it is ideal when the patient is on the go or has limited access to water. For the creation of ODTs, several patented and unpatented preparation techniques or technologies are available. Promethazine (PT), a first-generation H1 receptor antagonist, is a medication used to treat motion sickness that is also used as an antihistamine and antiemetic. Due to PT's 2.2-hour half-life and first-pass hepatic degradation, its 88 percent oral bioavailability has been decreased to 27%. As a result, efforts have been undertaken to create tablets that dissolve quickly using the direct compression technique. The tablets were created using the quality by design (QbD) method to successfully transfer technology. Nowadays, risk management for successful QbD has become essential for product approval as the FDA evaluates the execution and effectiveness of the procedure, formulation design as detailed in the application, and QbD.

Keywords- Formulation design technology, H1 receptor antagonist, Oral disintegrating tablets, Promethazine, Quality by design approach (QbD)

INTRODUCTION

Patients continue to choose the oral route for medicine delivery over other available dosage forms. The research assumes that over 50% of patients will favor ODTs over other solid oral dosage forms in the future. Because it has several advantages over other routes, taking medication orally is the preferred method [1]. Due to the extremely high risk of aspiration and difficulty swallowing, ODTs are recommended for asthma patients [1, 2]. In addition to the restrictions described above, it is desirable when the patient is on the go or has limited access to water [2]. It is recommended since it is simple and accessible to a variety of patients.

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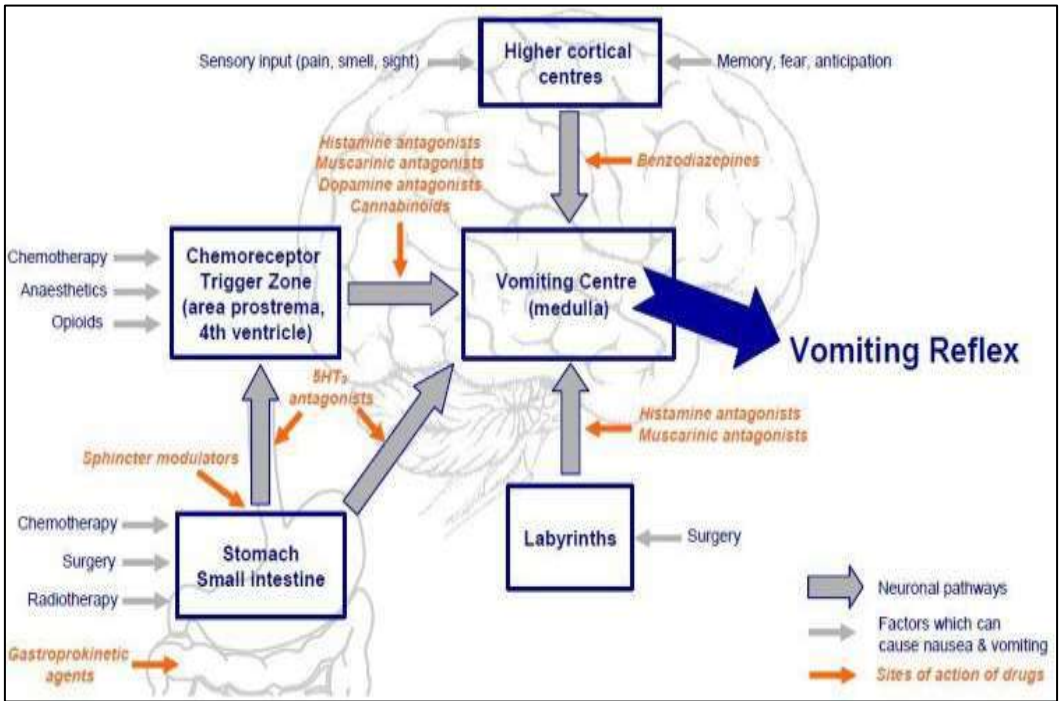

Figure 1: *Medication for nausea and vomiting.*

For the creation of ODTs, several patented and unpatented preparation techniques or technologies are available (Fig. 1). In addition to more traditional methods like tablet molding, direct compression, mass extrusion, spray drying, sublimation, and cotton candy process, patent-protected methods like Zydis technology, Orasolv technology, Durasolv technology, Wowtab technology, Flash tab technology, Flashdose technology are used. These strategies are shown in Table 1.

Table 1: *Tablets that dissolve in the mouth using patented technology.*

Sr. No.	Techniques	Involved Molecule	Process	Firm
1	WOWTAB®	Famotidine	Direct compression	Yamanouchi Pharma Technologies, 1050 Arastradero Road, Palo Alto, CA, USA
2	ORASOLV®	Paracetamol	Direct compression	Cima Labs, Inc., 10000 Valley Hill Road, Eden Prairies, MN, USA
3	DURASOLV®	Zolmitriptan	Direct compression	Cima Labs, Inc., 10000 Valley Hill Road, Eden Prairies, MN, USA
4	FLASHTAB®	Ibuprofen	Direct compression	Prographarm, haueauneuf- En-Thymeraia, France
5	LYOC®	Phlorglucinol hydrate	Lyophilization	Farmalyoc, 5AV Charles Marting, Maisons- Alfort, France
6	QUICKSOLV®	Risperidone	Lyophilization	Janssen Pharmaceutica, 1125 Trenton-Harbourton Road, Titusville, NJ, USA
7	ZYDIS®	Loratidine	Lyophilization	R. P. Scherer, Frankland Road, Swindon, UK
8	FLASHDOSE®	Tramodol hydrochloride	Cotton Candy Process	Fuisz Technologies, 14555 Avion At Lakeside, Chantilly, VA, USA

As the size of the SPH microparticle reduced, it was discovered that the micro particle's tensile strength was very low or even decreased. The produced ketoprofen-loaded FDTs' tensile strength and period of disintegration were both significantly influenced by the size of the SPH microparticle [4]. However, in certain instances, it was discovered that when the size of the microparticle was reduced, the tensile strength of the produced FDTs rose. The microparticle, which ranged in size from 75 to 106 µm, was found to disintegrate in less than a minute. It was recommended that the ideal microparticle size should fall between 75 and 106 µm. It has been recommended to employ single disintegrants or to utilize a mixture of disintegrants in a patent that has been published [5].

It can also be made better by adding more insoluble inorganic excipients and calcium salts such as dibasic calcium phosphate [6]. Components including flavoring agents, compressible sugars, binders, sweeteners, and surfactants are considerably soluble. The time it takes for the tablets to dissolve depends on the weight ratio between the water-soluble and water-insoluble excipients. Organic filler or insoluble inorganic salt are examples of excipients. As the proportion of insoluble components decreases, it has been claimed that the disintegration time would increase extremely quickly. The proportion of insoluble inorganic components employed in conjunction with disintegrants, they observed, had an impact on how easily manufactured FDTs disintegrated. The ideal excipients for this are those that are naturally water soluble [6, 7].

A key property of oral medication delivery systems is their palatability. In addition to maintaining the mechanical strength needed for tablet manufacture and distribution, it also disintegrates at a quick rate. It causes ODTs to continue to expand, which allows them to disintegrate at a very high and quick rate [8, 9]. Different kinds of sweetening and flavoring additives are needed to cover up the drug's harsh taste. It depends on how well-liked or effective ODTs are, which is further decided by the intended patients. ODTs have a quicker or faster beginning of action as a result of their diffusion in the upper section of the GIT [8]. One of the greatest coating methods for effectively disguising the bad taste of APIs is known as Coacervation. It has been claimed that utilizing monoglycerides is a unique way to disguise the taste of macrolides. In addition to masking the flavor, it accelerates the pace at which the APIs dissolve and disintegrate. The choice of ingredients used to make ODTs relies on how unpleasant or bitter the medications taste. Various techniques, including solvent evaporation and solvent extraction, are employed to improve the flavor of drugs. For the microencapsulation of medications, several pH-sensitive acrylic polymers, including Eudragit L-55, Eudragit E, and Eudragit RL, are used. These polymers are used to mask the disagreeable taste of the medication. The flavor of the medication is covered up using the microencapsulation technique. Several taste masking techniques are used to hide or disguise the unpleasant, bitter, or horrible taste of the medication [10].

The failure of therapy occurs when medications with limited bioavailability are partially lost through vomiting. Generally, nausea precedes vomiting, making it challenging to deliver medication with a glass of water. As a result, it is preferable to administer medications as fast-dissolving pills. The retention and absorption of the medicine are also impacted by dizziness, pregnancy, migraines, physiological processes including reduced stomach emptying, and other gastric problems [11, 12]. As a result, maintaining the oral dosage is necessary for absorption to prevent vomiting. The diaphragm must compress downward to violently vomit. As a result, there is poor bioavailability, which does not reduce the rate. The sphincter remains open when the abdominal muscles contract against a relaxed stomach. Emesis is only a symptom of changed physiological processes; it is not an illness. After oral dosing, this antiemetic has significant first-pass gastrointestinal activity [13].

MATERIALS AND METHODS

A first-generation antihistamine called promethazine (PT) is used to treat allergies, motion sickness, and nausea. Promethazine sulfoxide is the main product of PT metabolism, with desmethylpromethazine and a hydroxy metabolite coming in second and third place. Croscarmellose sodium, Lactose monohydrate (Pharmatose 200), Magnesium Stearate, Microcrystalline Cellulose, and PT for this current work were supplied by Abbott Pharma, India, DFE Pharma, India, Peter Greven, India, and Merck, India, respectively.

Before formulating API, preformulation tests were carried out to determine the properties, identification, and stability of the medication in the formulation [14]. To determine the purity and describe the likely structural alteration of the drug sample, infrared spectroscopy was used to evaluate the PT sample. With the use of an infrared spectrophotometer and Fourier transforms, sample spectra were performed between 4000 and 400 cm^{-1}. To reduce peak interference caused by functional groups contained in KBr, a blank KBR spectrum was conducted. The drug sample and blank KBR were combined in a 1:9 ratio before being poured into the cavity. The change in the appearance of these mixes was examined physically. An early risk reduction method is a medication and excipient compatibility study. For 15 to 20 minutes, potassium bromide (KBr) was allowed to activate in a hot air oven [15]. Excipients that could interact with the drug ingredient are not allowed to be used. An early risk reduction method is a medication and excipient compatibility study. Excipients that could interact with the drug ingredient are not allowed to be used. PT was triturated at a 1:1 ratio with each excipient separately. The samples were kept at 40°C and 75% RH for 4 weeks. To verify changes in appearance, these mixes were physically evaluated [16].

Formulation and Development of PT-FDT by QbD Approach

QTPP (Quality target product profile) (Quality target product profile) an outline of the qualities of a drug product's quality that should ideally be attained to assure the intended quality, taking into account the medication product's safety and efficacy. Avomine® (oral tablet), a chosen RLD (Reference Listed Drug), was used to identify QTPP for tablet formulation and development [17]. Table 2 provides a summary of the main QTPP constituents, including identification, mode of administration, dose, dosage form, dissolution, and disintegration.

Table 2: *QTPP of a tablet.*

QTTP Component	Focus	Explanation
Physical		
Route of administration	Oral	Requirement for pharmaceutical equivalence: same method of administration
Dosage form	Tablet	Requirement for pharmaceutical equivalence: identical dosage form
Dosage strength	25mg	Requirements for pharmaceutical equivalents: same strength
Therapeutic moiety release/delivery	Fast dissolving tablet	To fulfill label claims comparable to the references
QTTP Component	**Focus**	**Explanation**
Chemical		
Assay	Not less than 90% and not more than 110% of the labeled amount of PT	Required to maintain safety and efficacy.
Disintegration time	NMT 30 sec	The drug profile is important to achieving bioequivalence (BE).
Dissolution Profile: pH 6.8 USP Type II, Volume:900mL, Speed: 50 rpm, Time points(min): 5, 10, 15, 20, 30 and 45	NLT 80% (Q) labeled amount of PT dissolved in 15 minutes.	To achieve bioequivalence, the pharmacological profile is crucial (BE). A similar drug release profile with a reference product is sought after to guarantee bioequivalence since in vitro drug release serves as a proxy for in vivo performance.
Biological		
Intended use	Intended to treat people	Therapeutic equivalence requirement:

	with non-valvular atrial fibrillation and lower their risk of stroke and systemic embolism.	Same indication.
Packaging and Storage		
Container closure system	The container closure mechanism was determined to be appropriate for this pharmaceutical medication.	Required to maintain tablet integrity throughout transportation and to reach the desired shelf-life.
Storage condition	Away from direct sunshine, in a cool, dry environment, and at room temperature.	Room temperature in a cool, dry location away from the sunlight.

Innovator Product Characterization

The RLD selected was Avomine®. The analysis of RLD was carried out. Product identification of RLD is given in Table 3.

Table 3: *Product identification of RLD Avomine®.*

Parameter	Description
Batch No.	31171043
Mfg. Date	SEP.2020
Exp. Date	AUG 2022
Label claim	Each tablet contains 25 mg of PT

Table 4: *PT-FDT CQAs (Critical Quality Attributes).*

Characteristics of an Effective Drug Product	Focus	Is it CQA?	Explanation
Disintegration time	similar to RLD	**YES**	To be comparable with the innovator Avomine ®
Dissolution	NLT 80% of the Labeled amount of Drug should dissolve in 15 min	**YES**	To achieve bioequivalence, the pharmacological profile is crucial (BE). Since in vitro drug release serves as a proxy for in vivo performance, a reference product's drug release profile should be the goal to verify bioequivalence.
Palatability	Sweet taste	**YES**	Patient compliance

Table 5: *Overview of the preliminary risk analysis.*

Low	An acceptable level of danger. No more research is required.
Medium	Risk acceptance It could be necessary to do further research to lower the danger.
High	Risk cannot be tolerated. To lower the danger, further research is required.

Table 6: *Initial risk analysis.*

Drug Product CQAs	Level of CCS	Level of Magnesium Stearate	Hardness
Disintegration time	High	Low	High
Dissolution	Low	Medium	High

Identification of CQA's

CQAs was identified based on the effect of quality attribute safety, and efficacy of the formulation on a patient. Table 4 shows the CQAs for PT tablets.

Initial Risk Analysis

To find any possible interactions between the medicine, excipients, different unit activities, and key features, a preliminary risk assessment was conducted. The goal was to identify the damaging event, its origin, the likelihood that it will occur, its effect, and a method for detecting it [18].

DESIGN OF EXPERIMENT (DOE)

Excipient selections were made following preliminary tests using various ratios. The CQAs were influenced by the CCS level, magnesium stearate content, and tablet hardness. The optimization experiments were chosen from randomized blind batches (Table 5 & 6). The optimization investigations used a Box-Behnken design [19]. Researchers looked at how excipients affected CQAs for medicinal products at different concentrations. Magnesium stearate (B), CCS (A), and hardness (C) were chosen as independent variables (Table 7).

Table 7: *Coded levels for Box-Behnken design.*

Sr. No	Independent Variable	Level		
		-1	0	+1
1	Level of CCS (%)	4	5	6
2	Magnesium stearate (%)	0.5	1	1.5
3	Hardness (N)	50	100	150

Characterization of Tablet

Pre-Compression Characterization

Bulk Density

A compound's bulk density varies greatly depending on the method of crystallization, grinding, or formulation. It is determined by using a large funnel to pour the pre-sieved powder into a graduated cylinder, then determining the extent and weight using the formula in equation 1.

$$\text{Bulk density} = \frac{\text{weight of powder}}{\text{bulk volume of powder}} (1)$$

Tapped Density

A graded tape is used to calculate the tapped density, which may then be estimated using equation 2.

$$\text{Tapped density} = \frac{\text{weight of powder}}{\text{tapped volume of powder}} (2)$$

The volume of the powder bed must achieve a minimum volume in a cylinder with a specified powder mass and a mechanical extraction device that runs for a predetermined number of taps (10.500.1250). It is possible to gauge thread density.

Carr's Index

Bulk density and tapped density measurements are used to calculate Carr's index. Carr's index is determined using the following equation (3).

$$CI = \frac{(Dt-Db)}{Dt} x100 (3)$$

Where, D_t = Tapped density and D_b = Bulk density

Hausner's Ratio

The flowability and packing capacity are shown by Hausner's ratio. The capacity of materials to flow and pack is satisfactory when Hausner's ratio is near 1. The following equation 4 was used to compute Hausner's ratio.

$$\text{Hausner's ratio} = \frac{Dt}{Db} (4)$$

Where, D_t = Tapped density and D_b = Bulk density

Table 8: *Acceptable limits for flow properties.*

Parameter of Compressibility	Flow Nature	Hausner's Ratio
1 – 10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very Poor	1.46 – 1.59
> 38	Very very poor	> 1.60

Tablet Evaluation

Weight Variation

It is ideal for the weight of each pill in a batch to be consistent. If there is any weight variance, it should be within the permitted ranges (Table 8). Each trial's 20 core pills were weighed on a calibrated scale to check for weight fluctuation and compare the results to the acceptable thresholds [20] (Table 9).

Table 9: *USP's guidelines for acceptable variations in tablet weight.*

The Average Tablets Weight (mg)	Maximum Percentage Difference Permitted
130 or less	±10
130-324	±7.5
More than 324	±5

Hardness and Thickness

The only dimension linked to the procedure is the tablet's thickness and hardness. The Erweka Hardness Tester was used to determine the tablets' hardness and thickness.

Disintegration Time

The disintegration time of 6 core tablets of each trial was checked and compared with the limit mentioned in USP.

In-Vitro Dissolution Studies

With sodium phosphate buffer pH 6.8, 50 rpm, and USP type II equipment, in vitro release tests of produced PT-FDT were carried out at 5, 10, 15, 20, 30, and 45 min (ELECTROLAB). To keep the sink condition, an aliquot (5 ml) was removed and replaced with a new medium. With the use of a double-beam UV visible spectrophotometer and a dissolving media used as a blank, filtered samples were properly diluted before being examined at 249 nm. Using a calibration curve created from a reference standard, the quantity of drug contained in the samples was determined. RLD and the drug product were contrasted to evaluate the drug release profile and physicochemical characteristics of the drug product [21].

Updated Risk

Based on the CQAs and initial risk assessments for CMA's the above risk for DOE will be updated. Further control strategy will be developed for the product followed by continuous product development [22].

Design Space

It has a huge selection of verified process parameters that guarantee quality. The alternative draws attention to the intricate relationships between input variables and consciously makes a connection between the established hierarchy of a design space and the DoE's behavior, which is mostly made up of interactions between input variables. A design space may be built for one unit operation, a few unit operations, or the full method [23].

Control Strategy

The range of recognized and proven process parameters used for quality control makes up the design space. You may set up a design room for the whole process, a few units, or one single unit.

Stability Study

According to ICH recommendations, the stability study was conducted on the improved

formulation at 40°C and 75% RH for three months. A dissolving study was conducted to test the tablets [24].

RESULTS AND DISCUSSION

Infrared spectroscopy was used to investigate the PT sample to determine its purity and if any, any structural modifications. The material was examined between 4000 and 400 cm-1. The FTIR spectrum displayed fundamental peaks in the functional group-corresponding region. The presence of primary peaks in the spectrum proves that the sample used for testing was the PT shown in Fig. 2. The 10 g/ml solution's UV spectra were captured between 400 and 200 nm. At 249 nm, the greatest absorption was discovered. The absorbance values were measured using a double-beam UV spectrophotometer at various doses (Fig. 3). Fig. 4 shows a graph of absorbance vs. concentration. These graphs, which follow Beer-law, and Lambert's were linear in the concentration range of 2–25 µg/ml.

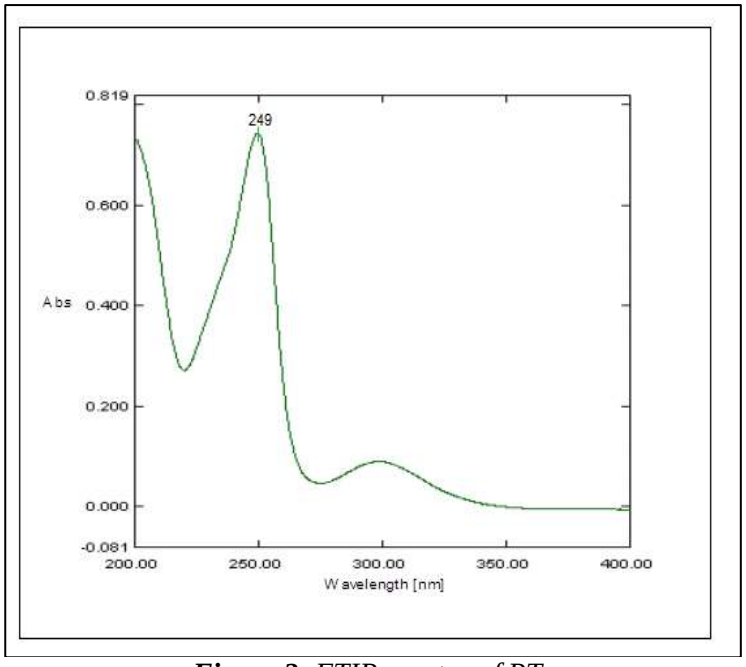

Figure 2: *FTIR spectra of PT.*

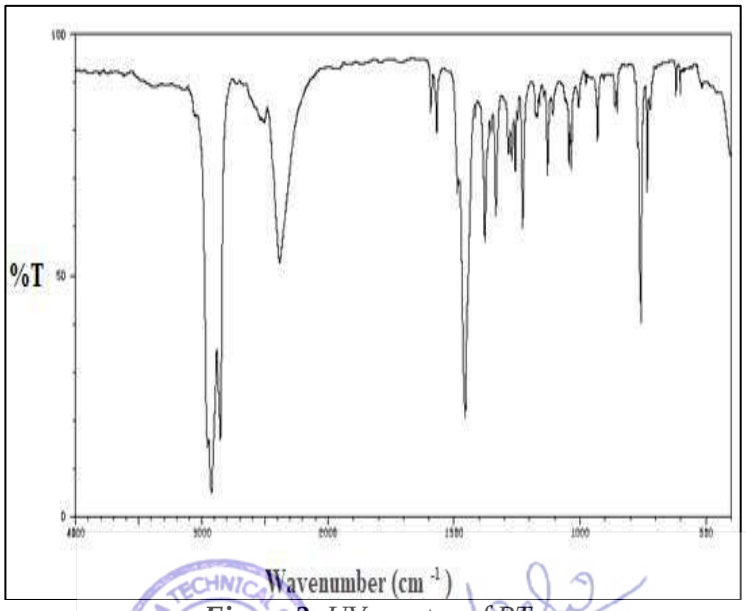

Figure 3: *UV spectra of PT.*

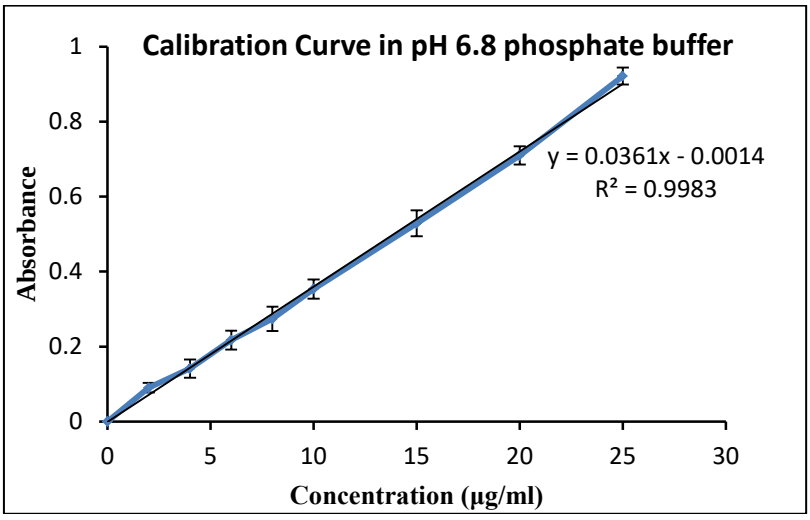

Figure 4: *Calibration curve of PT in pH 6.8 phosphate buffer.*

The physical examination of drug-excipient compatibility showed no change in the appearance of the drug with all the excipients in dry form as represented in Table 10. Thus all the selected excipients were compatible with the drug.

Table 10: *Drug and excipient compatibility study.*

Sr. No.	Sample	Initial Assessment	40°C/75%RH and After (4 weeks)
1	PT	White to off-white powder	White to off-white powder
2	PT+ Lactose monohydrate	White to off-white powder	White to off-white powder
3	PT+ CCS	White to off-white powder	White to off-white powder
4	PT+MCC	White to off-white powder	White to off-white powder
5	PT+ Mg stearate	White to off-white powder	White to off-white powder

Table 11: *According to the experiment's design, tablet batches (DoE).*

Run	Factor 1 A: Level of CCS (%)	Factor 2 B: Level of Mg Stearate (%)	Factor 3 C: Hardness (N)
1	6	1	50
2	4	1.5	100
3	4	0.5	100
4	5	1.5	50
5	5	0.5	50
6	4	1	50
7	6	0.5	100
8	6	1.5	100
9	5	0.5	150
10	5	1.5	150
11	6	1	150
12	4	1	150

An examination was conducted before and after compression. The bulk density, tapped density, Carr's index, and Hausner's ratio of each trial batch were evaluated and contrasted with USP standards. All batches of powder were discovered to have fair to good flow qualities based on their micrometric properties. The tablets' disintegration time, weight fluctuation, hardness, thickness, and diameter were assessed, and the results were good compared to the goal characteristics (Table 11). The angles of repose, Carr's index, and Hausner's ratio have corresponding ranges that indicate good-excellent, good, and fair-good flow characteristics [25].

Studies on *in-vitro* dissolution: Each trial batch's PT-FDT dissolution was conducted individually under prescribed protocol, and drug release profiles were computed using a standard absorbance (Fig. 5). The batch with greatest r2 (0.9991) value and release kinetics that was closest to those of Avomine® was deemed to be the optimal batch. When compared to Avomine®, it too exhibited a similarity factor of 57 (f2=57).

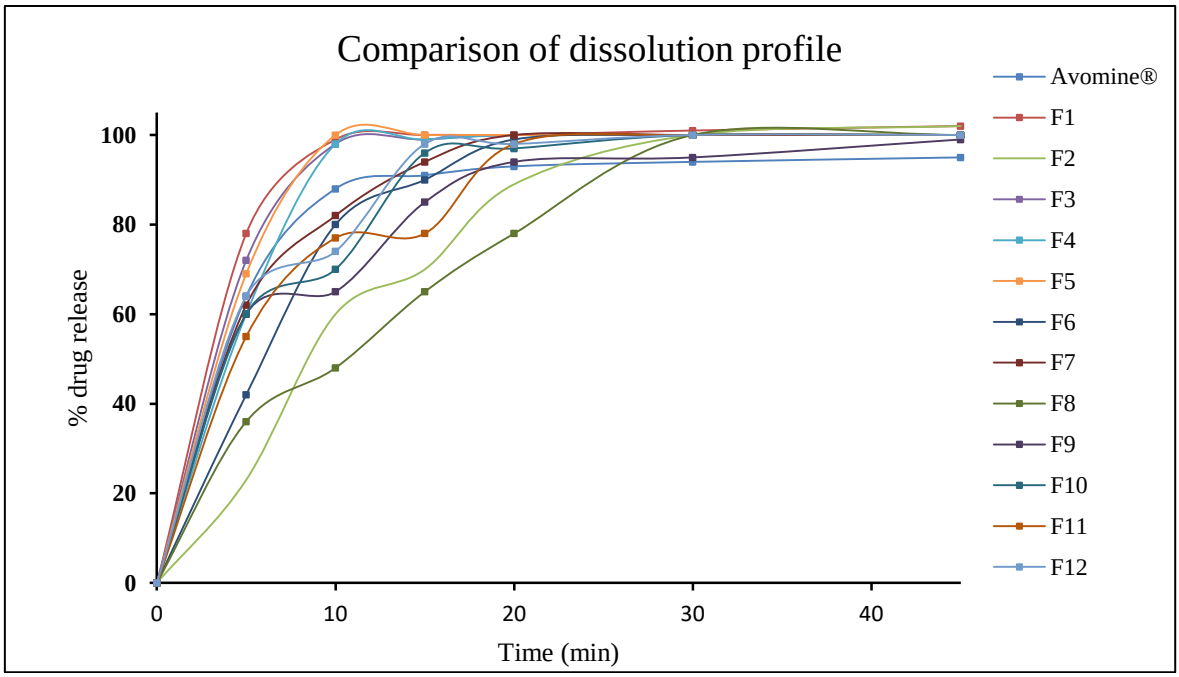

Figure 5: *Comparison of DoE batch in-vitro dissolution profiles.*

Assessment of Design of Experiment

The time it took for a tablet to completely dissolve was determined as Y1 (time taken to dissolve tablet entirely), and the dissolution rate (%DR) at the end of five minutes was computed as Y2 (dissolution rate). The %DR at 5 min was selected as the Y2 since there was a significant difference between the values that were gathered. Equations (1) and (2) were utilized in this design to determine the importance of each coefficient on main effects and interaction terms using p values and the appropriate polynomial model (2). The effect of the associated independent variable(s) is significant if the p-value is less than 0.05, which makes the corresponding coefficient more significant. A regression study of the rates of disintegration and dissolution's determination coefficients (R2) (0.99) showed that this design's strong correlation between the independent parameters accounts for 99% of the overall variations. A positive value for a response in the regression equation indicates a synergistic influence (direct link), whereas a negative value for a response indicates an inhibitory action (inverse relationship) on the regression model [26].

$$Y_1=8.8475-1.58875A-0.50875B+4.56C+0.5375AB+0.695AC+0.15BC-1.2675A^2-1.3775B^2 \quad (1)$$

$$Y_2=50.75+5A-1.75B-15C+2.25AB+10.75AC+0.25BC+7.5A^2+1.5B^2 \quad (2)$$

The average disintegration time and drug release at 5 minutes (Y1 and Y2), as shown in eqs. (1) and (2), were 8.84 and 50.75, respectively. It was discovered that the answer Y1's positive coefficient of C was bigger than its negative coefficient of A, showing that the degree of CCS has an inverse relationship with DT whereas hardness has a direct relationship with DT. The lower values of the AB, BC, and AC coefficients suggest that the interaction between the A, B, and C variables may have a less significant effect on the disintegration time. Greater influence on drug release at 5 minutes is indicated by a greater coefficient of C in equation 2. The amount of medication released at 5 minutes decreased with tablet hardness, whereas reaction Y2 was directly correlated with CCS level. A positive AC coefficient in Y2 meant that CCS and hardness affected drug release behavior more than BC and AB. The effects of the amount of CCS, magnesium stearate, and toughness on Y1 disintegration time (Fig. 6 (a-c)) and Y2 (% release of drug at the end of 5 min) were determined using their respective 3D response surface plots. While 3D response surface graphs are more useful in understanding the main and interaction effects of the independent variables, contour plots emphasize comparison implications through a visual depiction of the response values [27].

The time needed to dissolve the pill reduced as the level of super disintegrant rose. The tablet's hardness had a significant impact on how quickly it disintegrated. Increased disintegration time was seen as the hardness increased. The disintegration was not significantly impacted by the amount of magnesium stearate since the variable was low risk. Hardness and the level of magnesium stearate both harmed the percentage of DR, whereas the level of CCS had a positive impact (Fig. 6 (d-f)) [28].

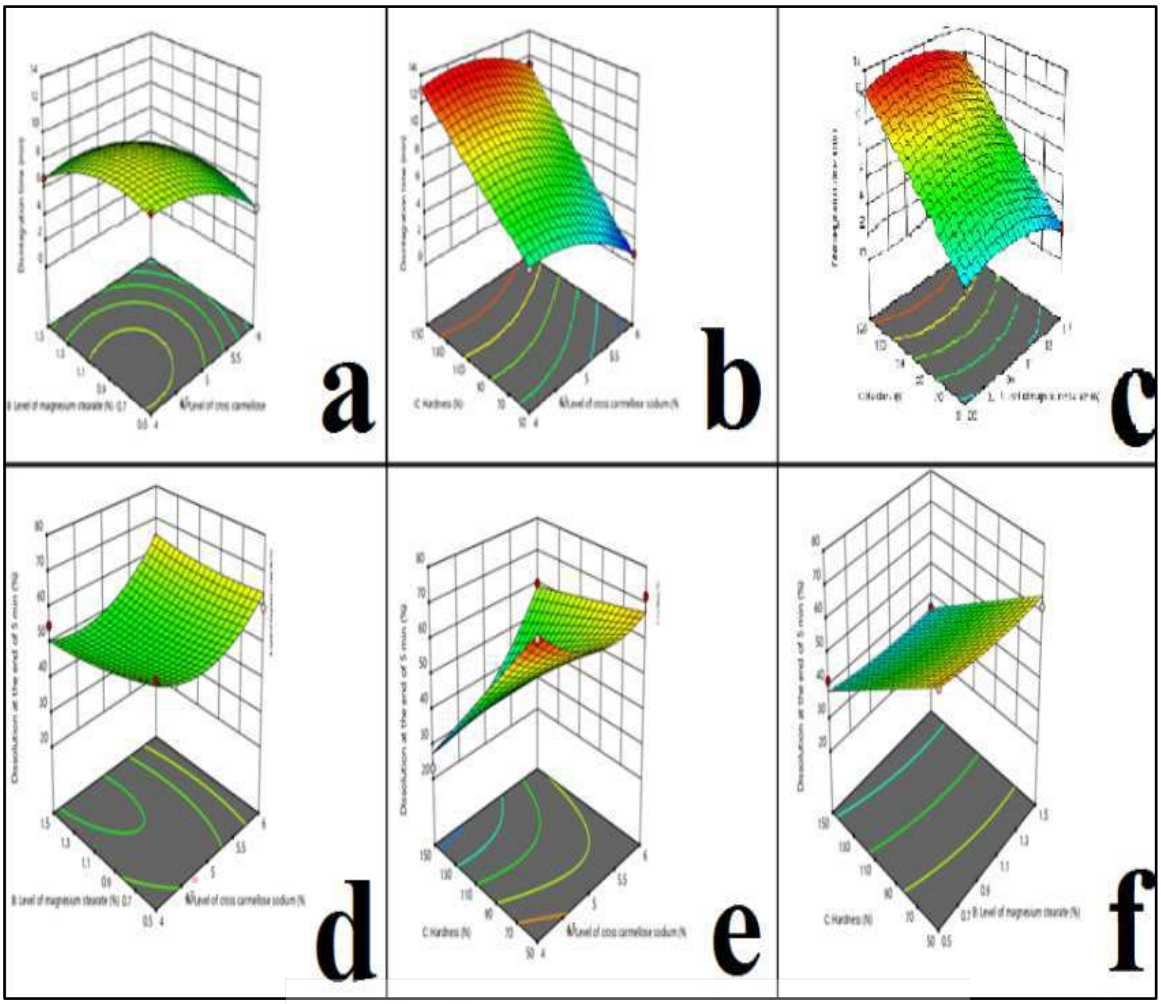

Figure 6: *Plots in 3D showing how independent factors affect (a–c): Disintegration time Y1 (d-f): drug release percentage Y2 (end of 2 minutes).*

Design area: To find the factors that would have the biggest impact, a Box Behnken factorial design was performed. Based on the desirability and overlay plot, where the design space is shown by the yellow portion of the plot, the Design Expert software (V. 11.0.3.0) assessed the proposed concentrations of the independent variables (Fig. 7).

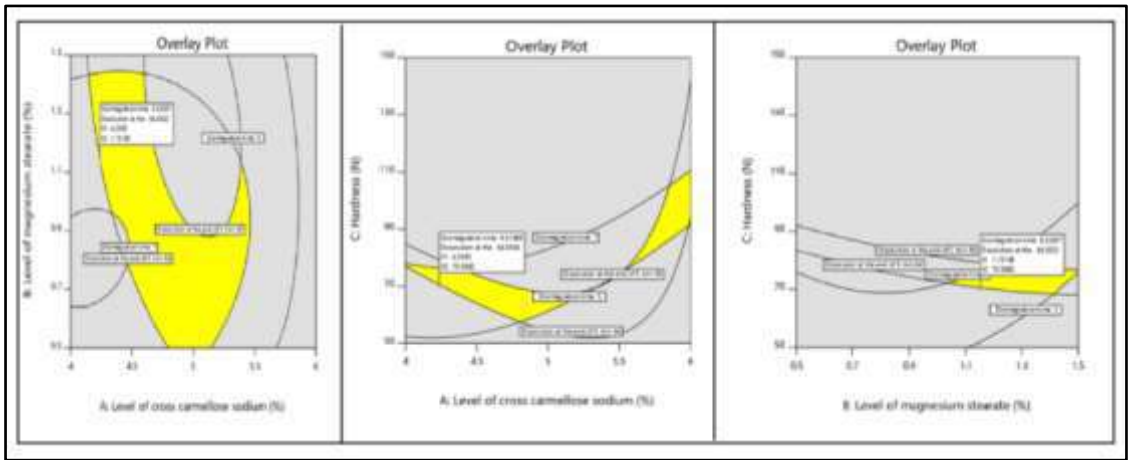

Figure 7: *Overlay plot of design space.*

The yellow region represents the simultaneous effects of independent factors on dependent variables (Fig. 7). According to the objectives shown in this multi-criteria decision technique employing QbD [22-25], the replies are within those targets.

Updated CQA risks include: The risk reduction and control plan are made up of several quality assurance methods based on knowledge about the product and the manufacturing process. All parameter values inside the design space's yellow zone suggest that they largely satisfy the QTPP. As a result, the initial risk was reduced from high to low in the revised risk assessment (Table 12).

Table 12: *Updated risk assessment for CQAs.*

Drug Product CQAs	Level of CCS	Level of Magnesium Stearate	Hardness
Disintegration time	Low*	Low	Low*
Dissolution	Low	Low*	Low*

To guarantee that a product would be regularly produced at the specified quality, a control strategy was created. The controls included a product specification process control and monitoring program and were based on an understanding of the product, formulation, and process. Variability-causing factors that affect product quality were found, correctly comprehended, and then managed. The final control method was used and is depicted in Table 13.

Table 13: *Control strategy for PT FDT.*

Measures	Range Studied	Set Point	Proposed Operating Range	Purpose of Control
Level of CCS	4-6%	5%	4.23-5.45%	To fulfill the criteria of IR tablet and drug release comparable to RLD
Level of magnesium stearate	0.5-1.5%	1%	0.50-1.42%	To fulfill the criteria of IR tablet and drug release comparable to RLD
Hardness	50-150N	100N	55.80-89.23N	To fulfill the criteria of IR tablet and drug release comparable to RLD

Stability Studies: To investigate the effects of storage conditions on the dissolution profile, the optimized batch was placed in stable storage for duration of three months. We compared drug release characteristics to the initial release. The stability investigation revealed that the release profile of the formulation did not significantly alter, indicating that the therapeutic product had not degraded (Fig. 8).

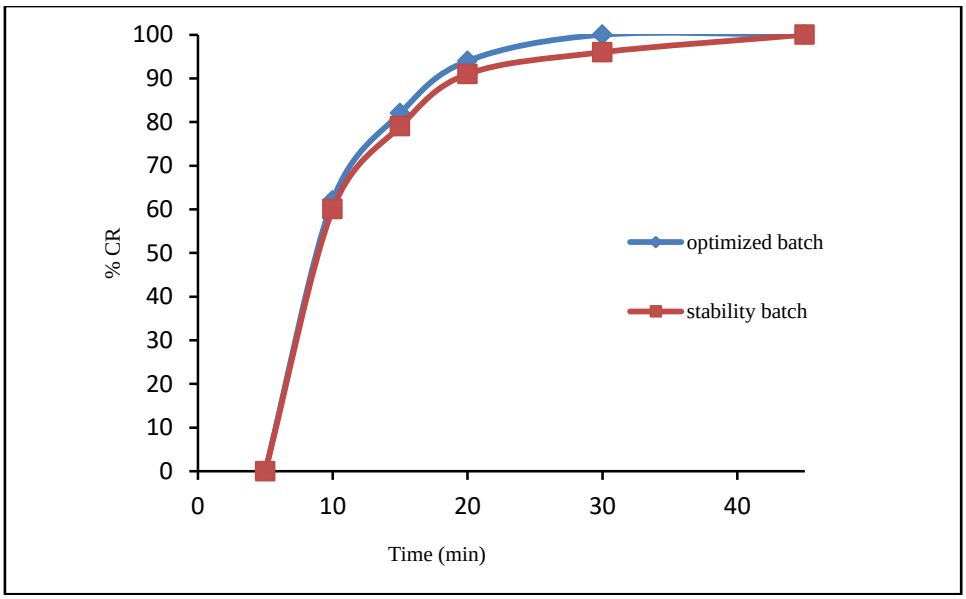

Figure 8: *% cumulative release of PT from optimized and stable batch.*

CONCLUSION

The creation of the PT-FDT formulation and in-vitro testing are the subjects of the current work. The development of a fast-dissolving tablet was accomplished thanks to the use of several core tablet compositions. Drug-excipient compatibility studies and the PT employed in the study both demonstrated compliance and no physical change. The batches' % medication release was calculated, and the results were compared to the intended target profile. The QbD technique was used to optimize the formulation for each parameter. For pharma products, important quality characteristics, QTPP design, identification of CQA, risk evaluation of CMA of PT formulation variable, and CPP were completed. For pharma product CQA, CMA, formulation variable, and CPP, updated risk assessments were created, and the risk was lowered from high to low. Comparison with commercial formulation was discovered to be within predetermined limits. A stability analysis was conducted, and the results revealed that the release profiles of the stability batch were equivalent to those of the first batch and that the pace and extent of the dissolution remained unchanged after the stability period.

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A Comparative Study on Antidiabetic Activity of *Gymnema Sylvestre*, Saxagliptin, Insulin and Alloherbal Combination in Alloxan Induced Diabetic Rats

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ABSTRACT

Aim of the study **:** In this study, we evaluated and compared the effect of *Gymnema sylvestre*, Saxagliptin, Insulin and Alloherbal combination (*Gymnema sylvestre* & Saxagliptin) on hyperglycemia, plasma lipid profile, liver enzymes in Alloxan induced diabetes mellitus in rats.

Method**:** The antidiabetic activity (along with other parameters) of *Gymnema sylvestre* (100mg/kg), Saxagliptin (25mg/kg), Insulin (Human Actrapid; 10U/kg), Alloherbal combination (Gymnema sylvestre; 100mg/kg & Saxagliptin; 25mg/kg) was investigated in alloxan induced diabetes in rats. These drugs were administered once a day, for 14 days and blood glucose levels were measured on 0, 7 and 14th day. At the end of treatment various biochemical estimations & histopathological examination of pancreas were also carried out.

Result: The statistical data indicated, 14 Days oral administration all drugs included in study showed significant ($P<0.05$) decreased in blood glucose, total cholesterol, triglycerides, LDL; SGPT and SGOT level , along with significant increase in HDL; But not better than Alloherbal combination.

Conclusion : Present research findings provide experimental evidence that the combination of allopathic hypoglycemic drug; Saxagliptin with hypoglycemic herbal drug; *Gymnema sylvestre* provides effective and rapid glycemic control on diabetes mellitus and it could be considered for further evaluation in clinical studies and drug development.

Key words: *Gymnema sylvestre*, Saxagliptin, Insulin, Alloxan, Alloherbal combination, Diabetes

1. INTRODUCTION

Diabetes mellitus (DM) It is a metabolic disorder characterized by hyperglycaemia, (fasting plasma glucose ≥ 126 mg/dL and/or > 200 mg/dL 2 hours after 75 g oral glucose), glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement

membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.**[1]**

***Gymnema sylvestre*[2]** is a perennial woody vine native to Asia (including the Arabian Peninsula), Africa and Australia. It has been used in Ayurvedic medicine. Common names include gymnema,**[3]** Australian cowplant, and Periploca of the woods, and the Hindi term *gurmar*, which means "sugar destroyer".**[4][5][6]** It has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes.**[7]**

Saxagliptin a Dipeptidyl Peptidase-4 (DPP-4) inhibitor are the newer class of compounds that was approved in 2006 for the treatment of T2DM. Their primary mechanism of action is through inhibition of degradation of incretins, such as glucagon like peptide-1 (GLP-1) and Gastric Inhibitory Polypeptide (GIP)**[8]**

Human Actrapid is a fast-acting insulin. Onset of action is within ½ hour, reaches a maximum effect within 1.5–3.5 hours and the entire duration of action is approximately 7–8 hours.The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver. [9]

This work reviews and comparatively analyzes the herbal, allopathic and biologic treatments to cure the problems in health care. It suggests the adoption of the concept of integrative medication and health care that connects mainstream allopathic medical treatment, herbal therapies and biologics, which will select the best, scientifically validated therapies out of the systems.

2. MATERIALS AND METHODS:

2.1 Drugs and Chemicals:

Alloxan monohydrate obtained from Dolphin pharmacy instruments Pvt. Ltd. Mumbai. *Gymnema sylvestre* obtained from Inlife pharma Pvt. Ltd. Saxagliptin obtained from CTX Lifesciences Pvt. Ltd. Gujrat. Human Actrapid Insulin 40IU/ml obtained from novo nordisk®

2.2 Animals & Housing Condition :

Albino Wistar Rats of (180-200gm) were selected for experimental study. The animals were kept and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50± 15%and 12 hrs. light/dark cycle. They were allowed free access to food (standard pellets) and water *ad libitum.* Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethics Committee of YSPM's, YTC, Faculty of Pharmacy, NH4 Wadhe, Satara, Maharashtra, India.

2.3 Induction of Diabetes:

Albino Wistar Rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg/day). Alloxan monohydrate solution of 150mg/kg/day prepared in 0.9% NaCl solution and was administered within 5 minutes at a dose of 150-mg/kg/day intraperitoneally. All the animals except control group were i.p. administered with Alloxan at a dose of 150mg/kg once a day for 2 days. After 72 hours of Alloxan administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with a blood glucose of 250- 350mg/dl) were taken for the experiment. **[10]**

2.4 Blood Glucose level & Body Weight Determination:

Blood samples were drawn from tail tip of rats. Fasting blood glucose estimation were done on 0^{th}, 7^{th}, & 14^{th} day of the study. Blood glucose estimation was done by ACCU-CHECK Active Glucometer using glucose test strips. For body weight determination, all experimental animals were weighted on 0^{th}, 7^{th}, & 14^{th} day of the study. The body weights were recorded at recording time in the morning mentioned by Al-Attar and Zari **[11]**. Furthermore, for any signs of abnormalities throughout the duration of investigation, the rats were continuously observed.

2.5 Biochemical Estimation:

After Fourteen days, rats were fasted for 8 h. Rats were anesthetized using diethyl ether and samples of blood were obtained from retro-orbital plexus. These Blood samples were withdrawn for estimation of Blood glucose level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL), Liver function test (Alkaline phosphatase, AST;SGOT, ALT;SGPT, Total Protein etc.).

2.6 Histopathological Examination:

After blood collection, all rats were sacrificed with high dose of anaesthesia and dissected; pancreatic tissues were isolated and fixed in 10% formalin. Fixed pancreatic tissues were dehydrated and embedded in paraffin. All tissues were sectioned at 4 µm. The routine process of staining was applied using hematoxylin and eosin stains **[12]**. The pancreatic sections were evaluated by light microscopy using Motic basic biological microscope BA210. Motic imaging software was used to evaluate the histological profile of pancreatic sections in all groups.

2.7 Experimental Design:

2.7.1 Acute Toxicity Study:

Acute toxicity study was carried out for Gymnema sylvestre by adapting fixed dose method of CPCSEA, OECD guidelines no. 423. Healthy Albino Wistar rats of either sex were randomly divided into 4 groups with 3 animals in each group. The animals were kept fasted overnight providing only water, after which the *Gymnema sylvestre* were administered orally with Starting dose is selected from one of four fixed levels 5, 50, 300, and 2000 mg/kg body weight by intra gastrictube. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily there after, for a total of 14 days. Animals are observed for general neurological & behavioural or autonomic profile. **[13]**

2.7.2 Hypoglycemic Evaluation:

For Hypoglycemic evaluation, Albino Wistar Rats were used and divided into five groups of six animals in each group. Animals were kept fasted overnight (18hrs) before treatment.

Group I - (Control) rats received vehicle that was Distilled water (10ml/kg p.o.).

Group II - (Test1) rats received *Gymnema sylvestre* (100mg/kg p.o.)

Group III - (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV - (Test3) rats received Insulin (1U/100gm SC).

Group V - (Test4) rats received *Gymnema sylvestre* (100mg/kg p.o.) and Saxagliptin (25mg/kg p.o.) in combination.

Blood glucose was estimated on 0, 30, 60, 90, 120 min of the treatment using the ACCU-CHECK Active Glucometer.

2.7.3 Oral Glucose Tolerance Test:

For OGTT evaluation, Albino Wistar Rats were used and divided into five groups of sixanimals in each group. Animals were kept fasted overnight (18hrs.) before treatment.

Group I- (Control) rats received Glucose (2gm/kg p.o.)

Group II- (Test1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group III- (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV- (Test3) rats received Insulin (1U/100gm SC).

Group V- (Test4) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.) andSaxagliptin (25mg/kg p.o.) in combination.

Glucose (2gm/kg p.o.) was administered to all the rats after Half hour of administration of differentdrug treatments. Blood glucose was estimated at 0, 30, 60, 90 & 120 min after different drug treatment using the ACCU-CHECK Active Glucometer.

2.7.4 Antidiabetic study by different drug treatment :

After 72 hours of Alloxan (150mg/kg/day i.p.) administration, rats with moderate diabetes havingglycosuria and hyperglycemia (i.e. with a blood glucose of 250-350 mg/dl) were taken for the experiment. The Albino Wistar rats were divided into six groups of six rats in each group. All the animals were fasted overnight (18hrs.) before the treatment of test drug.

Group I- (Normal Control) rats received only vehicle that is Distilled water (10ml/kg/day)

Group II- (Toxic Control) rats received Alloxan Monohydrate (150mg/kg/day)

Group III- (Test 1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group IV- (Test 2) rats received Saxagliptin (25mg/kg/day p.o.)

Group V- (Test 3) rats received Insulin (1U/100gm/day SC).

Group VI- (Test 4) rats received *Gymnema sylvestre* leaves extract (100mg/kg/day p.o.) and Saxagliptin (25mg/kg/day p.o.) in combination.

2.8 Statistical Analysis :

All values of results were presented as mean ± standard error of mean (SEM). The statistical analysis involving one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

3. RESULTS

3.1 Hypoglycemic Effect of different drug treatment in Normal Rats:

The results from the study clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 90 and 120min as compared with normal control group.

Table 1: Hypoglycemic Effect of different drug treatment in Normal Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	NormalControl	67±1.78	70±1.89	66±1.88	67±3.10	66±2.29
II	Test group 1 (*Gymnema sylvestre*)	73±1.73	71±2.11	69±2.36	65±1.92	60±3.15
III	Test group 2 (Saxagliptin)	81±2.43	71±2.39	63±2.01	57±2.36	55±1.87
IV	Test group 3 (Insulin)	90±2.46	68±1.89	53±2.79	56±2.30	48±3.48
V	Test group 4 (G.S + Saxagliptin)	71±2.03	65±2.76	63±3.07	60±2.88	58±2.15

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.2 Effect of different drug treatment on the Oral Glucose Tolerance Test in Normal Rats:

The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2g/kg p.o.) significantly after 120 min of administration, as compared with control group.

Table 2: Effect of different drug treatment on the Oral Glucose Tolerance Test in NormalRats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	Control (Glucose)	80±1.54	78±2.55	130±1.67	110±1.89	94±1.56
II	Test group 1 (*Gymnema sylvestre*)	85±1.25	82±1.99	120±3.89	109±2.56	90±1.59
III	Test group 2 (Saxagliptin)	88±1.55	83±2.45	110±1.98	95±1.22	85±3.68
IV	Test group 3 (Insulin)	87±1.98	70±3.56	82±1.36	62±2.66	41±3.02
V	Test group 4 (G.S + Saxagliptin)	90±1.5	83±3.01	111±2.65	95±1.98	82±1.75

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.3 Effect of different drug treatment on Body Weight of Diabetic Rats:

At the end of study after 14 days, body weight was significantly decreased in toxic control groupas compared with normal control group & significantly increased in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group as compared with toxic control group. But Insulin treated group shows marked rise in body weight than the Normal control group.

Table 3: Effect of different drug treatment on Body Weight of Diabetic Rats

Group no.	Treatment Groups (n=6)	Body Weight of Animals (gm)		
		0^{th} day	7^{th} day	14^{th} day
I	Normal control	210±1.33	215±1.89	224±2.01
II	Toxic control	216±1.65	196±2.96	180±3.69
III	Test group 1	228±2.63	232±3.06	238±1.32
IV	Test group 2	237±3.01	242±1.65	249±2.36
V	Test group 3	241±2.65	252±3.11	266±1.158
VI	Test group 4	248±1.65	253±2.36	259±3.05

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.4 Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

A marked rise in fasting blood glucose level was observed in toxic control group as compared with normal control group. *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) treated group which produced a significant reduction in blood glucose level as compared with toxic control group; But not better than their combination. Where the Insulin treated group shows Goodcontrol of Hyperglycemia until 7th day then it shows mild Hypoglycemia on 14th day.

Table 4: Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)		
		0th day	7th day	14th day
I	Normal Control	80±1.89	80±1.045	82±2.07
II	Toxic control	321±2.89	326±1.22	330±1.65
III	Test group 1 (*Gymnema sylvestre*)	285±1.59	221±1.03	157±2.67
IV	Test group 2 (Saxagliptin)	299±2.86	191±1.09	144±1.75
V	Test group 3(Insulin)	325±1.65	195±2.69	65±1.44
VI	Test group 4 (G.S + Saxagliptin)	316±1.07	165±1.76	115±2.66

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.5 Effect of different drug treatment on Biochemical Parameters in Diabetic Rats:

Serum Lipid Profile:

After 14 days of treatment period it was observed that increased level of Total Cholesterol, TG, LDL, VLDL, & decreased HDL level in toxic control group as compared with normal control group. Animals treated with *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showedsignificant reductions in Total Cholesterol, LDL, VLDL, TG & significant increased level in HDLas compared with toxic control group; But not better than their combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) showed reduction in values than normal control values.

Table 5 : Effect of different drug treatment on Biochemical Parameters in Diabetic Rats

Group no.	Treatment Groups (n=6)	Total Cholesterol (mg/dl)	Tri-Glycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
I	NormalControl	78.4±1.54	122±2.03	11.9±1.98	39±1.09	24±1.45
II	Toxic control	102±2.13	480±1.98	38±3.54	35±4.03	96±1.84
III	Test group 1 (*Gymnema sylvestre*)	87±3.05	153±2.03	18.3±1.04	37±4.65	30.7±2.51
IV	Test group 2 (Saxagliptin)	100±2.65	140±3.98	24.8±1.75	34±4.03	28±1.33
V	Test group 3 (Insulin)	66±2.68	201±1.66	21±3.78	30±4.65	40.2±1.21
VI	Test group 4 (G.S + Saxagliptin)	83±1.03	120±2.78	19±1.98	39±3.98	24±4.01

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followedby Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.6 Effect of different drug treatment on Liver Function Test in Diabetic Rats :

After 14 days of treatment period it was observed that increased level of Bilirubin, SGPT, SGOT,TP & ALKP in toxic control group as compared with normal control group. Animals treated with*Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showed significant reductions in AST; SGOT, ALT; SGPT & ALP as compared with Toxic control group; But not not better thantheir combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) shows reduction in values than normal Control values.

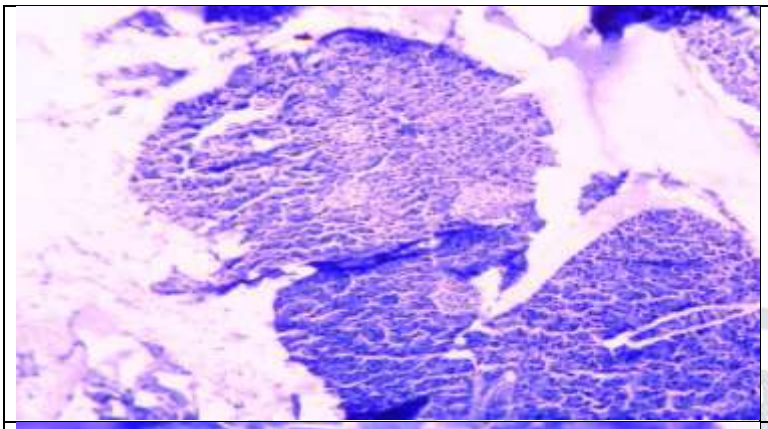
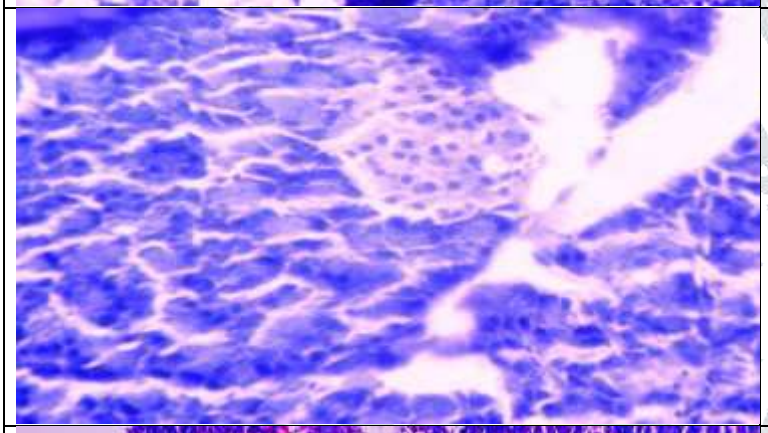
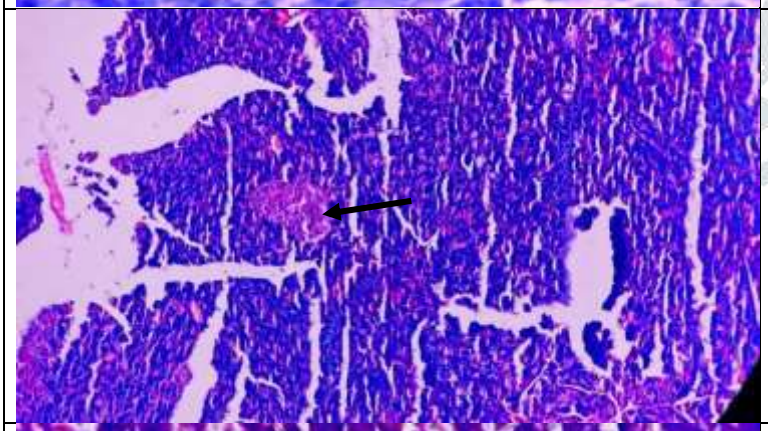
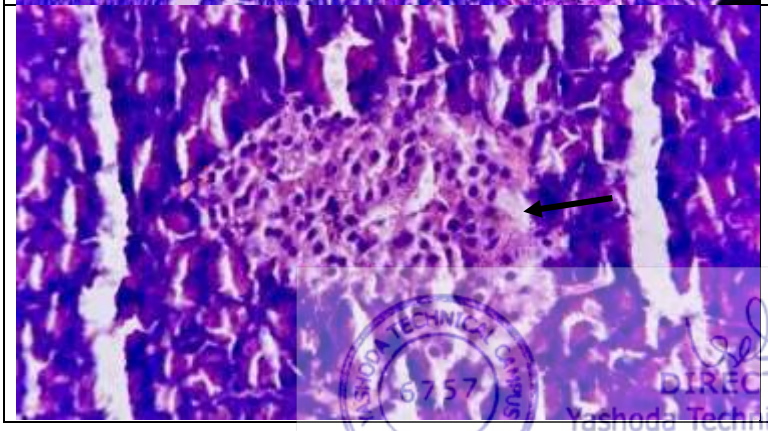
Table 6 : Effect of different drug treatment on Liver Function Test

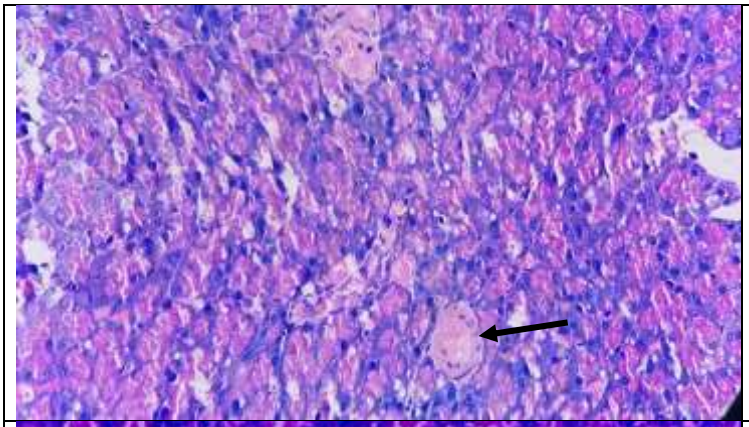
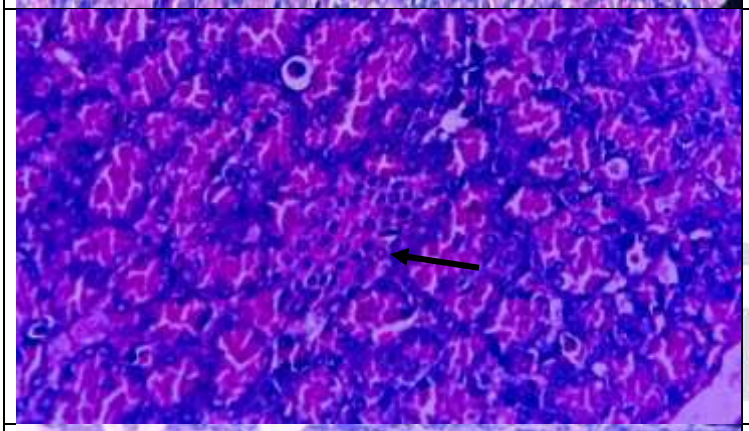
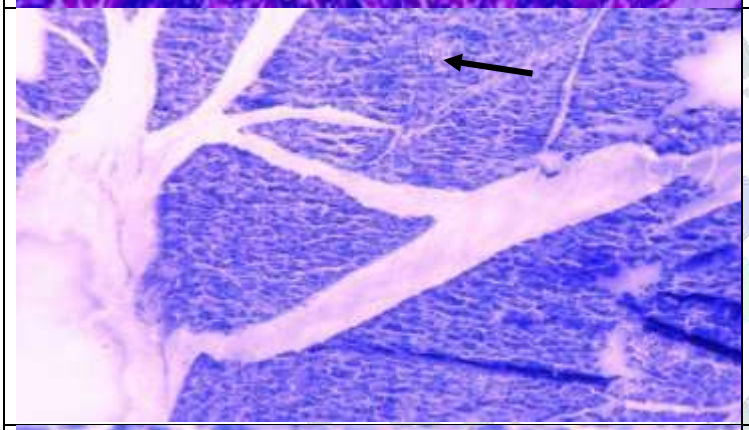
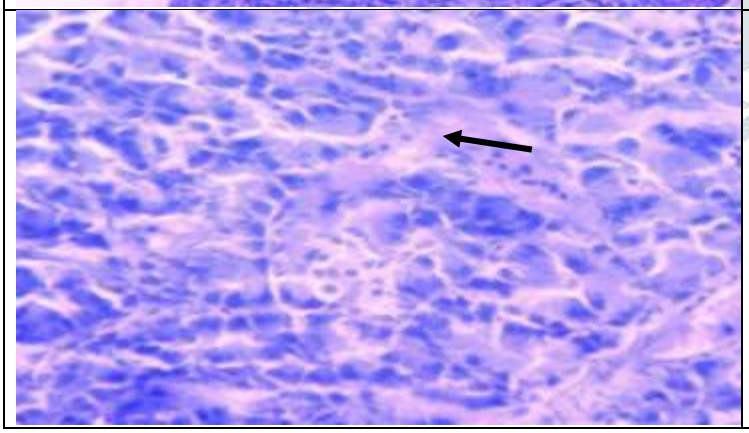
Group no.	Treatment Groups (n=6)	Bilirubin mg/dl	SGPT U/L	SGOT U/L	TP mg/dl	ALKP U/L
I	NormalControl	0.74±1.33	43±1.6	36±3.06	5.8±2.09	126±1.48
II	Toxic control	1.12±2.35	59±1.59	42±3.65	7.5±4.89	190±2.89
III	Test group 1 (*Gymnema sylvestre*)	0.83±1.78	46±2.13	36±3.60	6.5±4.21	110±2.44
IV	Test group 2 (Saxagliptin)	0.93±2.54	38±3.45	31±1.09	6.9±4.01	102±1.98
V	Test group 3 (Insulin)	0.6±1.08	34±2.40	29±1.88	4.9±4.02	97±3.48

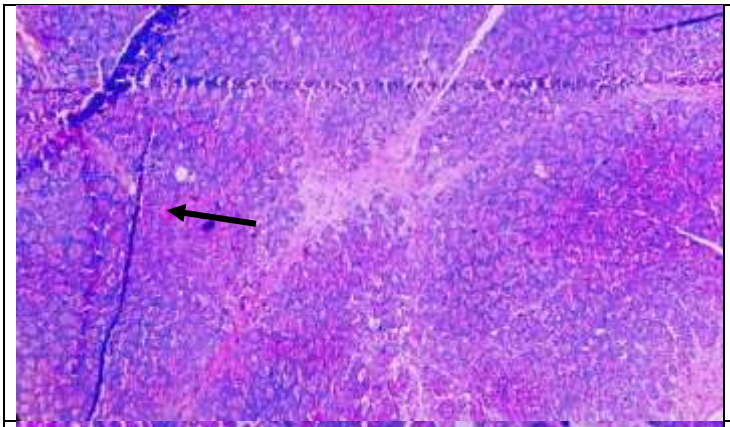
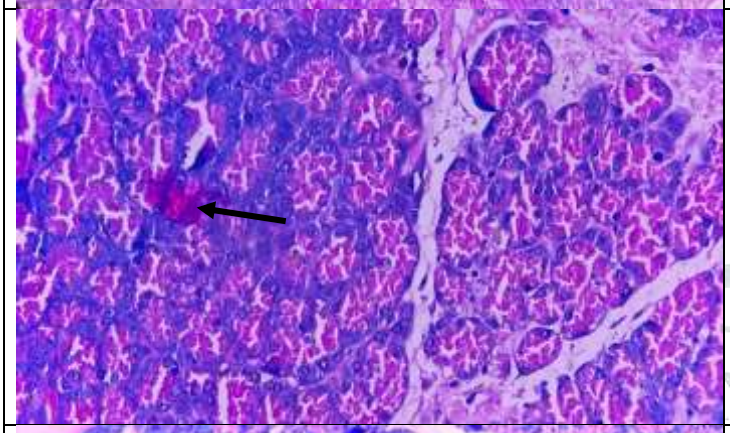
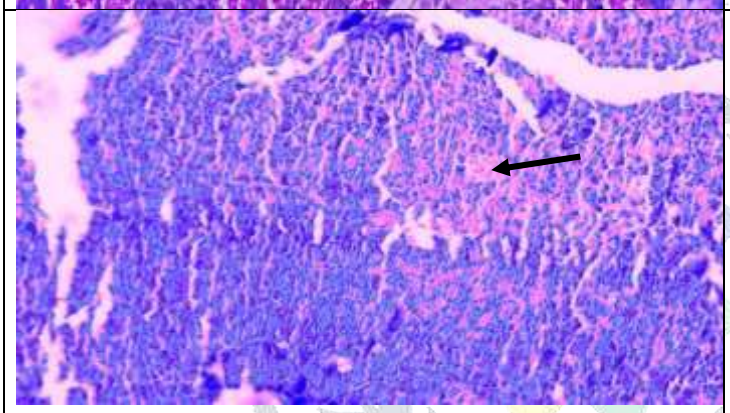
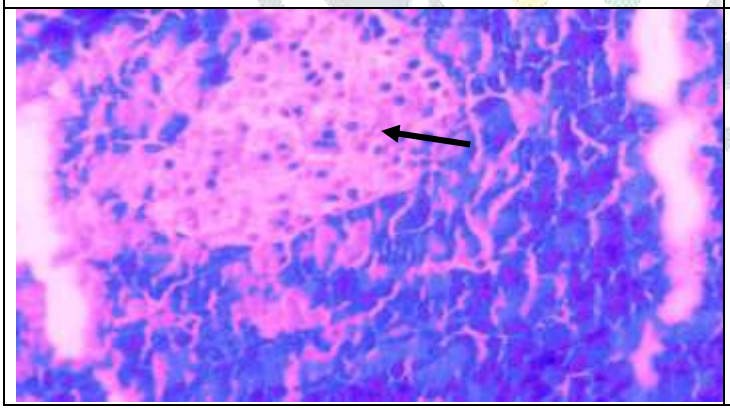
VI	Test group 4(G.S + Saxagliptin)	0.79±1.03	39±2.03	30±2.78	6.9±3.33	118±4.11

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

Pancreas Histopathology

	**Normal control Pancreas** –H & E stain 10X
	**Normal control Pancreas** –H & E stain 40X
	**Alloxan inducer group** - Photograph showing necrosis of islets of Langerhans (black arrow) H & E stain 10X
	**Alloxan inducer group** - Photograph showing necrosis of islets of Langerhans with cellular infiltration H & E stain 40X

	**Test group 1 –** Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 10X
	**Test group 1 –** Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 40X
	**Test group 2** – Photograph showing necrosis of islets of Langerhans (black arrow),H & E stain 10X
	**Test group 2** – Photograph showing necrosis of islets of Langerhans with cellular infiltration (black arrow) H & E stain 40X

	**Test group 3** – Photograph showing Acinar Hemorrahage (black arrow) H & E stain 10X
	**Test group 3** – Photograph showing Acinar hemorrhage and degeneration ofislet of Langerhans with Hyalination (Black Arrow), H & E stain 40X
	**Test group 4** – Photograph showing islets of Langerhans (black arrow) H & E stain 10X
	**Test group 4** – Photograph showing islets of Langerhans with Acinar degeneratiom H & E stain 40X

4. DISCUSSION :

4.1 Acute oral toxicity, Hypoglycemic study, OGTT Study & Body Weight Determination:

Globally, the rapid increase the incidence of DM poses a demand for the quest of novel therapeuticdrugs necessitates addition of alternative medicine. As a result number of studies has been conductedto assess the utility of herbal and allopathic medicine in DM. The present study was undertaken to evaluate the Antidiabetic activity of *Gymnema sylvestre,* Saxagliptin, Insulin and Alloherbal combination against Alloxan Induced Diabetic Albino Wistar Rats.

The Acute oral toxicity was performed according to OECD guideline 423. In this study we observed that the *Gymnema sylvestre* was safe to use in animals. There was no change in neurological, behavioural or autonomic, no lethality or toxic reactions were found with the selecteddoses (5, 50, 300 and 2000mg/kg/day p.o.) until the end of study period. Therefore 100 mg/kg was selected for all in vivo experiments as minimal dose.

The results of Hypoglycemic study have shown that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 120min as compared with normal control group.(Table 3).

OGTT for nondiabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925).**[14]** The Oral glucose tolerance test in nondiabeticc rats, blood glucose level was significantly greater in the glucose loaded control group. The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2gm/kg p.o.) after120 min of administration, as compared with control group. (Table 4).

Induction of diabetes by Alloxan leads to loss of body weight due to increased muscle wasting and loss of tissue proteins as well as due to destruction of pancreatic cells; insufficient insulin prevents the body from getting glucose from the blood into the body's cells to use as energy & when this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight.)**[15]** whereas body weight of animals significantly increased in *Gymnema sylvestre*, Saxagliptin and their combination treated group as compared with toxic control group. But treatmentof Insulin shows marked rise in body weight.).**[16]** (Table 5).

4.2 Alloxan-Induced Rodent Model of Diabetes & Antidiabetic effect of different drug treatment:

Alloxan has two distinct pathological effects: Alloxan is a toxic glucose analogue it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induceROS formation leading to demolition of pancreas β-cells & selective necrosis leading to hypoinsulinemia and hyperglycemia.

The results of the antidiabetic study reduced blood glucose level Significantly on 7^{th} & 14^{th} days when animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) as compared to toxic control groups (Table 6)

There are some possible mechanisms by which the *Gymnema sylvestre* leaves and especially Gymnemic acids from G. sylvestre exert its hypoglycemic effects are: 1) it increases secretion of insulin, 2) it promotes regeneration of islet cells, 3) it increases utilization of glucose: it is shown toincrease the activities of enzymes responsible for utilization of glucose by insulin dependant pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and 4) it causes inhibition of glucose absorption from intestine).**[17]**

Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormonesin the body. It is this increase in incretin hormones that

is responsible for the beneficial actions of saxagliptin, including increasing insulin production in response to meals and decreasing the rateof gluconeogenesis in the liver).**[18]** Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP and the degradation of GLP-1).**[19][20]**

The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver).**[21]**

4.3 Biochemical Parameters Analysis:

In Alloxan induced diabetes mellitus showed improvement in biochemical parameters after the treatment of Drugs.

In the result of **lipid profile**, marked decrease in total cholesterol, LDL, VLDL and triglycerides was observed, while increase in HDL cholesterol which reduces the risk of atherosclerosis has been observed in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated diabetic rats, which suggest that HDL is inversely related to the total body cholesterol as compared with toxic control group (Table 7).

These results could thus reflect the ability of *Gymnema sylvestre*, Saxagliptin improve the tissue sensitivity to insulin. Thus reducing the hormone sensitive lipase activity and increasing the lipoprotein lipase activity, resulting in a decrease of lipolysis these leading to hypolipidemic activity.

In the present study, rats treated with G. sylvestre post Alloxan-diabetic induction showed a significant decrease in triglyceride, cholesterol and LDL and showed a significant increase in HDLas compared to that of untreated diabetic rats. Decreasing levels of triglyceride, cholesterol and LDLand increasing level of HDL might be due to an increase in insulin which caused an increased activity of lipoprotein lipase (Facilitated chylomicron transport through cell membranes) and a decreased activity of hormone-sensitive lipase (converted neutral fats into free fatty acids). This result was in agreement with Daisy et al. (2009) **[22]** and Aralelimath and Bhisea (2012) **[23]** who reported that increasing insulin secretion after administration of G. sylvestre extract led to a decreaseof cholesterogenesis and fatty acid synthesis.

Our study results elucidated that, saxagliptin improve lipid status in rats, via significant reduction of Total Cholesterol, LDL and Triglycerides. In line with these results are also the results of other research groups. Possible explanation for beneficial lipid effects of DPP4 inhibitors may be connected to its stimulating effect on the activated proteine-kinase pathway, which leads to increasein glucose and lipid catabolism. **[24]** On the other hand, no improvement in HDL parameters was achieved in our study, which is in correlation with the findings of Saad et al. **[25]**

In our study the result of Insulin treatment is accordance with Ibrahim Aslan et.al. (2013) **[26]** whichshows Total cholesterol (TC), triglyceride (TG) and very low-density lipoprotein cholesterol (VLDL-C) levels were significantly decreased while HDL-C levels were significantly increased after insulin treatment.

In **liver function test**, animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group showed significant reductions in Bilirubin, ALT; SGPT, AST; SGOT, Total Protein & ALKP as compared with toxic control group (Table 8). This demonstrated the hepatoprotective activity could be related to reduced blood glucose level due to different treatment groups.

Evidence from studies about heme oxygenase (HO) system**[27][28]** might also support the increasedrisk of bilirubin with T2D. Increased activity of HO could elevate the heme catabolic products suchas carbo monoxide, iron, and bilirubin. **[27]** HO-1 has been reported as a strong positive predictor ofmetabolic inflammation among obese insulin-resistance individuals and animals.**[28][29]** The higher bilirubin levels might be a biomarker of oxidative stress and inflammation in diabetes

Glucose level might be decreased in treated diabetic rats as a result of decreasing gluconeogenesis that was indicated by low levels of ALT;SGPT and AST;SGOT in treated diabetic rats compared tountreated diabetic rats(Toxic Control Group). This result was in agreement with Shanmugasundaramet al. (1983)**[30]** who reported that administration of dried leaf powder of G. sylvestre decreased glucose levels as it controlled gluconeogenic enzymes (ALT and AST) and increased glycogen levels in liver, kidney and muscle.

Total proteins were found to be significantly increased in diabetics as compared to Normal controls.Competition between serum albumin and hemoglobin could be a factor for the negative correlationbetween them, besides preventing other proteins from glycation and altering the diabetic complications. Similar findings have been reported by other studies.**[31] [32] [33] [34]** Increase in total proteins may be due to the elevation of acute phase proteins, globulins, fibrinogen and compounded by decrease in the fractional synthetic rate of albumin due to insulin resistance/deficiency (F A Nazki 2017)

4.4 Histopathological Examination:

In histopathological study (Table 19), the fine section of Normal Control diabetic rat's pancreas onmicroscopic examination using H & E stain, 10X & 40X showed the presence of islets of Langerhans, blood vessels, connective tissues, inter and arrangement of islets of Langerhans was normal with tightly arranged cells and even distribution throughout the lob-necrosis.

Also, Pancrease Exocrine portion predominantly and composed of lobules, each of which is surrounded by connective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts. Adequate islets of beta and alfa cells was seen. No evidence of stromal Infiltrationwas seen.

In toxic control group i.e Alloxan inducer group Histopathological report shows that necrosis of islets of Langerhans was shown with cellular infiltration. It also Shows Exocrine portion predominantly and composed of lobules formed by acinar structure, each of which is surrounded byconnective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts.

There is No evidence of islets of beta and alfa cells are seen, which complete destruction of alpha and beta cells.

In Saxagliptin treated diabetic rats, Photograph showing necrosis of islets of Langerhans with cellular infiltration (The migration of cells from their sources of origin), Where In Insulin treated rats showing Acinar hemorrhage

and degeneration of islet of Langerhans with Hyalinization (process of conversion of stromal connective tissue into a homogeneous, acellular translucent material that could provide insights into the prognosis of pathological lesions.) was seen. So it can be concluded that Insulin treatment does not provide protection to the pancreatic cells.

In *Gymnema sylvestre* and *Gymnema sylvestre* with Saxagliptin treated groups it was observed thatalthough the gap between the islets was more than lesser number of islets as compared to Normal control group, it was significantly much better than the toxic control group. By showing Acinar degeneration it is concluded that, the dose of *Gymnema sylvestre* had slight protection to the cells. Because Necrosis formed after Acinar degeneration which was shown in Toxic control group. Thus the histopathological examination revealed good protective property of this herbal drug alone and with combination.

5. CONCLUSION :

In conclusion, it can be stated that the use of Combination of *Gymnema sylvestre* and Saxagliptin produces more beneficial effect than using alone. Gymnemic acids from G. sylvestre exert its hypoglycemic effectsby increasing secretion of insulin, increasing utilization of glucose or inhibiting glucose uptake from intestine; Whereas Saxagliptin exerts its hypoglycemic effect by this increase in incretin hormonesand increasing insulin production in response to meals and decreasing the rate of gluconeogenesis inthe liver.

Use of *Gymnema sylvestre* and Saxagliptin in combination shown beneficial effects in reducing theelevated blood glucose level as well as gained body weight, hypoglycemic potential, significant oral glucose tolerance & normalization in altered biochemical parameters of Alloxan induced diabetic rats.

From the present results it can be concluded that using herbs with allopathic drugs may produce the synergistic effect. Hence, For further use of these combination clinical studies are needed.

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Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Rizatriptan in Immediate Release Dosage Form

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Abstract:
In this research work of life cycle management of analytical RP-HPLC method development for assay of Rizatriptan in immediate release dosage form, the RP-HPLC assay method was developed and validated for Rizatriptan. Stress study is also carried out. The chromatographic conditions will be as, Symmetry C-18, 150 mm x 4.6 mm, 5 µm column at 30°C temperature by using a mobile phase [Mixture of 100 mL Acetonitrile, and 900 mL Buffer solution] at a flow rate of 1.8mL/min, and UV detection at 225 nm and run time is 15 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.
***Key words*: Rizatriptan, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form**

Materials and Methods:
Introduction:
Rizatriptan benzoate is N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine. It is an anti migraine drug, which selectively activates 5-HT1B/1D receptors. Physical properties are white to off white crystalline powder, soluble in water, melting point 178–180_, and stable under ordinary condition. The some published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.

Structure of Rizatriptan benzoate

Experimental:

Materials:

1) Risatriptan (Rizatriptan Benzoate): - Working standard and its claimed purity was 98.20%.
2) Risatriptan (Rizatriptan Benzoate) Tablet (label claim 5 and 10 mg) and placebo, which was prepared and supplied by Instavision lab.

- Reagents and Chemicals:
  1) Acetonitrile: -HPLC grade, Rankem, India.
  2) Methanol: - HPLC grade, Rankem, India.
  3) Milli-Q water: - It was purified by Millipore Corporation's system.
  4) Acetic acid: - Reagent Grade, Merck, India.
  5) Hydrochloric acid: - Reagent Grade, Merck, India.
  6) Sodium hydroxide: - Reagent Grade, Merck, India.
  7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
  8) Sodium Perchlorate :- Reagent Grade, Merck, India.
  9) Triethylamine :- Reagent Grade, Merck, India.

Instruments, Apparatus and equipment:

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Crome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.

4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipments, Germany

➢ Chromatographic system:

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).

The published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan.

➢ Selection of Buffer in Mobile Phase: -

Sodium perchlorate and 1ml Triethylamine in 1000ml water and adjusted pH 2.00, 3.00, 4.00 and 5.00 with 10% Acetic acid was used to optimize the peak shape and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Mobile Phase: -

Acetonitrile was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Column:-

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of degradation products and impurities, a reversed phase C_{18} column was preferred over other columns to separate all polar impurities as Symmetry C-18, 150 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

➢ Selection of Diluent / Solvent for extraction:-

Different solvents were tried including single solvent and combination of solvents like Acetonitrile and methanol in different concentrations, But Risatriptan (Rizatriptan Benzoate) tablet gets dissolved in Solvent Mixture: [Bufferl: Acetonitrile 90:10] and hence mobile phase is used as diluent

Various Method screening Trials has been taken using following different compositions.

➢ Table for trials:

Sr.No.	Trails Taken	Observation	Remarks
1	Buffer pH2.00 : Methanol (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer4.00 : ACN (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
3	Buffer: Acetonitrile (70:30 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Peak observed	Not Satisfactory
4	Buffer: Acetonitrile : Methanol (80:10:10 v/v), Flow rate 2.0 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
5	Buffer: Acetonitrile : Methanol (90:05:05 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Tailing observed	Not Satisfactory
6	Acetonitrile : Buffer (10:90 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Good peak shape observed and separated from benzoic acid peak	Satisfactory

Reason for validation: Non-Pharmacopeial method.

Design of experiment (DOE):

A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of buffer ad organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. Molarity of buffer Sodium Perchlorate conc. 1gm +/- 0.01g
2. Triethylamine conc. 1mL +/- 0.01mL
3. pH of buffer pH 4.3 +/- 0.2
4. Buffer ratio 900 mL +/- 90mL

5. Acetonitrile 100 mL +/- 10mL
6. Flow rate 1.8 +/-0.2mL
7. Column temp 30+/- 5 °C

Method Validation:

- Standard preparation:

Weigh and transfer about 100 mg of Risatriptan (Rizatriptan Benzoate) reference standard to a 100 mL volumetric flask and dissolve and dilute up to the mark with mobile phase, further dilute 10mL solution to 100mL with mobile phase.

- Sample preparation

Weigh accurately not less than 20 tablet crush and weigh powder equivalent to 100mg of label amount into 100 mL volumetric flask add about 75 mL of mobile phase, sonicate at for about 15 min with intermittent shaking, keep achieve room temperature make up to volume with mobile phase, further dilute 5mL solution to 50mL with mobile phase.

- Buffer Preparation

Added 1 gm of sodium perchlorate and 1ml triethylamine in 1000ml water and adjust pH 4.30 with 10% Acetic acid.

- Mobile phase Preparation

Mix 100 ml of Acetonitrile and 900ml of buffer solution, sonicate and filter through 0.45µ membrane filter and degas.

- Diluent/Blank Solution:
  Use mobile phase as blank.
- Optimized HPLC Parameters:

Instrument	: Agilent Liquid Chromatography with PDA detector
Column	: Symmetry C-18, 150 mm x 4.6 mm, 5.0 µm
Flow Rate	: 1.8 mL/min
Injection volume	: 20 µL
Column temperature	: 30°C
Sample cooler Temperature	: Ambient
Detection	: 225 nm
Run time	: 15 minutes

System Suitability Test:

Sr. No.	Parameters	Risatriptan (Rizatriptan Benzoate)
1.	Peak area	*5670421*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	5.312
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.11*

Specificity:

Specificity Part-I: Interference from blank, benzoic acid and placebo

- Procedure

Prepare blank preparation, prepared placebo preparation, standard preparation, and sample preparation for 5 and 10mg tablet as per the method.

- Benzoic acid solution preparation

Transfer accurately measured quantity of acetic acid 50 mg and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , mix and make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

- Placebo preparation

Weighed accurately placebo equivalent to 100 mg of Risatriptan (Rizatriptan Benzoate) and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , sonicate at for about 15 min with intermittent shaking, keep to achieve room temperature make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

Observation: No interference seen

Specificity Part-II :Forced degradation

Sr. No.	Stress type	% Degradation	Observation
1	Untreated sample	----	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate).
2	Heat degradation (Solid state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
3	Heat degradation (Solution state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
4	Photolytic degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
5	Humidity degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
6	Acid dégradation	5.86%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
7	Base degradation	10.15%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

Sr. No.	Stress type	% Degradation	Observation
8	Peroxide degradations	16.53%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

Linearity and Range:

Linearity Level	Standard concentration	Concentration of Risatriptan (Rizatriptan Benzoate) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	50.20	2952527	0.9997
Level – 2	80%	75.30	4051177	
Level – 3	100%	100.40	5405053	
Level – 4	120%	125.50	6390534	
Level – 5	150%	150.60	7457580	

Precision:

Sample Preparation	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution -1	99.67
Test solution -2	99.59
Test solution -3	99.55
Test solution -4	99.38
Test solution -5	99.48
Test solution -6	99.69
Mean	99.56
Standard Deviation	0.12
Relative Standard Deviation (%)	0.12

Intermediate precision:

Analysis performed during method precision study Analyst: Analyst-I HPLC ID No.: EAR040 Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number. : 0402471K	
Sr. No.	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution-1	99.67
Test solution-2	99.59
Test solution-3	99.55
Test solution-4	99.38
Test solution-5	99.48
Test solution-6	99.69
Analysis performed during intermediate precision study HPLC ID No.: EAR039 Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number : 0502481L	
Test solution-1	99.44
Test solution-2	99.56
Test solution-3	100.05
Test solution-4	99.42
Test solution-5	99.64
Test solution-6	99.83
Mean of twelve samples	99.66
Standard Deviation	0.24
Relative Standard Deviation (%)	0.24

Robustness:

- Change the flow rate of Mobile Phase:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 1.6 mL/ min.	1	99.65
	2	99.38
Mean		99.55
Standard deviation		0.12
Relative standard deviation (%)		0.12
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 2.0 mL/ min.	1	99.21
	2	98.99
Mean		99.44
Standard deviation		0.24
Relative standard deviation (%)		0.24

- Change in the Mobile Phase composition ±10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component +10%	1	99.78
	2	99.52
Mean		99.55
Standard deviation		0.13
Relative standard deviation (%)		0.13
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59

	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component -10%	1	99.11
	2	99.68
Mean		99.52
Standard deviation		0.20
Relative standard deviation (%)		0.20

➢ Change in the Temperature of the Column ±5°C:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Temperature of Column oven +5°C	1	*99.05*
	2	*100.65*
Mean		*99.63*
Standard deviation		*0.46*
Relative standard deviation (%)		*0.46*
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Temperature of Column oven -5°C	1	*101.01*
	2	*100.65*
Mean		*99.88*
Standard deviation		*0.60*
Relative standard deviation (%)		*0.60*

➢ Change in buffer component of mobile phase ±10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*

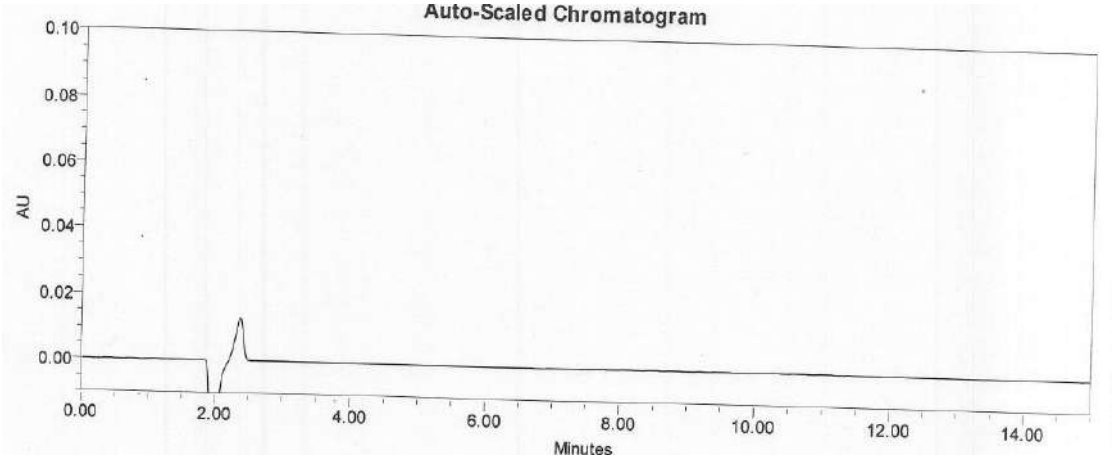
	5	*99.48*
	6	*99.69*
Change in Buffer component +10%	1	*100.25*
	2	*100.01*
Mean		*99.70*
Standard deviation		*0.29*
Relative standard deviation (%)		*0.29*
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Buffer component -10%	1	*99.65*
	2	*100.15*
Mean		*99.65*
Standard deviation		*0.23*
Relative standard deviation (%)		*0.23*

- System suitability parameters:

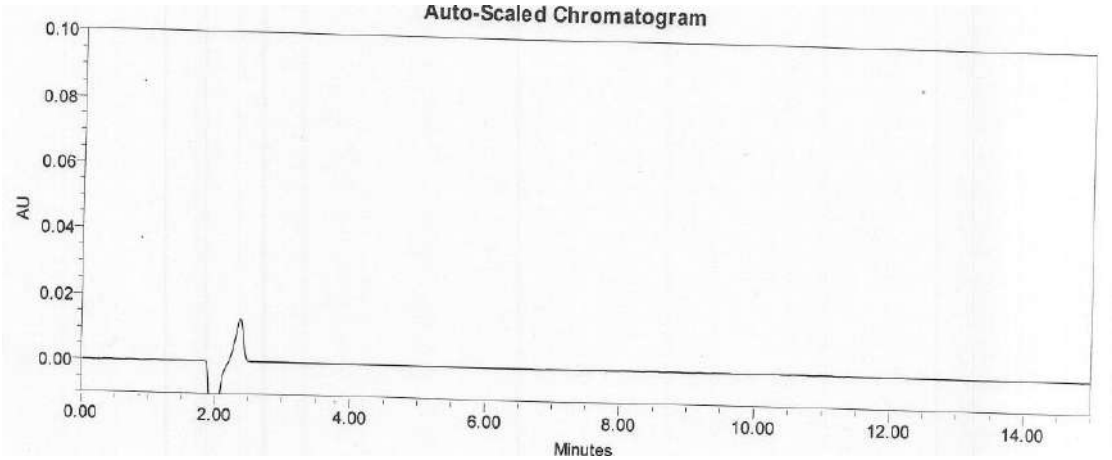
Parameter			Theoretical Plates	Tailing Factor	%RSD
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	Specificity				
	1.1	Specificity-Part-A	8529	1.02	0.11
	1.2	Specificity-Part-B	8313	1.05	0.15
2	Linearity and Range		8204	1.02	0.18
3	Accuracy study (Recovery)		8735	1.02	0.23
4	Precision				
	4.1	Method precision (Repeatability)	8526	1.04	0.12
	4.2	Intermediate Precision (Ruggedness)	8431	1.06	0.24
5	Robustness				
	5.1	Change flow rate by ± 10% (1.6 ml/minute and 2.0 ml/minute).	7952 8358	1.09 1.24	0.12 0.47
	5.2	Change the column temperature by ± 5°C (25°C and 35°C)	7952 8878	1.08 1.01	0.62 0.19
	5.3	Change the mobile phase Organic components by ± 10%	7952 6754	1.09 1.11	0.58 0.39
	5.4	Change the mobile phase Buffer components by ± 10%	7952 8886	1.09 1.31	0.58 0.79

Reason for validation: Non-Pharmacopeial method.

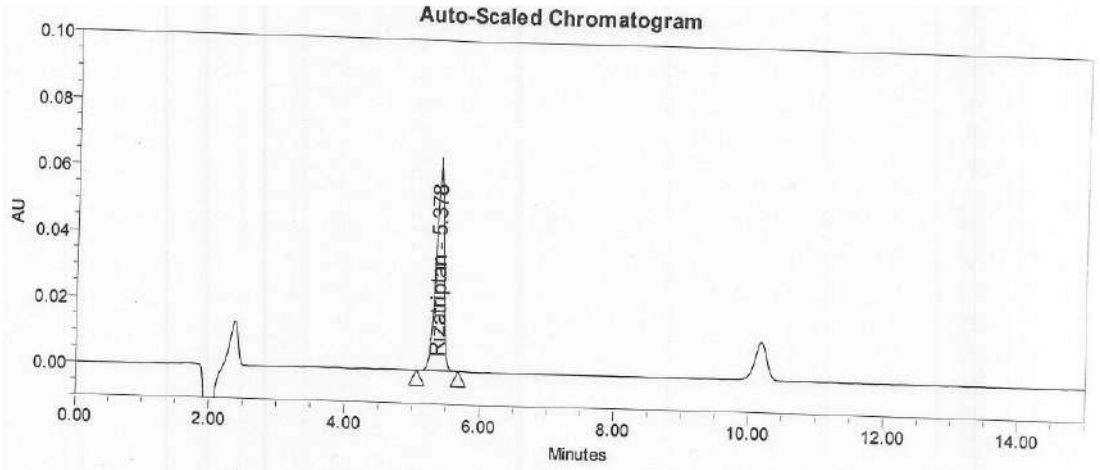
The results of analysis in terms of % label claim were found to be 98.00 to 102.00 for formulation analyzed.
Chromatograms:



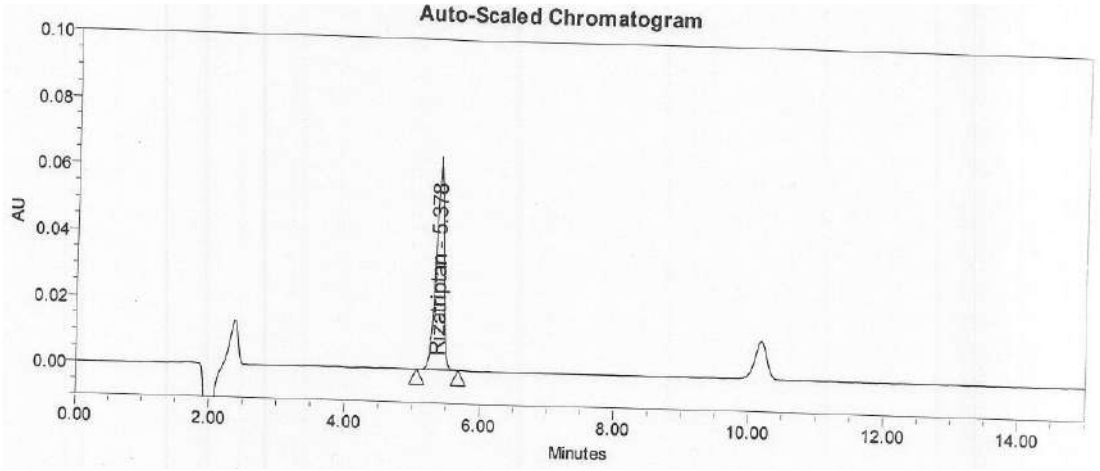
Blank



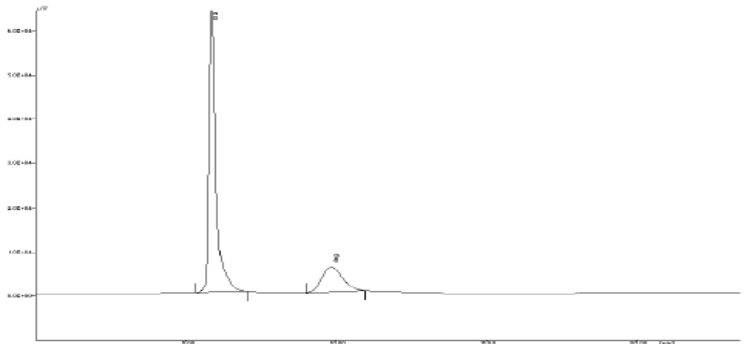
Placebo



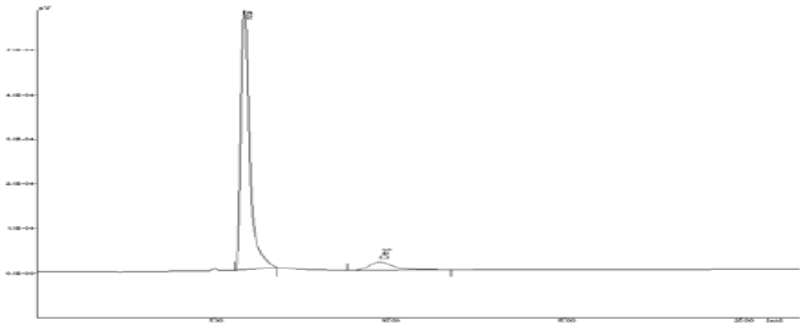
Standard



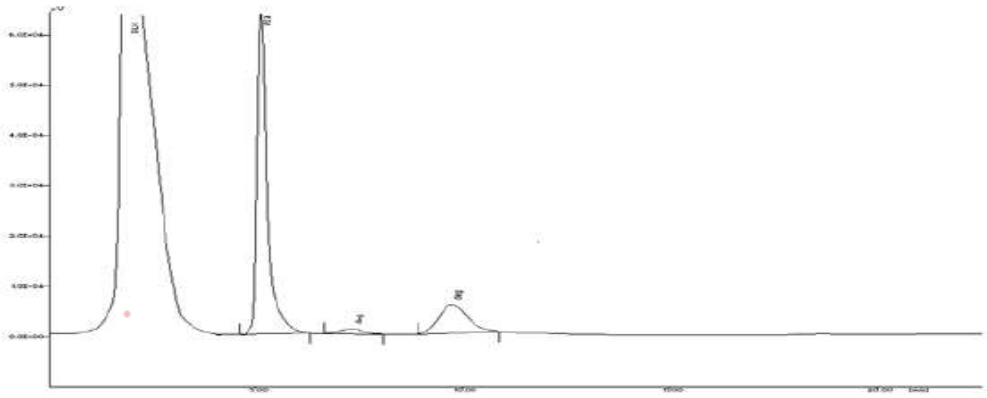
Sample



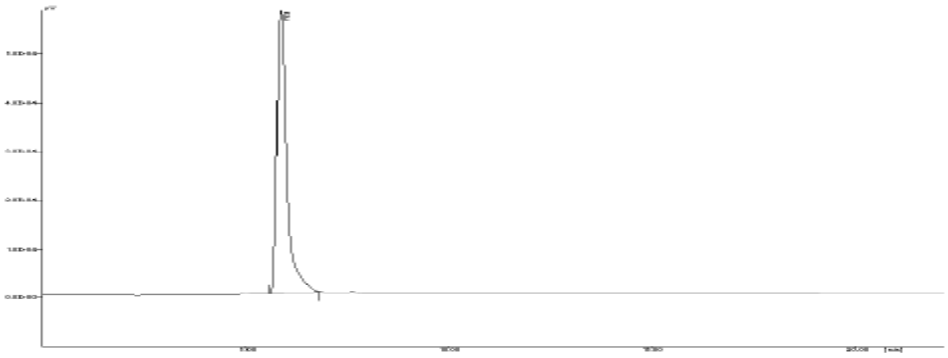
Chromatogram of Base degradation sample for Risatriptan (Rizatriptan Benzoate) tablet



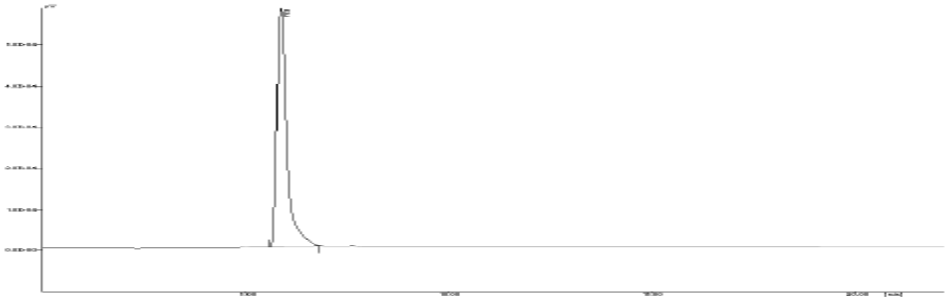
Chromatogram of Acid degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



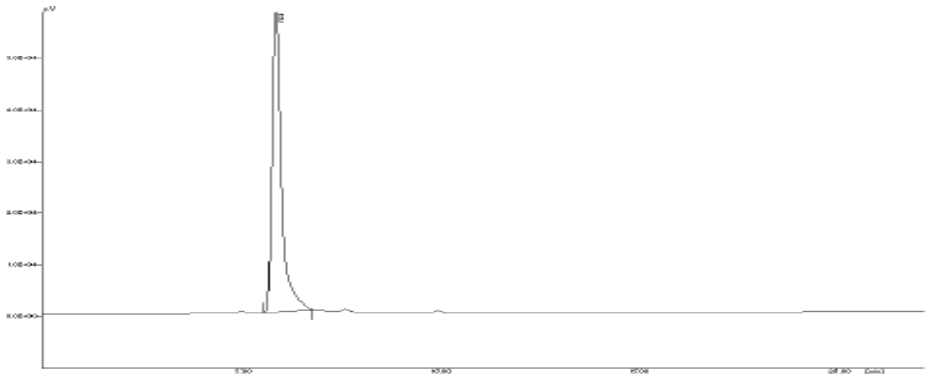
Chromatograms of per oxidation degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of thermal-degraded-Solution state sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of photo-degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of Humidity degradation sample for Risatriptan (Rizatriptan Benzoate) tablet

Result and Discussion:

- Specificity Part-I

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- Specificity Part-II

- Degraded impurities in all sample preparation are well separated from the main peak.
- Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.

Hence the method can be termed as specific

- Linearity and Range

The areas obtained are directly proportional to the concentration of analyte in the sample. Hence the method considered as linear in the range considered.

- Accuracy

The recovery at each level and mean recovery meets the established acceptance criteria.
Hence, the method can be termed as accurate in the considered range.

- Precision

The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- Filter media interference

The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- Robustness

No significant changes observed in system suitability parameters.
Hence, the method can be termed as robust.

- System Suitability

The mean values of system suitability parameters are well within acceptance criteria, hence the method is suitable
Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:

The proposed method for determination of Risatriptan (Rizatriptan Benzoate) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

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Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Abilify Disc melts in Immediate Release Dosage Form

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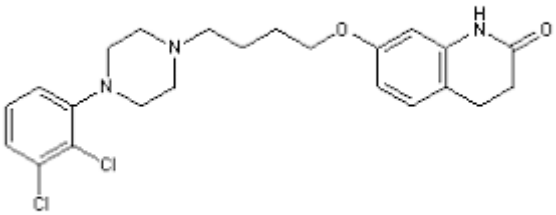
Abstract: In this research work of life cycle management of analytical RP-HPLC method development for assay of Ability Disc melt in immediate release dosage form, the RP-HPLC assay method was developed and validated for Ability Disc melt. Stress study is also carried out. The chromatographic conditions will be as, mobile phase is 0.05M Phosphate buffer pH2.0, Methanol, Acetonitrile in a ratio of 5:3:2, column used is Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm, Flow Rate1.5 ml/min, Injection volume 20 µl, Detection wavelength is 215 nm and run time is 25 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Ability Disc melt in immediate release dosage form.

Key words: Abilify Discmelt, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form

Introduction:

Abilify Disc melt (Aripiprazole)) is a typical antipsychotic agent, which is used in a treatment of schizophrenia, bipolar I disorder and acute treatment of manic and mixed episodes [1-3]. It is also used in Tourette's disorder in pediatrics patients (16-18 years) in the dose range 5-20 mg/day patient weight less than 50 kg. It has chemical name 7-(-4-(4-2-3- dichlorophenyl)-1-piperazinyl)butoxy)-3,4-dihydrocarbostyr il (Figure 1). It is effective in the treatment of both negative and positive symptoms of schizophrenia disorder. This agent belongs to the class of benzioxazole with dose 10-15 mg/day. It has partial agonist effect towards 5- HT1A receptor, dopamine D2 receptor and antagonistic effect on 5- HT2 receptor. Its sides effects including weight gain, QTc prolongation and hyperprolactinemia [4]. On the basis of literature survey few analytical methods reported for the detection of Aripiprazole in pharmaceutical dosage forms and biological fluids include high performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), capillary electrophoresis and spectrophotometric methods have been describe for the determination of Aripiprazole in pharmaceutical preparations [5,7]. The purpose of the current study was to develop a validated HPLC method for determination of Aripiprazole in bulk and pharmaceutical dosage form, to achieve more accuracy, specificity and precision. The method validation was preformed according to ICH guidelines. The method designed for estimation of Aripiprazole is more superior than previously reported methods and water is used as major part of solvents and less use of hazardous organic solvents.



Structure of Abilify Discmelt (Aripiprazole)

Experimental:

- **Materials:**

1) Abilify Discmelt (Aripiprazole): Working standard and its claimed purity was 98.20%.
2) Abilify Discmelt (Aripiprazole): Tablet (label claim 10mg) and placebo, which was prepared and supplied by Instavision lab,.

Note: No any known Impurity reported.

- **Reagents and Chemicals:**

1) Acetonitrile: -HPLC grade, Rankem, India.
2) Methanol: - HPLC grade, Rankem, India.
3) Milli-Q water: - It was purified by Millipore Corporation's system.
4) Acetic acid: - Reagent Grade, Merck, India.
5) Hydrochloric acid: - Reagent Grade, Merck, India.
6) Sodium hydroxide: - Reagent Grade, Merck, India.
7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
8) Potassium di-hydrogen orthophosphate - AR Grade, Merck, India.

- **Instruments, Apparatus and equipment:**

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Chrome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.
4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipment's, Germany

- **Chromatographic system:**

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).The published methods of analysis for determination and separation of Abilify Discmelt (Aripiprazole) in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Abilify Discmelt (Aripiprazole) .

- **Selection of Buffer in Mobile Phase: -**

0.05M Phosphate buffer pH2.0 with orthophosphoric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (50:30:20) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Mobile Phase: -**

Different ratios of Acetonitrile and Methanol was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Methanol: Acetonitrile) [50:30:20 v/v] after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Column:-**

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Hypersil ODS C-18, 250 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

- **Selection of Diluents / Solvent for extraction:-**

Different solvents were tried including single solvent and combination of solvents like ACN: Water, Methanol: Water, in different concentrations, But Abilify Discmelt (Aripiprazole) tablet gets dissolved in Methanol. Hence first stock was prepared in methanol and followed by second dilution done in diluents as [Methanol: Acetonitrile: Buffer 30:20:50] same as that of mobile phase to reduce the peak shape related problems.

The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria, Various Method screening Trials has been taken using following different compositions.

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- **Table for trials:**

Sr. No.	Trails Taken	Observation	Remarks
1	Buffer : Methanol (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer : Acetonitrile (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Peak observed later.	Not Satisfactory
3.	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 2.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
4	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Tailing observed	Not Satisfactory
5	Buffer : Acetonitrile : Methanol (50:20:30 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Good peak shape observed	Satisfactory

Reason for validation: Non-Pharmacopeia method.

Design of experiment (DOE):
A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. KH_2PO_4 conc. 0.05M +/- 0.01
2. pH of buffer pH 2.0 +/- 0.2
3. Buffer ratio 500 mL +/- 50mL
4. Methanol 300 mL +/- 30mL
5. Acetonitrile 200 mL +/- 20mL
6. Flow rate 1.5 +/-0.2mL
7. Column temp 40+/- 5 °C

Method Validation:

- **Standard preparation:**

Weigh and transfer about 30 mg of Abilify Discmelt (Aripiprazole) reference standard to a 200 mL volumetric flask. Add about 140 mL of Methanol, sonicate to dissolve, make up the volume with solvent Methanol. Further dilute 10 mL of this solution and make up the volume 25 mL with mobile phase. (60 ppm)

- **Sample preparation**

Weigh accurately tablet powder equivalent to 15mg transfer into 100 mL volumetric flask add about 75 mL of methanol , sonicate at for about 45 min with intermittent shaking, keep to achieve room temperature make up to volume with methanol. Centrifuge the solution at 3500rpm for 20 minutes and further dilute 10mL of the supernatant to 25mL with mobile phase. (60 ppm)

- **Mobile phase Preparation**

Mixture of (Buffer:- 0.05M Phosphate buffer pH2.0) 500 mL buffer, 300 mL methanol, 200 mL Acetonitrile and filter through 0.45µ membrane filter and degas.

- **Blank Solution:**

Use mobile phase as blank.

- **Optimized HPLC Parameters:**

Instrument : Agilant Liquid Chromatography with PDA detector
Column : Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm
Flow Rate : 1.5 mL/min
Injection volume : 20 µL
Column temperature : 40°C
Sample cooler Temperature : Ambient

Detection : 215 nm
Run time : 25 minutes

System Suitability Test:

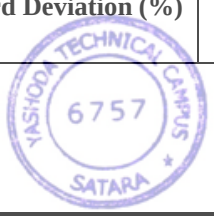
Sr. No.	Parameters	Abilify Discmelt (Aripiprazole)
1.	Peak area	*1183271*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	8.212
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.35*

Linearity:

Linearity Level	Standard concentration	Concentration of Abilify Discmelt (Aripiprazole) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	30.08	592833	0.9999
Level – 2	80%	48.12	946507	
Level – 3	100%	60.15	1186807	
Level – 4	120%	72.18	1425522	
Level – 5	150%	90.23	1765958	

Precision:

Sample Preparation	% Assay of Abilify Discmelt (Aripiprazole)
Test solution -1	99.57
Test solution -2	99.49
Test solution -3	99.40
Test solution -4	99.28
Test solution -5	99.59
Test solution -6	99.39
Mean	**99.45**
Standard Deviation	**0.12**
Relative Standard Deviation (%)	**0.12**



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Intermediate precision:

Analysis performed during method precision study

Analyst: Analyst-I HPLC ID No.: ASR34

Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number. : 12058H

Sr. No.	% Assay of Abilify Discmelt (Aripiprazole)
Test solution-1	99.57
Test solution-2	99.49
Test solution-3	99.40
Test solution-4	99.28
Test solution-5	99.59
Test solution-6	99.39

Analysis performed during intermediate precision study
HPLC ID No.: ASR34
Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number : 05482J

Test solution-1	99.64
Test solution-2	99.53
Test solution-3	100.02
Test solution-4	99.32
Test solution-5	99.62
Test solution-6	99.82
Mean of twelve samples	**99.56**
Standard Deviation	**0.21**
Relative Standard Deviation (%)	**0.21**

Robustness:

- **Change the flow rate of Mobile Phase:**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.30 mL/ min.	1	99.55
	2	99.30
Mean		**99.45**
Standard deviation		**0.12**
Relative standard deviation (%)		**0.12**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40

	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.70 mL/ min.	1	99.11
	2	98.95
Mean		**99.35**
Standard deviation		**0.22**
Relative standard deviation (%)		**0.22**

- **Change in the Mobile Phase composition ±10%:**

Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition +10% {higher organic- Buffer: MeOH: ACN) (500:330:220)}	1	100.14
	2	100.69
Mean		99.69
Standard deviation		0.48
Relative standard deviation (%)		0.48
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition -10%. {Buffer:MeOH:ACN) (500:270:180)}	1	99.98
	2	101.01
Mean		99.71
Standard deviation		0.56
Relative standard deviation (%)		0.57

- **Change in the Temperature of the Column ±5°C:**

Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**

Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column +5°C	1	99.66
	2	99.87
Mean		99.53
Standard deviation		0.18
Relative standard deviation (%)		0.19
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column -5°C	1	100.14
	2	99.68
Mean		99.57
Standard deviation		0.26
Relative standard deviation (%)		0.27

- **Change in the pH of the Buffer +0.1Unit:**

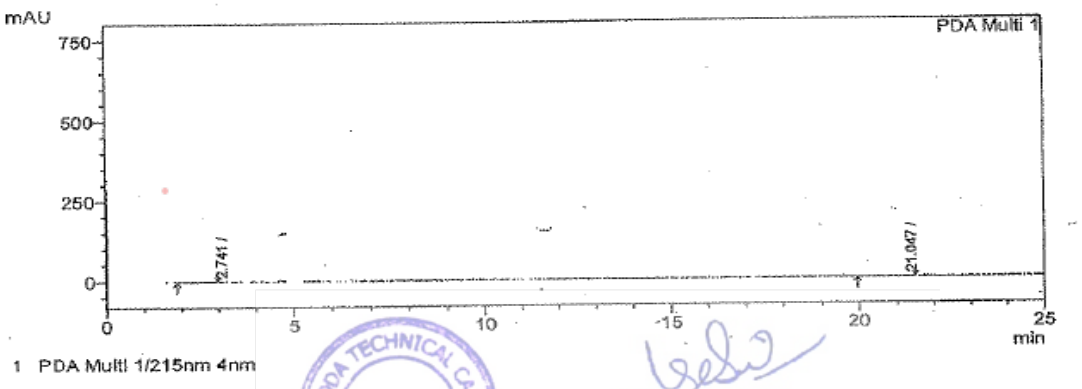
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by + 0.1 unit	1	101.01
	2	100.65
Mean		99.80
Standard deviation		0.65
Relative standard deviation (%)		0.65
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57

	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by - 0.1 unit	1	99.98
	2	100.15
Mean		99.61
Standard deviation		0.30
Relative standard deviation (%)		0.31

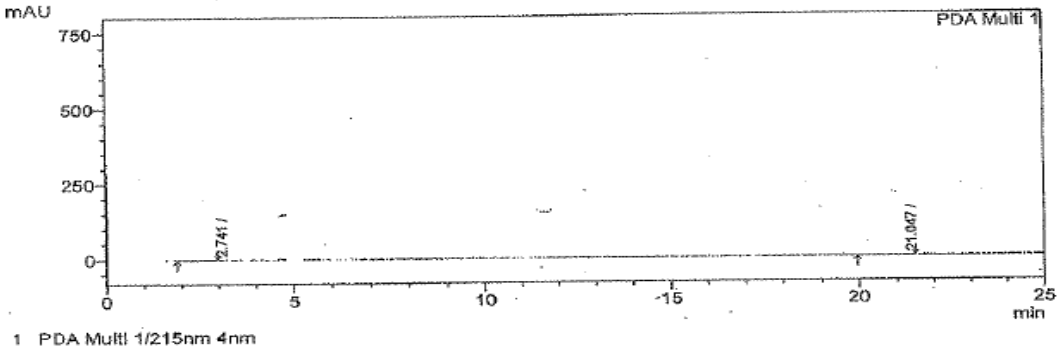
- **System suitability parameters:**

Parameter			**Theoretical Plates**	**Tailing Factor**	**%RSD**
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	**Specificity**				
	1.1	Specificity-Part-A	8952	1.07	0.13
	1.2	Specificity-Part-B	8213	1.05	0.15
2	**Linearity and Range**		8201	1.03	0.17
3	**Accuracy study (Recovery)**		8135	1.02	0.16
4	**Precision**				
	4.1	Method precision (Repeatability)	8546	1.04	0.14
	4.2	Intermediate Precision (Ruggedness)	8451	1.06	0.12
5	**Robustness**				
	5.1	Change flow rate by ± 10% (1.3 ml/minute and 1.8 ml/minute).	8212 8672	1.09 1.01	0.60 0.46
	5.2	Change the column temperature by ± 5°C (35°C and 45°C)	8014 7753	1.08 1.28	0.60 0.85
	5.3	Change the mobile phase Organic components by ± 10%	7845 8911	1.09 1.20	0.68 1.02
	5.4	Change the mobile phase Buffer pH by ± 0.1 Unit	8162 6785	1.11 1.29	0.57 1.36

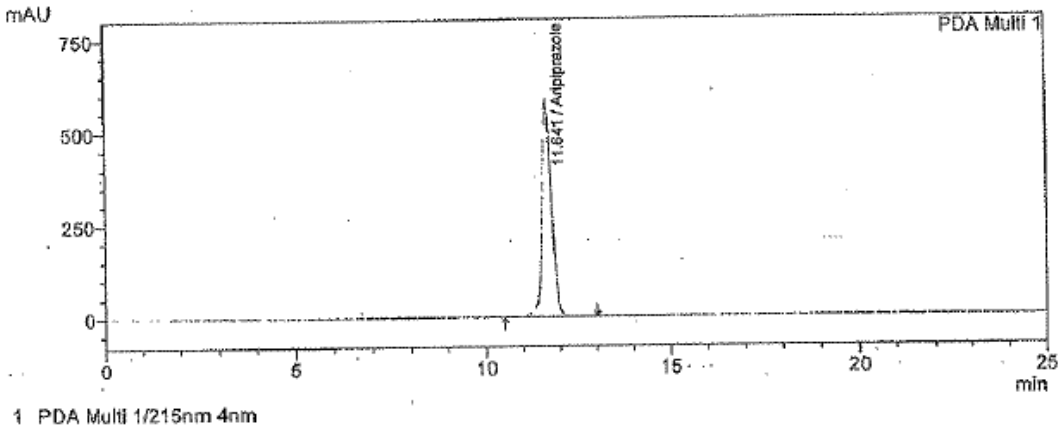
Chromatograms:



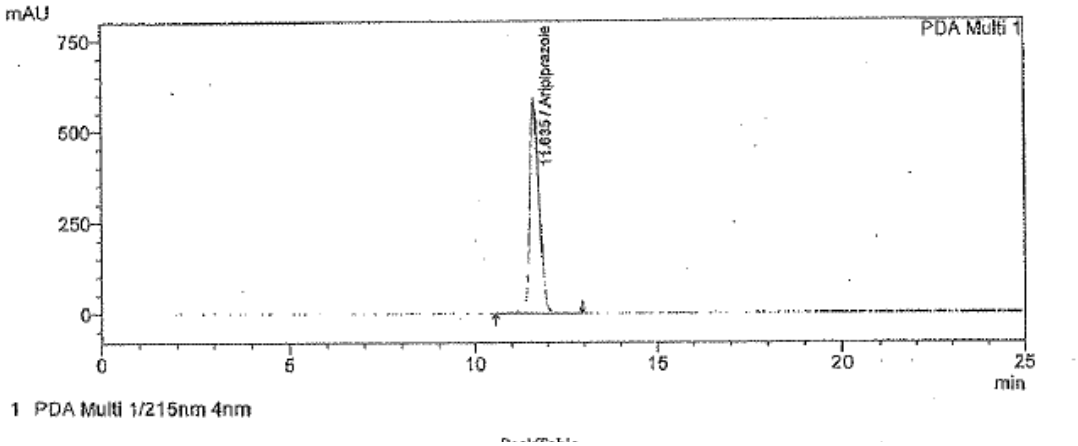
Blank



Placebo



Standard



Sample

Result and Discussion:

- **Specificity Part-A**

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- **Specificity Part-B**
  - Degraded impurities in all sample preparation are well separated from the main peak.
  - Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.
  - Hence the method can be termed as specific

- **Linearity and Range**

The areas obtained are directly proportional to the concentration of analyze in the sample. Hence the method considered as linear in the range considered.

- **Accuracy**
  The recovery at each level and mean recovery meets the established acceptance criteria. Hence, the method can be termed as accurate in the considered range.

- **Precision**
  The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- **Filter media interference**
  The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- **Robustness**
  No significant changes observed in system suitability parameters. Hence, the method can be termed as robust.

- **System Suitability**
  The mean values of system suitability parameters lay well within acceptance criteria, hence the method is suitable. Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:
The proposed method for determination of Abilify Discmelt (Aripiprazole) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

Acknowledgement:
I am thankful to my guide Dr. Rahul V. Mayee, Dr. Ajit Ekal, Managing Director, Instavision laboratory and Services, Satara for their technical help in this project. I am also thankful to our Principal YSPM's YTC, Faculty of Pharmacy Dr. V. K. Redasani and Management for their motivation and support.

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FORMULATION AND EVALUATION OF ALOEVERA AND VITAMIN E PEEL OF MASK

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ABSTRACT

Peel off mask is the type of dosage form which is gently applied onto the facial skin surface and is peeled off after few minutes of its application. It is used as the remedy to treat facial skin related problems such as wrinkles, ageing, acne and mainly used to open the closed pores due to deposition of dust. Aloe vera and Vitamin E is being added as an active ingredient in this formulation. Dosage formulations of peel of mask made in type of three methods. Further preparation peel of mask evaluated physical properties (organoleptic, homogenicity, P^H, spredability test, peeling time, irritation test). The results showed that the first method shows most of the evaluation parameters compatible during 2 weeks of storage.

Keyword: Aloe vera, Cosmetic, Peel of mask, Antioxidant

INTRODUCTION

Peel of face mask makes skin healthy People make many attempts to get beautiful and fresher skin. But due to dust, pollution, unhealthy eating habits and poor daily routine, many skin related problems erupt. People use beauty products and home remedies to avoid these problems. In this regard, a peel-off mask can also be a better option as it removes the skin dirt from the inside and also destroys dead skin cell. The peel-off mask also reduces many skin problems and keeps the skin healthy and fresh **[1]**.

Applying a face mask and using cosmetics to maintain beauty has been a beauty ritual practiced since ancient times. In fact, we know that the first cosmetic product ever made was a face mask. Face masks can nourish, cleanse, moisturize and tone the

skin while also providing essential active ingredients for skin care **[2]**.

Indian women prepare different kinds of face masks at home. You can find many ready-made Ubtan powders on the market where you simply need to mix them with water or milk and apply them to your face. Ayurveda beauty care has not changed much since its introduction some five thousand years ago, proof of its effectiveness, safety and ease of use.

Roman women regularly used face masks as part of their beauty routine. Oils, honey, vinegar, basil juice and goose fat were popular ingredients. They also used some rather exotic ingredients such as placentas or stools of animals like kingfishers and cows **[3]**.

The pale look continued to be popular well into the times of Elizabeth I. By now, women had resorted to even more dangerous means to reach their desired skin color. They used hitherto unknown toxins such as white lead mixed with honey and olive oil to whiten their skin. But even this was not enough for some, and the practice of bloodletting continued. Fortunately, less invasive methods weren't completely discarded. Face masks made of egg whites and lemon juice were also used by some to brighten and nourish the complexion and achieved much safer results.Face masks in particular are available in all different types: creams, gels, powders or sheets. There are masks that choose to follow a more scientific and "chemical" approach and others that are rooted in a natural and holistic approach to skin care **[4]**.

MATERIALS AND METHODS

Carbopol 934 grade dissolved in water for 24 hr and after swell the carbopol is continuous stir with mechanical stirrer. Polyvinyl alcohol dissolved in warm water in water bath gently with continuous mechanical stirring and allows swelling for 2-3 hours then add methyl paraben. To this Disodium edetate (Solubilised in 1M NaOH) were loaded gently & dissolved. Drug solution along with carbopol and PVP were added slowly in swelled NaCMC (Sodium-carboxymethylcellulose) under continuous stirring. Citric acid added to the obtained solution to maintain PH and added in propylene glycol. Talcum powder mixed it to give the formulation opacity. Aloe extract dissolved in Rose water and added the vitamin E in continuous stirring. Final volume was made up with the purified water. After addition of whole ingredient, stirred continuously until a smooth dispersion obtained. Prepared formulation filled in collapsible tube for further analysis **[5, 6]**.

Table 1: Ingredients for the Peel of Mask

Sr. No.	Ingredients	Manufactures
1.	Disodium edentate	S.D. FINE MUMBAI
2.	Polyvinyl Alcohol [PVP]	
3.	Carbopol [934grade]	
4.	Sodium carboxymethylcellulose	
5.	Methyl paraben	
6.	Propylene glycol	
7.	Talcum powder	
8.	Citric acid	
9.	Aloe extract	
10.	Vitamin E	
11.	Rose water	
12.	Water	

Figure 1: Mechanical stirrer

RESULT

The Aloe vera and vitamin E peel off mask was found to successful with good results. The peel off mask showed a good spredability. The formulation showed a good peel off property on human skin without causing skin irritation. The formulation showed good stability results and was found to be stable till room temperature.

The entire required ingredient to prepare peel of mask Aloe vera powder and vitamin E from the aloe Vera powder extract and vitamin E capsule. It was prepared according to the procedure and evaluated by performing the above test like spredability, stability, appearance etc.

EVALUATION [7-10]

1. ORGANOLEPTIC CHARACTER

The consistency and the colour was checked visually the odour was evaluated manually by smelling the product. The organoleptic character include its color, odor, feel and consistency which were evaluated manually for its physical properties **(Table 2)**.

2. PHYSICAL STABILITY

Observation of physical stability at room temperature by observing organoleptic during storage. This formulation was

performed to see the stability on formulations at low and high temperature of prepared peel off mask. Six cycle between refrigerator temperature (4°C) and accelerated temperature (40°C) with storage at each temperature for not less than 24 hours performed. The formulation was found to be stable at these temperatures were subjected to Freeze thaw stress test found stable **(Table 3)**.

3. IRRITATION TEST

This parameter checked with patch test. Irritated skin at the patch site may indicate an allergy

Mark an area (1sq.cm) on the left-hand dorsal surface. Definite quantities of prepared peel of mask were applied to the specified area and time was noted. Irritancy was checked if any for regular intervals up to 24 hrs and reported **(Table 4)**.

4. PEELING TIME

The peel gel was applied on the skin surface uniformly. The peel was allowed to dry. After 15 min the peel was removed from the skin surface. It was observed that the peel was removed easily without breaking **(Table 5)**.

5. HOMOGENISITY TEST-

Test Homogeneity testing is investigated by applying a peel-off mask to a glass object or transparent material, then observing the composition of coarse or inhomogeneous particles and recording them. The preparation must show a homogeneous order and should not show any coarse grains **(Table 6)**.

6. SPREDABILITY TEST -

The spreading capacity of peel of mask formulation was measured 48 hr after preparation by measuring the spreading diameter of 1 gm of the gel between two 20×20cm glass plate after 1 min. The mass of the upper plate was standardized at 125g **(Table 7)**.

The following equation was used for the purpose:

S=m*l/t

Where,

S = the spredability of the mask formulation

m = the weight (g) tied on the upper plate

l =the length of the glass plates

t = the time taken (second)

7. P^H MEASUREMENT

The p^H value of topical peel off mask was determined by using digital p^H meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurements of p^H of the formulation were done in triplicate and average values were calculated **(Table 8)**.

Table 2: Organoleptic Properties of the Peel of Mask

PARAMETER	OBESERVATION
Colour	Yellowish Green
Odour	Pleasant
Consistency	Smooth
Nature	Semisolid

Table 3: Stability Study of the Peel of Mask

Stability	Preparation		
	1 gm	2gm	2.5gm
1day	Stable	Stable	Stable
1week	Stable	Stable	Stable
2week	Stable	Stable	Stable

Table 4: Results of Irritation Tests

Formulation	Observation		
1%	Non irritant	Non irritant	Non irritant
2%	Non irritant	Non irritant	Non irritant
2.5%	Non irritant	Non irritant	Non irritant

Table 5: Results of Peeling Time Study

Formulation	Time
1%	12minute,10 second
2%	14 minute, 37 second
2.5%	15 minute,25 second

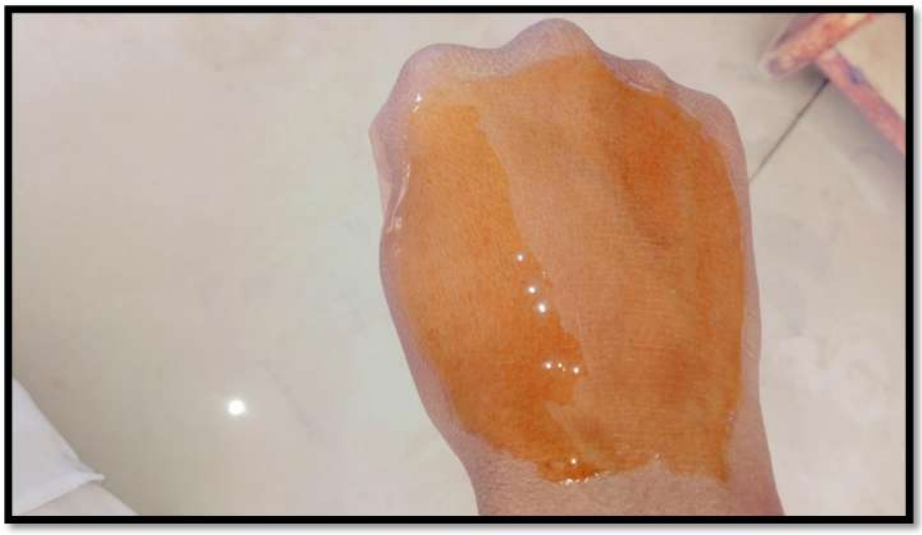

Figure 2: Applied peeling property

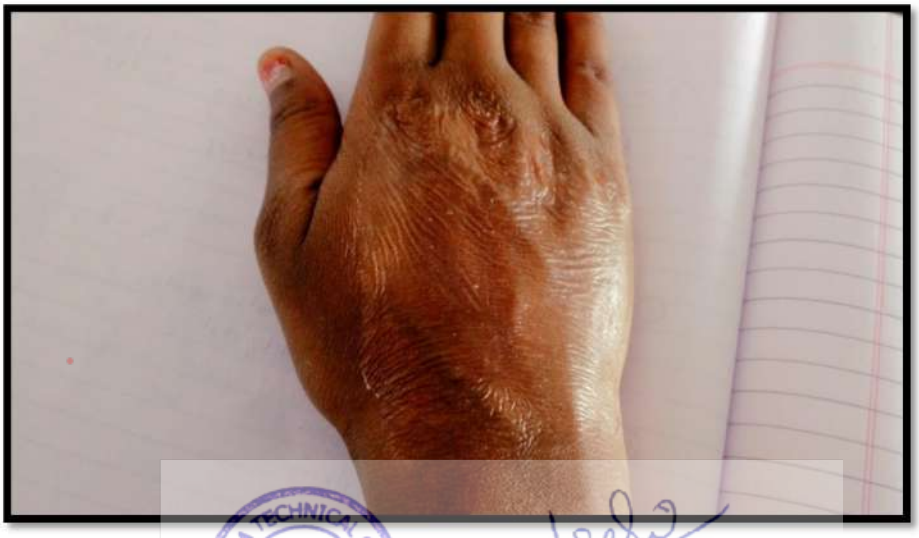

Figure 3: Dried peeling property

Table 6: Homogeneity of the Formulation

Formulation	Observation		
1%	Gel	Gel	Gel
2%	Gel	Gel	Gel
2.5%	Gel	Gel	Gel

Table 7: Spreadability Study of the Formulation

Formulation	Observation		
1%	0.900 cm^2	1.8 cm^2	2.3 cm^2
2%	0.92 cm^2	1.5 cm^2	2.2 cm^2
2.5%	0.7 cm^2	1.57 cm^2	2.0 cm^2

Table 8: pH evaluation of the Peel of Mask

Formulation	P^H Observation		
	1Day	1week	2week
1%	5.3	6.0	6.8
2%	5.5	6.2	7.2
2.5%	5.1	6.5	7.5

Figure 4: Formulation of Peel of mask of aloe vera and vitamin E

Figure 5: Packing and Labeling of Final Product

CONCLUSION

The Aloe vera and vitamin E peel off mask was found to successful with good results. The peel off mask showed a good spredability. The formulation showed a good peel off property on human skin without causing skin irritation. The formulation showed good stability results and was found to be stable till room temperature.

The entire required ingredient to prepare peel of mask Aloe vera powder and vitamin E from the aloe Vera powder extract and vitamin E capsule. It was prepared according to the procedure and evaluated by performing the above test like spredability, stability, appearance etc.

Aloe vera and peel of mask was prepared and evaluated by doing various test. evaluation tests were carried out and confirmed the product sensitivity and appearance. Aloe vera and Vitamin E peel of mask successfully passed all tests such as Organoleptic characters, Physical stability, Irritation Test, Peeling Time, Homogenisity Test, Spredability Test and PH Measurement.

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Building a Self-Driving Autonomous Car Model Using the Raspberry Pi Processor and Computer Vision Methods

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ABSTRACT

The development of autonomous self-driving vehicles has attracted a lot of attention in the transportation industry recently. The building of a self-driving autonomous automobile model employing a 4B 8GB RAM Raspberry Pi processor and computer vision algorithms is demonstrated in this project. The vehicle has a Raspberry Pi camera in the front for object recognition and an ultrasonic sensor in the back for obstacle recognition. Using image processing methods, the suggested model is capable of detecting objects, lanes, and traffic signals. The vehicle is also capable of making choices depending on the information detected and controlling the vehicle accordingly. Future smart cars that are predicted to be driverless, effective, and crash-avoidant are autonomous vehicles. Automakers have started working in this area to realize the potential and address the issues that are currently present to achieve the desired results. The field of autonomous automation is of interest to researchers and much in this field has been done, of which the present paper has a detailed timeline. This article can help to understand the trends in autonomous vehicle technology for the past, present and future. Since 1920 we see a dramatic change in autonomous vehicle technology when the fest radio-controlled vehicles were designed. In subsequent decades, we see fairly autonomous electric cars powered by embedded circuitry on roads. By the 1960s autonomous vehicles with similar Electronic Guide Systems came into the picture. By the 1980s vision guided SC autonomous vehicles was a major step in technology. Various semi-autonomous features introduced in the modem cars such as Laredo, automated braking and adaptive cruise control are based on such systems. The future of autonomous vehicles is extensive network-aided systems in conjunction with vision-driven features. By the advent of the next decade, most companies will launch fully autonomous cars. The autonomous vehicle future is an ambitious era of safe and comfortable transportation.

Keywords- Circuit, Lane detection, Predictive modelling, Self-driving, Smart discrimination

INTRODUCTION

The development of self-driving autonomous cars has become a viable alternative for improving transportation safety and efficiency. Autonomous vehicles have the potential to eliminate human error and make driving more convenient for individuals. We propose a self-driving autonomous car model employing a 4B 8GB RAM Raspberry Pi processor and computer vision algorithms in this research. The car is programmed to identify traffic signs, lanes, and objects on the road and make appropriate decisions [1].

LITERATURE VIEW

Rasheed Hussain and Shefali Zeadally's [2] article, "Autonomous Cars: Research Results, Issues, and Future Challenges," is available online. The recommended system is an autonomous vehicle prototype with several objectives, such as object detection, path detection, and traffic signal detection using the Raspberry Pi's processor, camera, and ultrasonic sensor for the aforementioned functions. Ultrasonic sensors are used to steer clear of obstacles and detect them. Traffic signals, signs, and walkways are all detected by a camera. Motor drives enable the direction-changing,

starting, and stopping of motors.[3] A Unified Map-Based Autonomous Driving System for Unknown Environments by Jongwon Choi [4]. Autonomous vehicles have made significant advancements and will be key components of future intelligent transportation networks. These cars must have the ability to independently adhere to traffic laws while avoiding Short Term Traffic Prediction for Enhanced Autonomous and Connected Cars using Edge Computing- Hui-Nien Hung, Shun-Ren Yang, Yu-Ju Su, Yao-Yuan Chang [5]. Future intelligent transportation systems will be greatly aided by the development of self-driving cars, which have advanced significantly. For these cars to be successfully deployed on real roads, they must be able to autonomously travel along collision-free paths while obeying traffic laws. Instead of using prebuilt maps of highways and traffic signals to identify barriers, other cars, traffic signs, and pedestrians, we propose methods and systems that use a single map made using a variety of onboard sensors. The suggested map not only provides details about nearby physical obstructions but also virtual ones like traffic. Based on realistic road data and typical sensor accuracies, simulations are run across driving distances of about 150 km and the navigation system's layout is explored. Positioning errors less than 10 m (standard deviation) in size are seen. The synchronization error between measured and mapped data must be continuously assessed to reach this accuracy. The newly released Navistar GPS-based navigation technology is perfect for completing current, commercial automotive navigation systems. The path planner may effectively discover collision-free paths while adhering to traffic regulations using this map. The proposed algorithms were tested on a commercial vehicle and proven in a variety of scenarios, including the 2012 Hyundai autonomous ground vehicle competition [6].

COMPONENTS

Power Supply

The power supply circuit has two additional pins for attaching a transformer. These pins charge the battery and supply the required DC voltage to the bridge rectifier, which has a PIV (Peak Inverse Voltage) rating of 1000V. To generate a smoother DC waveform, the rectified pulsing DC output is sent to a 1000uF capacitor. This smoothed DC voltage is then sent into the 7805 IC, which produces a stable 5V output, and the 7812 IC, which produces a stable 12V output. In addition, a 470-ohm resistor is included in the circuit to manage the current (Fig. 1)[7,8].

***Figure 1**: Battery for power supply.*

Raspberry pi

The central component employed in the project is a compact single-board computer with dimensions like a credit card. This computer is specifically designed and programmed to perform image recognition tasks, enabling it to analyze and compare images. Once the analysis is complete, the computer executes an algorithm to determine the most appropriate course of action based on the input image. In essence, it leverages its training and processing capabilities to swiftly respond to the given visual information (Fig. 2) [9].

***Figure 2**: Raspberry Pi microprocessor.*

Motor Drive

The purpose of this circuit is to control the movement of the model by driving two DC motors. The motor driver utilized in this circuit is the L293D, capable of independently operating two DC motors simultaneously. Input signals for controlling the motor driver are received from the GPIO (General Purpose Input/Output) pins, specifically pins 12, 16, 20, and 21. These input signals dictate the desired movement and speed of the motors, allowing for precise control and coordination (Fig. 3) [10].

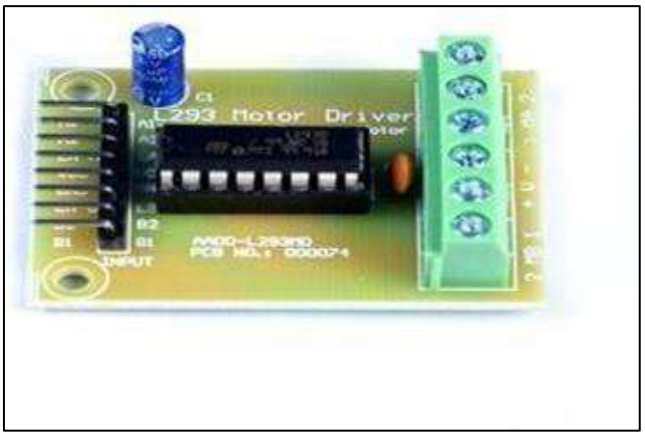

***Figure 3**: Motor drive to control motors.*

Ultrasonic Sensor

It is used to calculate distance. The ultrasonic trig and echo pins are linked to Raspberry Pi GPIO pins 17 and 18, respectively. (Fig. 4) [11].

***Figure 4:** Ultrasonic sensor for sensing.*

Camera

The camera is linked to the raspberry pi via USB and will capture images from its surroundings and send them to the raspberry pi for processing (Fig. 5).

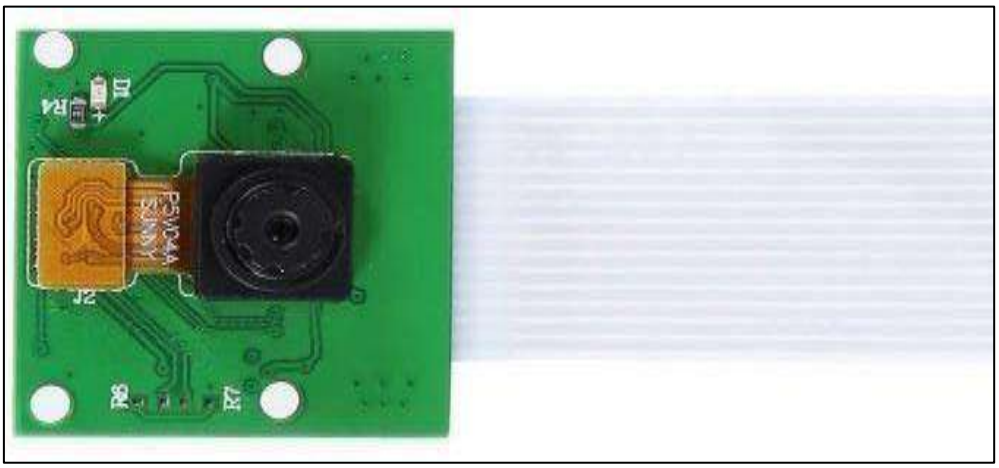

Figure 5: *Camera for detection.*

PROPOSED SYSTEM

The present suggested system employs a pattern-matching technique in which cameras detect a unique pattern that would be printed on the roads. This pattern will be captured by the camera and processed using a Raspberry Pi to command the automobile to travel in the given direction. The camera will also capture surrounding images to determine different obstructions adjacent to it; if the obstacles get too close or are going to collide with the vehicle, the vehicle will stop until the obstacle close to it moves. Special patterns will be deployed alongside the route to detect the type of road ahead. The Pi Camera will be installed on the vehicle. It will be connected to Raspberry Pi 3B+. The camera will capture all the images and send the data to the Raspberry Pi for processing. The Raspberry Pi will be powered by a 5V power supply. We will install the Raspbian Stretch OS. The Genny editor will be used for programming purposes. Python will be used as the programming language. The L293D motor driver will start the dc motors based on the Raspberry Pi instructions [12].

OPERATING METHODOLOGY

Computer Vision

Vision in computers because there was insufficient time to build it properly, a computer vision algorithm was not implemented in the core algorithm of this project. However, there existed a camera with which some testing was conducted, as well as an algorithm written in Python to work with OpenCV. This algorithm developed by a member of the computation team working on this was able to detect the presence of traffic signals and to detect some of them in real-time; however, even though this was possible for a small number of signals, it proved difficult to work with all of them because they were too numerous and would significantly slow down the main programme. It was planned to use a database confined to the signals that the vehicle would encounter, as well as a neural network that would learn the signals and then not need to search for matches, but none of these was eventually implemented. A couple of MATLAB programmes were created for road detecting one for detecting road lines in a normal road and another for detecting asphalt based on textures and/or colour [13-16].

Path Planning

Path planning entails developing a series of states from the car's present state to the next objective state, which does not describe how the car's states evolve. Path planning is typically divided into global and local paths [KAM04]. A global path is generated before the car begins travelling using an offline global map of the environment in global path planning. A local path is built as the car is travelling utilising online local maps of the surroundings, allowing the automobile to deal with moving obstacles.

Path planning methods can be divided into two categories: graph search-based and interpolating curve based [17].

BLOCK DIAGRAM

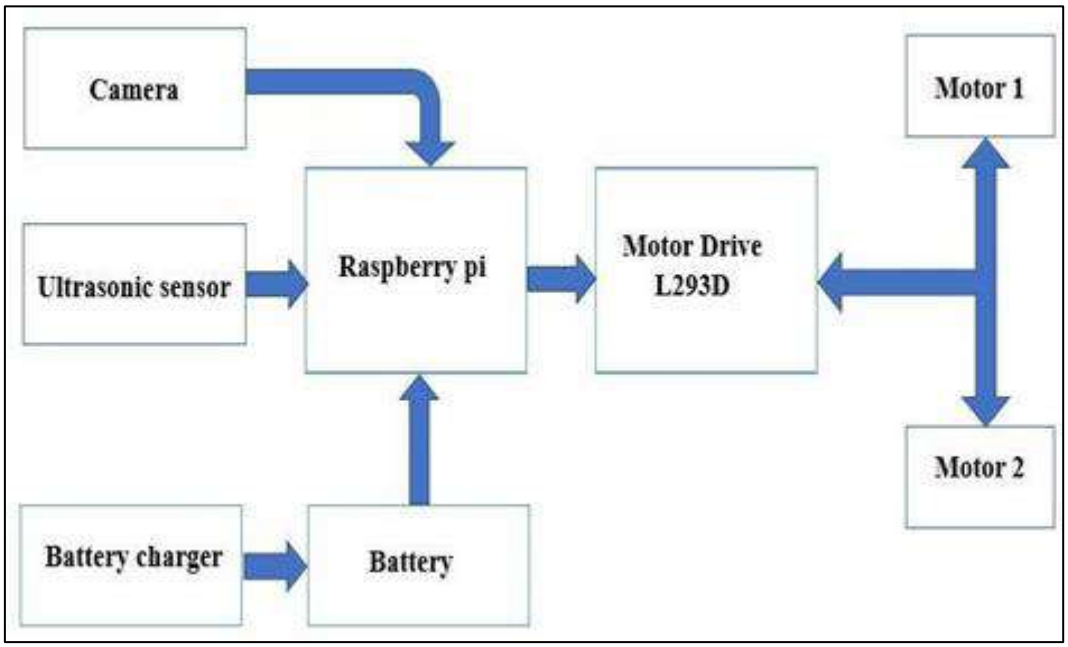

***Figure 6**: Block diagram of construction for the autonomous car.*

The proposed system is a prototype function of an autonomous car with multiple objectives, like object detection, path detection and traffic signal detection. The above functions are implemented with a Raspberry Pi processor, camera and ultrasonic sensor. The ultrasonic sensor is used to detect obstacles and avoid collision. The camera is used to detect paths, traffic signs and signals. The motor drive is used for direction change and start and stop of motors (Fig. 6, 7).

***Figure 7**: Autonomous car with connection.*

CONCLUSION

A self-driving autonomous car model using a 4B 8GB RAM Raspberry Pi processor and programs for computer vision was presented in this project. The vehicle had an ultrasonic sensor for the back to identify obstacles and a Raspberry Pi camera for the front to detect objects. The suggested model could recognize objects, lanes, and traffic signs along the way and make decisions depending on the information it had picked up. Obstacles in the back of the car could be found and avoided with the help of the back ultrasonic sensor. The outcomes demonstrated that the suggested model was successful in following the path with high accuracy and without requiring human intervention. As technology advances around the world, self-driving cars will become the dominant means of transportation in the future. The legal, ethical, and societal consequences of self-driving cars revolve around concepts such as liability, responsibility, and efficiency. Automobile cars will benefit the economy through increased fuel efficiency, the environment through reduced carbon emissions, society by increased togetherness, and the law through simplified liability systems. These concepts, however, concentrate on two essential features of autonomous vehicles: how they work and how they are secured. As technology advances, security technologies for self-driving cars will develop to combat hackers, improve internal system accuracy, and prevent accidents. When all of these technologies are at their peak, civilization will be one step closer to the ideal of flying vehicles that most of us dreamed of as children.

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Smart EV Charging Station With ON Grid Green Power & Wireless Charging

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ABSTRACT

Emerging nations like India are deploying electric vehicle (EV) technology and phasing out the use of fossil fuel-powered vehicles as part of their effort to tackle climate change and rising urban pollution. In April 2017, the Indian government declared that all EVs would be sold on the market by 2030. Also followed is the promotion of the FAME (faster adoption and production of electric vehicles) program. The infrastructure for electric charging is a crucial part of the ecosystem for electric mobility. The market for EV charging stations must grow and accept EVs at the same rate. EVs are constrained by their speed and range. The key to successful electric vehicle use is the network of charging stations that are available. Electric vehicles (EVs) will be smoothly incorporated into the transportation system, which is one of the key elements of future smart city planning. The primary energy source for EVs is a charging station, and a city's accessibility to EVs depends on the station's location. They should be placed thoughtfully so that an electric vehicle (EV) can access a charging station within driving distance and travel anywhere in the city once it has been recharged. In this article, we formulate the Electric Vehicle Charging Station Placement Problem, in which we want to reduce the overall construction cost while keeping in mind the drivers' convenience and the charging station coverage requirements. We examine the problem's characteristics, particularly its hardness.

Keywords- Power supply, Powerful vehicle, Renewable energy, Solar energy, Wireless

INTRODUCTION

Due to growing awareness of the benefits of living sustainably, the adoption of electric vehicles as gas-powered vehicles substitutes has grown quickly.

The grid has traditionally been used for charging electric vehicles. However, due to technological advances in solar energy, it is now possible to recharge electric vehicles using solar-powered chargers. These clean solar chargers supply clean electricity to electric vehicles while also benefiting the environment. Furthermore, the availability of these charging stations would inspire people to reassess their transportation preferences and switch to zero-emission vehicles.

As electric vehicles become more affordable each year, investors have begun to invest in charging infrastructure grid supply for widely available automobiles, to design and create a solar-powered charging station, to gather electric vehicle power information, and put the charging station into operation with the capability of utilising solar energy when it is available and switching to grid supply otherwise. A charging station supplied by a traditional grid supply has numerous restrictions and disadvantages, thus we employ solar energy for charging. The switching circuit allows circuits to be switched, and the application of MPPT (maximum power point tracking) allows maximum solar energy to be tracked.

The market for electric cars (EVs) is gradually expanding, to fast recharge the car; the current conductive charging method requires high-power charging equipment or charging stations.

The different EV models' incompatible plug receptacles add to the inconvenience. As with wireless charging systems, several EV models can share the same charging

infrastructure.

85 million autonomous vehicles are projected to be in operation by 2035, which necessitates the development of wireless charging [1, 2]. WPT, as opposed to wires, makes the system simpler, automatic, secure, inexpensive, and more efficient. Wireless charger solutions, for example, enable automated charging while EVs are temporarily parked in parking lots.

SYSTEM DESCRIPTION

In this approach, the car is charged via the plug-in method. This has various shortcomings, such as the size of the battery. The battery's large size makes acceleration lessen. The battery is the major obstacle to bringing electric automobiles into the affordable price range. Smaller batteries would be much cheaper, thus wireless charging was used. Therefore, wireless charging is more user-friendly and has a faster charging rate because there is no need to plug a cable into the automobile [3].

METHODOLOGY

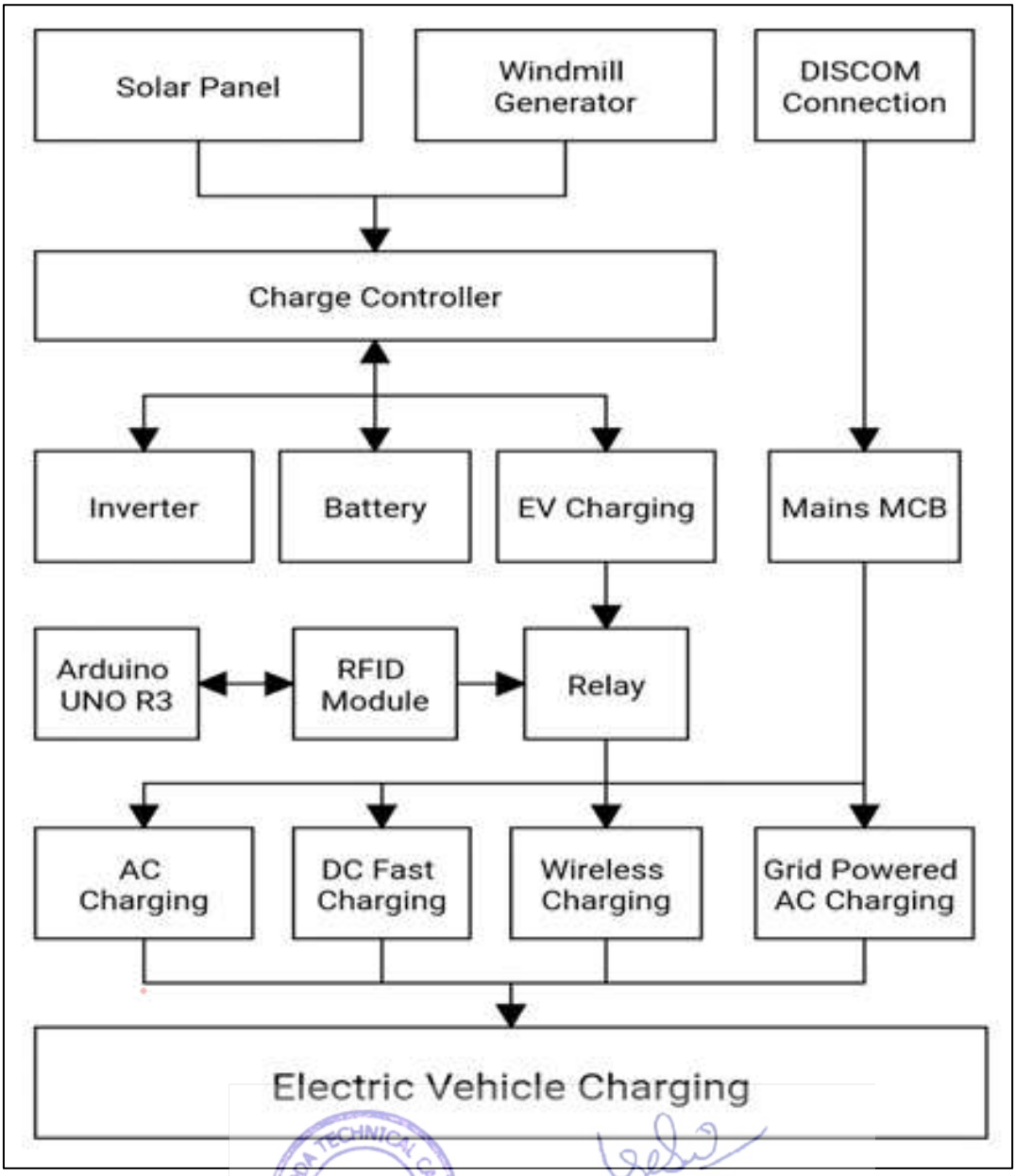

Figure 1: *Block diagram of charging methods.*

An EV charging station must include an inverter with a grid interface, an RCL filter, a transformer, a DC bus feed, and battery chargers. The two forms of renewable energy sources utilized in this project are solar energy and wind energy. Firstly, the components that convert natural phenomena into electrical energy, such as solar PV cells and wind turbines. Both solar PV cells and wind turbines frequently use Buck-Boost converters, which ensure that low voltage is increased into necessary high voltage so that the battery may be charged. Because this circuit only converts AC to DC in one direction, a rectifier with C smoothing is only used at wind energy sources (Fig. 1, 2).

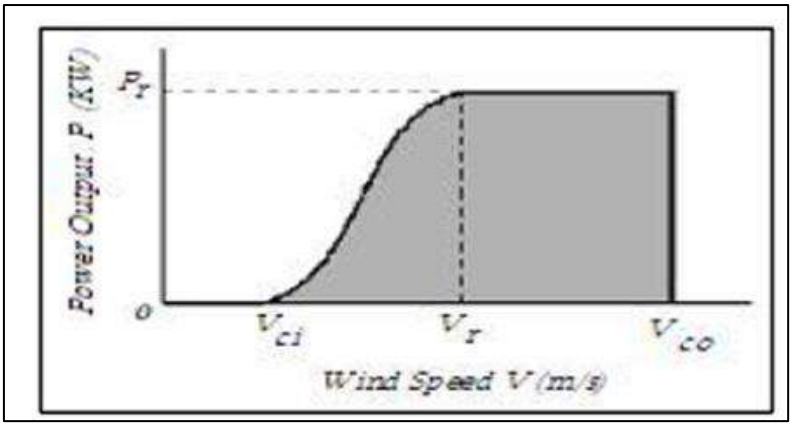

Figure 2: *Waveform of wind energy output.*

WORKING

Let us say that the transmitter has L1 turns, I1 current, and a magnetic field. A little amount of magnetic flux may flow through the receiver when the transmitter and receiver are close together [4, 5].

If the transformer has a primary and secondary side, charging without a WEVCS connection has a transmitter side and a reception side. A wireless charger for an electric vehicle's transmitter and receiver windings are equivalent to the primary (coil) and secondary windings of a transformer. However, wireless charging of electric vehicles shifts the alternating current (AC) parameters from low frequency 50Hz to high frequency, which does not occur in the transformer. Before the alternating magnetic field is formed, the transmitter coil gets high-frequency alternating current power. This field then stimulates the receiver winding, causing a voltage to appear on it. This voltage is utilized to charge the battery in the car [6].

The frequency of resonance between the transmitter and receiver must be maintained for wireless charging to function properly. Compensatory networks are introduced on both sides to keep the resonance frequency constant (Fig. 3).

Figure 3: Working project module.

Modes of Charging

Mode 1 is the cheapest and simplest method for charging at home, but it is also the slowest. Electric cars (EVs) include a built-in battery charger as well as connections that allow owners to plug their vehicles into a garage outlet. EVs can be charged using a typical 230 V household socket, although the available current is only 16 A, according to mode 1. Although charging periods vary widely from vehicle to vehicle, a tiny electric car's battery typically needs 7 to 15 hours to recharge to its full capacity. The EV is connected to the outlet using conventional industrial plugs and sockets in This method of charging is not allowed in the USA since it

Mode 2: This charging method uses single-phase or three-phase 230 V or 440 V alternating current mains with a maximum current of 32 A. Similar to Mode 1, no special plugs or sockets are required to connect EVs to garage wall boxes or charging stations located in public spaces, such as a restaurant, mall, city park, or even a place of employment. The circuitry required to carry out safety functions, such as i) confirming that the vehicle is connected properly, ii) routinely checking the continuity of the protective earth conductor, iii) energizing the system, and iv) de-energizing the system, is present in charging boxes and/or charging stations as well as the on-board battery charger. In general, Mode 2 charging is referred to as "opportunity charging" because it is frequently used by vehicles [7].

Mode 2: charging takes around 3-5 hours for a full charge of a compact car.

Mode 3: It is generally powered by a three-phase 440 V alternating current and employs specialized plugs and sockets to supply up to 63 kW to the onboard battery charger. In addition to Mode 2's security features, charging stations and onboard battery chargers adhere to the proper protocols for coordinating their operations. Because of the higher available power, a mode 3 charge can fully charge a small vehicle in under an hour. Electric buses, for example, require mode 3 charging, which is available in public and commercial areas, airports, and transportation hubs.

Mode 4: The charging station's rectifier converts alternating current mains power into direct current voltage. The EV receives up to 400 A from a special connector on an off-board battery charger. The Japanese standard "CHAdeMO" is the most extensively used mode 4 charging option. With a power output of up to 50 kW, it can charge a tiny car in under 30 minutes.

WPTS Technology for Electric Vehicle

In 1996 and 1997, General Motors released two EVs, the EV1 and the Chevrolet S-10 EV, which employed the Magne Charger, also known as J1773, which utilized the principle of inductive power transfer. In place of a plug, a "pad" carrying the primary coil is put into the EV's slot. The secondary coil is housed in the slot, and when combined with the pad, a Transformer for wireless power transfer (WPT) is produced. These pads did not perform as well as they could have. However, these pads required manual insertion into the EV, making them equally as problematic as conventional plugs.

However, with growing interest in e-mobility, there is a lot of research interest in making EVs a good option for future transportation.

WPT charging technology can positively affect people's attitudes towards EVs. It is expensive, has a limited driving range, and has a lengthy charging process. However, with the development of WPT technology for charge replenishment, an EV scan has become a desired option. WPT charging provides the advantage of being able to automate, simplify, and secure the charging process for users. The broad adoption of WPT charging infrastructure may also help reduce the size of the battery pack, increasing the efficiency of EVs. Traditional inductive chargers are incapable of handling all of this, necessitating WPT charging via vast air gaps and minimal human touch. GM, Qualcomm Halo, Delphi, and others are among the largest manufacturers [8].

SIMULATION

Dependence on renewable energy sources would be the key response to the crisis. As a result, this project includes a solar-powered grid-connected system with electric auto rickshaw charging capacity. To discuss the influence of solar energy, the study used correct result analysis.

Simulation of Solar System

A 6 kW solar system is selected for the performance investigation of a Solar Powered Grid Connected Charging Station. The infrastructure will be put in place at Chinnakada, Kollam, Kerala, India to make it easier for electric auto rickshaw drivers to charge their vehicles. Solar panels are used to offset the energy given to the load from the grid. The 6kW system was chosen based on the load profile of the available electric vehicles in the area. To meet future demand, the system's capacity can be increased. A simplified schematic diagram represents it (Fig. 4).

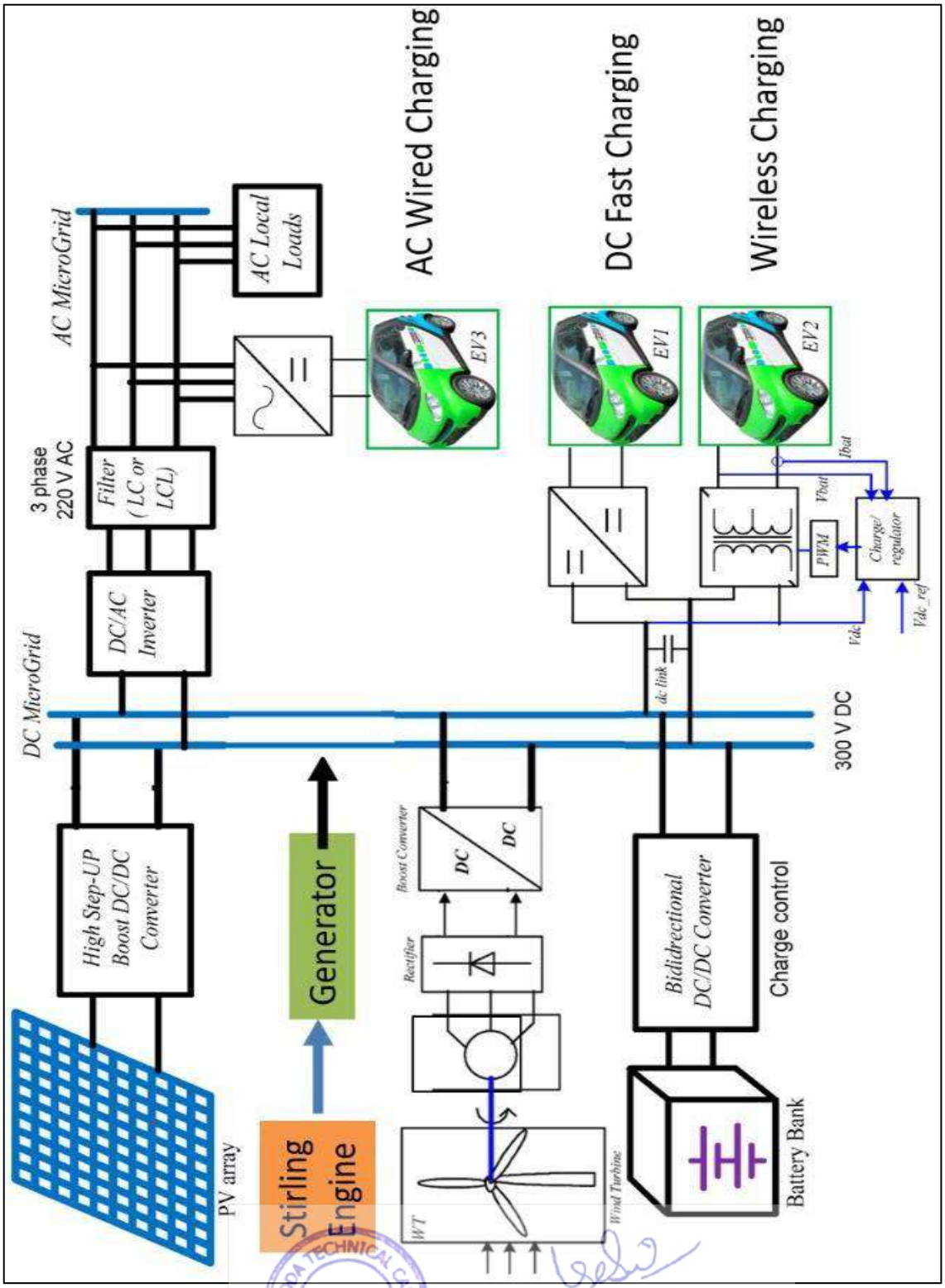

Figure 4: *Block diagram of inputs and outputs of charging.*

A PV array can produce the necessary demand. The system is connected to the grid, so any additional power requirements are satisfied by utilising the grid's supply of electricity. Three subsystems comprise the overall schema.

RESULT AND DISCUSSION

Inverter output signal while the battery is being charged and discharged then Charging and draining at the battery end A 440V AC supply is used as the device's input, and a smoothing capacitor linked in parallel with the circuit allows for variable output DC voltage [9, 10].

Observation

By raising the capacitor value, the output voltage is smoothed. The (Is) n = n=1, 3, 5 (4IO/n) cos(n/2) sin(nt-n/2) supply the output waveform's output voltage and current harmonics. As a result, decreases the output harmonics and becomes inversely proportional to the capacitor value.

However, there are two important factors to take into account while selecting a smoothing capacitor. The operating voltage must be larger than the no-load output value to function the rectifier, and the capacitance value, which determines how much ripple, is superimposed on top of the DC voltage. When the capacitance is too low, as with a 1 microfarad capacitor, it has no impact on the waveform of the output. The output voltage will be almost as smooth as pure DC if the smoothing capacitor is large enough (parallel capacitors can be utilized) and the load current is not too high.

Inverter Output for Charging

The output voltage, output current, and source current of the inverter are determined using Simulink while maintaining the value of the LC elements. The result therefore acquired is filtered with an LC filter to get rid of the harmonics. Under standard testing conditions, the specifications are - Hz or fifty hertz.

The output voltage and current frequency can be changed by altering the capacitor and inductor values. We can see from the Simulink findings above that the output voltage frequency rises while the output current falls under the influence of harmonics controlled by the LC filter. It is known that the increase in the capacitor value decreases the output waveform harmonics and hence the LC filter alongside the inverted produces a close-by ideal AC input for the charging station hub. The SCR firing angle (α) is changed to meet desired output load requirements for EV charging (Fig. 5, 6).

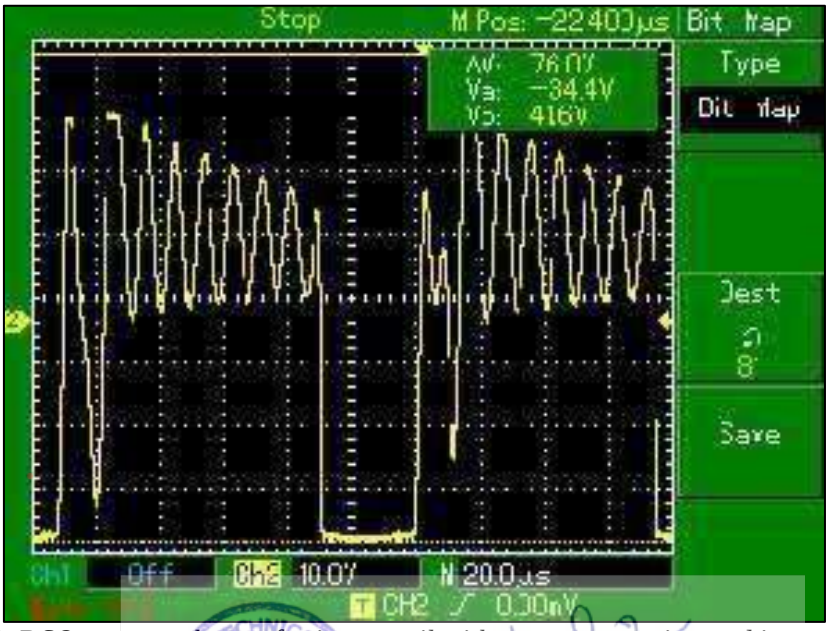

Figure 5: *DSO output voltage of primary coil without compensation and its parameters.*

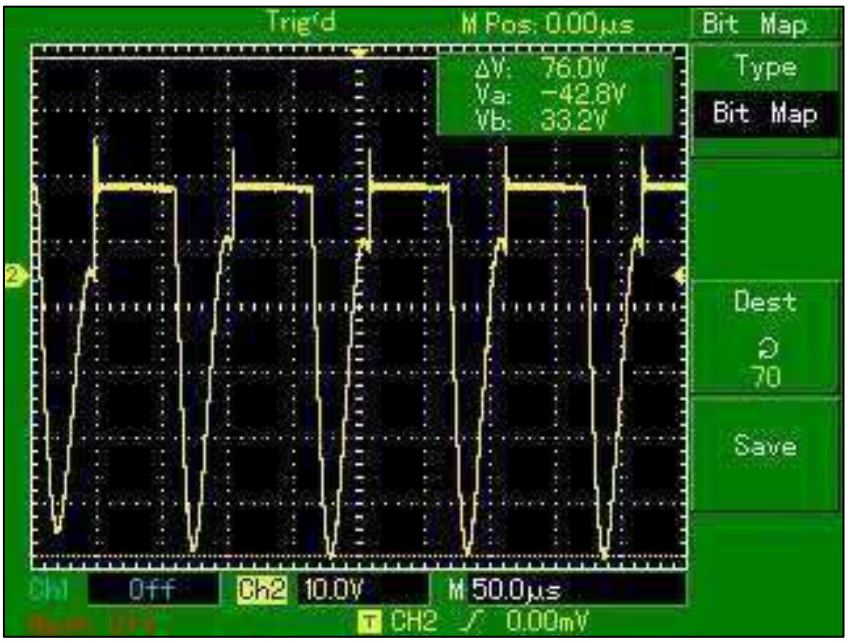

Figure 6: *DSO output voltage of secondary coil voltage without compensation and its parameters.*

Final Output

There are three kinds of standard EV charging stations. AC is used for level 1 and level 2 charging while DC is used for level 3 fast charging, which is primarily used in European nations (Table 1).

Table 1: *Charging station ratings.*

Parameters	Level 1	Level 2	Fast Charging
Voltage	120 V	220-240 V	200-450 V
Maximum Current	16 A	80 A	200 A
Current Type	AC	AC	DC
Power	1.4 KW	7.2 KW	50 KW
Maximum Output	1.9 KW	19 KW	150 KW
Charging Time	12 Hours	3 Hours	20 Minutes

CONCLUSION

Currently, the necessity to lessen emissions while conserving energy is the main factor driving domestic and international adoption of electric vehicles. Using renewable energy sources can save energy while reducing emissions. The development of renewable energy can considerably increase energy conservation.

- Solar-wind hybrid power plants, when combined with electric vehicles, will help to cut energy consumption and greenhouse gas emissions in the transportation sector.
- Fuel-based transportation has significantly increased pollution problems. To give a more beneficial option, the ICPT principle is employed to address the significant drawbacks associated with charging electric vehicles in the current situation. Thus, this project offers a template that may be applied to wireless charging at various parking lots.

Voltage changes as coil spacing changes. The model is scaled at a ratio of 100:1 with power levels up to 15 watts, and significant power is transmitted up to 35mm utilizing PP topology, which will increase efficiency when scaled in real-time.

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UTILIZATION OF M25 GRADE CONCRETE BY PARTIAL REPLACEMENT OF CUPOLA SLAG FOR COARSE AGGREGATE

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ABSTRACT

An abstract is a summary of entire paper should be written in Times new roman with font size- 10. The abstract The Indian steel industry has been growing rapidly over past few years, driven by increased demand from various sectors including construction, infrastructure and railways cupola slag is by product of steel industry and it is generally disposed into the landfill without treating properly. In this experimental study we are partially replacing the coarse aggregates by using cupola slag in (0%, 5%, 10%, 15%, 20%, 25%, and 30%) with water cement ratio of 0.45 in M25 grade concert. We tested compressive strength after 7 & 28 days of curing. It is observed that maximum compressive strength attain was 40.22 N/MM^2 at 25% for 28 days of curing at the same time it is observed that the compressive strength reduced up to 26.07 N/MM^2 at 30%. Also we observed that the minimum compressive strength attain was 25.70N/MM^2 at 5% .overall, the study concludes that the use of cupola slag as a partial replacement of natural coarse aggregates in m25 grade concrete can be a viable option provided that the replacement levels are kept below 30% and the mix design parameters are carefully optimised to maintain the desired strength and durability properties of the concrete.

Keywords: Concrete, Cupola Slag, Compressive Strength, Split Tensile Strength, M25 Grade Concrete.

I. INTRODUCTION

The construction industry in India has undergone significant growth over past few decades. The sector has been major contributor to the country's economic development and infrastructure development. In recent year the industry has witnessed significant modernization and technology upgrades, using conventional materials for the development of concrete to provide better strength durability and cost effectiveness is one of the upgrade construction industry is adopting now days.

[1]For the development of various infrastructure the main constituent is concrete with a typical density of 2400kg/M^3. This means for the given volume concrete will weigh more than any other construction material concrete is a composite material that contains of cement, water, and aggregates in the unit volume of concrete aggregates hold up to 50-60% of volume. The main source of aggregates in construction industry is from mining the mountains with the help of stone crusher.

[2]Which can have significant envormental impact, both in terms of immediate effects as well as the long term effect on the ecosystem. Extraction of aggregates from mountains can cause soil erosion, deforestation & habitat destruction which can harm the biodiversity of the area.

[3]The construction industry in India is expected to grow at a compound annual growth rate (CAGR) of 15.7% between years 2021-2026. As the construction industry grows the demand for the aggregates produced by the stone crusher industry is likely to increase as well. Using alternative source of aggregates, such as cupola slag can help reduce the dependence on natural source of aggregates and helps mitigation the envormental impact of the construction industry

[4]Cupola slag is a by- product of the steel industry which is generated during the melting of iron and steel in a cupola furnace. The chemical and physical properties of the slag can vary depending on the type of metal being melted, he temperature of the furnace. Also using cupola slag in concrete will help in reducing the self-weight of the structure as well as the overall cost of the structure.

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II. METHODOLOGY

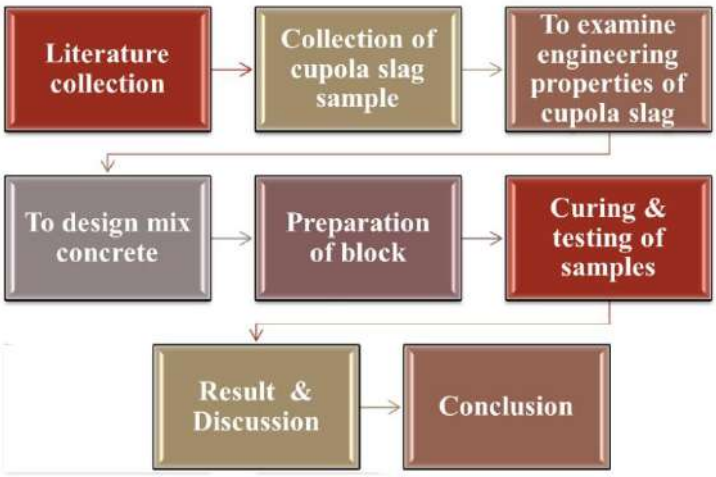

Figure 1: Methodology chart

III. OBJECTIVES

- To study engineering properties of cupola slag.
- To reduce waste generated from steel industries.
- To prepare concrete blocks by using slag as partial replacement for coarse aggregates.
- To compare with normal aggregat econcrete and cupola aggregate concrete.

IV. MODELING AND ANALYSIS

1 CEMENT – We used OPC cement of grade 53. The 53 Grade OPC has a higher strength concrete. As per BIS requirements the minimum 28 days compressive strength of 53 Grade OPC should not be less than 53Mpa.

2 FINE AGGREGATE – We used 4.75mm of fine aggregates in our design mix as shown in following image. Fine aggregates are an essential component of concrete because they help in filling the voids between the coarse aggregates, here are some reasons why we use fine aggregates in concrete.

3 COARSE AGGREGATE – We used 20mm sized coarse aggregate in our design mix the main functions of coarse aggregates in concrete are to provide strength and durability to the concrete. They help to distribute the loads evenly across the concrete structure and provide resistance good compressive strength to the concrete.

4 CUPOLA SLAG - Cupola slag is a material generated during the manufacturing process of steel, and it can used as partial replacement of the coarse aggregates, cement, as well as fine aggregates. In this experimental study we are replacing the coarse aggregates with cupola slag. We collected the sample of cupola slag from Jotirling founders, shiroli, Kolhapur. And graded them into the aggregates in size between 10mm – 20mm coarse aggregates.

Figure 2: Cupola Slag

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Cupola slag can possess physical properties similar to traditional coarse aggregates, such as suitable particles size distribution and sufficient strength while specific properties may vary depending on the characteristics of the slag obtained from the steel industry careful, grading & selection can ensure that cupola slag adequately perform as replacement, material.

V. RESULTS AND DISCUSSION

1. COMPRESSIVE STRENGTH :

The compressive test on both conventional concrete and cupola slag concrete is carried out in accordance with IS 516- 1999 standards. The test is conducted on concrete specimens of size 150mm x 150mm x 150mm. The specimen is placed at the centre of the compressive testing machine and the load is applied gradually till the specimen fails.

Figure 3: Compressive Strength testing machine.

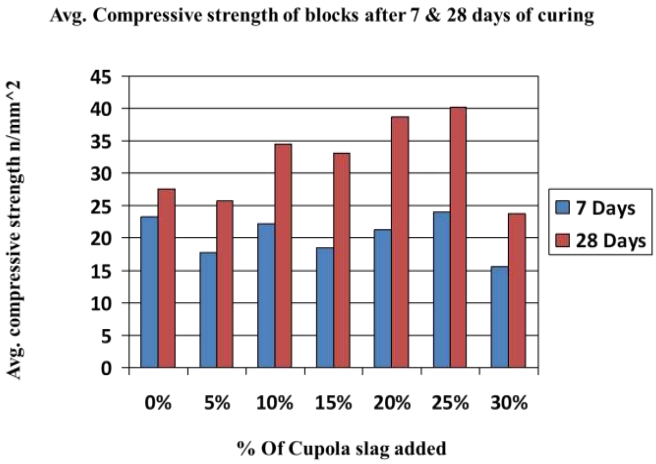

Figure 4 : Variation of compressive strength with variation % of cupola slag

Table 1: compressive strength of blocks after 7 & 28 days of curing

Sr.No.	% of Cupola	Avg. comp. strength 7 days curing N/MM²	Avg. comp. strength 28 days curing N/MM²
1	0%	23.33	31.7
2	5%	17.70	25.70
3	10%	22.15	34.5
4	15%	18.55	33.11
5	20%	21.33	38.14
6	25%	24.00	40.22
7	30%	15.6	26.07

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It can be observed from the results that the compressive strength of the concrete increased by use of cupola slag up to 25% as a partly replacement to coarse aggregate in all concrete mixes & it will be observed that decreases on further increase in cupola slag (30%) replacement in concrete samples. The above result are plotted graphically for curing period of 7 and 28 days.

2. SPLIT TENSILE STRENGTH

The test is conducted on concrete specimens of size 150 x 300mm.The cylindrical specimen is placed at the centre of the testing machine and the load is applied gradually till the specimen fails.

Figure 5: Casting of cylinders for spilt tensile test

Figure 6: Split Tensile Strength

Table 2: Split tensile strength of blocks after 7 & 28 days of curing

Sr. No.	% of Cupola	Avg. comp. strength 7 days curing N/MM²	Avg. comp. strength 28 days curing N/MM²
1	0%	2.47	3.67
2	25%	2.75	3.97

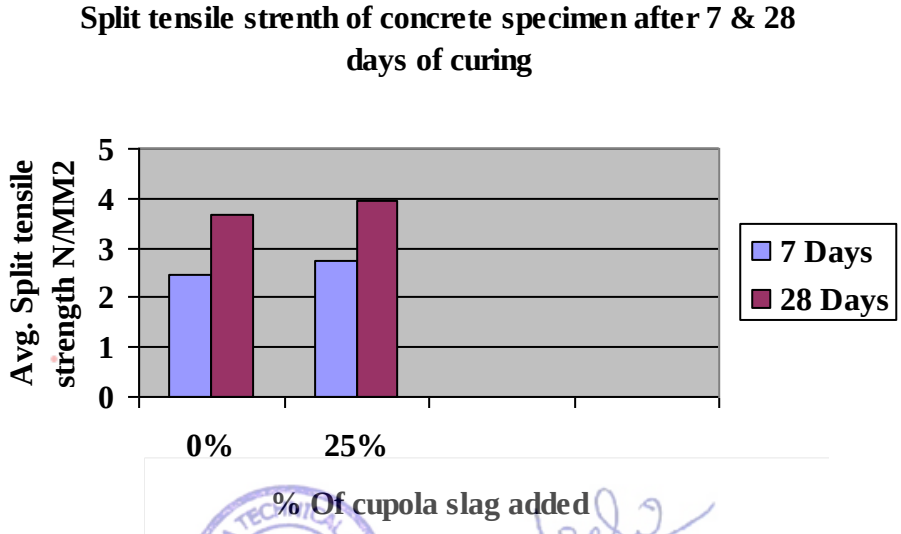

Figure 7: Variation of split tensile strength with variation of % cupola slag

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VI. CONCLUSION

Following are the conclusion of the experimental study:

- The maximum strength achieved by the blocks after 28 days of curing with 25% inclusion of cupola slag is 40.22 N/mm2.
- The utilization of cupola slag, characterized by its low specific gravity and light weight nature contribution to a reduction in the self-weight of the concrete member.
- Cupola slag is widely available at minimal or no cost rendering it an economically viable option for significant construction projects by substituting 25% of the coarse aggregates with cupola slag expenses can be minimised resulting in enhanced cost effectiveness.

VII. FUTURE SCOPE

- Use of cupola slag (25%) replacement percentage for enhancing mechanical properties.
- By optimizing cupola slag in the concrete as partial replacement it will help to reduce waste generated from steel industries.
- Long-term durability studies to assess the performance of cupola slag concrete over time.
- Implementation cupola slag aggregate in concrete is practically possible in real-world construction projects.
- Implementing cupola slag aggregate reduces global-warming and also gives strength.
- Economic feasibility analysis to evaluate the cost-effectiveness and viability of cupola slag concrete.

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PERFORMANCE EVALUATION OF SLUDGE BRICK WITH CONVENTIONAL BRICK

Mr. Sohel M. Shaikh[*1], Mr. Huzefa F. Tamboli[*2], Mr. Akshay U. Sawant[*3], Mr. Rohit S. Kamble[*4], Mr. Rohit S. More[*5], Mr. Saddam S. Kotwal[*6], Mr. P.G. Borate[*7]

[*1,2,3,4,5,6]Students, Department Of Civil Engineering, YSPM's Yashoda Technical Campus, Satara, India.

[*7]HOD, Department Of Civil Engineering, YSPM's Yashoda Technical Campus, Satara, India.

ABSTRACT

Waste is the product of the unwanted material that is from manufacturing Process from industry, from House, or other industries such as agricultural and chemical etc. Waste is harmfull for the environment. It produces many types of disease, infection and problem's to every living being so. The sludge disposal has major concern for human and animal and for every living being The sludge from the water treatment plant has problems of disposal.Therefore, these study is useful for stulge disposal in proper manner with ecofiendly use. So these study we utilize those studge waste (SW) produced from the water treatment plant as using as brick making material. For sludge brick the different proportion and material should use with sludge. Then tests is conducted like water absorption and compressive strength. As the conclustion the 20% sludge is usefull or acceptable to produce good quality brick from these the water treatment plant sludge is suitable ingredient or material for manufacturing of bricks with different proportion, material,mix and design.

Keywords: Sludge Waste, Sludge Brick, Compressive Strength, Water Treatment Plant Sludge, Proportionate Mix.

I. INTRODUCTION

Growth in industrialization and fast growing urbanization is causing major environmental problems. From these major concern is safe and sound disposal of solid sludge waste. There is a strong demand for reuse and effective disposal methods for sludge due to its increasing daily amount of generated sludge by the waste water treatment plants. Sanitary landfills are commonly used for disposal or sludge from sewage,rapid urbanization has made it difficult to find suitable landfill sites or land for disposal. Therefore, Sugar industries, paper pulp and Textile industry are three major agriculture-based industries in India which produce large quantity of solid,semi solid and liquid wastes after consuming greater amount of quality and fresh water. Textile mills are one of the oldest and big sectors in India. Every year textile exports generates large amount of revenues for Indian economy. The proposed method for the manufacture of energy efficient bricks using the sludge from textile industry, thus suggests a means for the waste disposal also. For the past thousands of years, water treatment plant sludge is almost similiar to bricks property. Its chemical composition is also same. Therefore these study shows that sludge is efficiency used as a replacement for brick clay. Throughout It is mainly focused on charactesristics present in brick and its influence on modyfing and thinking of the chemical -physical properties, throughout the concentrations, in water treatment plant sludge the following chemical presents such as Cu, Zn, Cr,Cd and Pb. The natural resources are used for manufacturing of sludge bricks, and as an alternative to conventional or cement bricks which helps in conservation of naturally resources and improves the environment.

1.1 Liquid sludge

The effluent coming from the industries are treated by flocculation process, during this treatment the sludge obtained is called as liquid sludge.

1.2 Semi solid sludge

This is the second form of sludge, obtained by dewatering of liquid sludge by passing through centrifuges at 1500 rpm, because of this high revolution the sludge gets dewatered and comes out as wet cake. This wet cake is called wet sludge.

1.3 Dry sludge

The sludge from the centrifuges is dried by spreading it over a large area, i.e., on the sludge drying beds in the presence of sunlight. Thus, wet sludge is converted into dry sludge in a period of 40 days. All the three forms of

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sludge which has been discussed above are being used as composite material and influence of each on compressive strength and other parameters have been discussed in subsequent articles.

II. OBJECTIVES

2.1 To check Partial replacement of soil by dry sludge as one of the ingredients in brick manufacturing process.

2.2 To reduce in cost of brick and reduction in impact on environment.

2.3 To find if the compressive strength of bricks made using sludge is compatible with conventional brick.

2.4 To examine the effect of dry sludge in brick properties.

III. METHODOLOGY

3.1 Sludge Collection: The collection of sludge can be done from water treatment plant so we got the liquid type sludge we take that sludge in bucket and then put it on the slab for drying of sludge. The waste sludge which is left over drying is allowed to dry for 10days to 14days.The drying process is completely natural.

3.2 Sludge Grinding: After the drying of sludge available water in sludge is evaporated in atmosphere by the sun heat so we get completely dry sludge but in dry sludge we get some lumps so we want to do sludge grinding for getting powdered sludge so we do sludge grinding process.

3.3 Analysis of properties of material used: Here we analyse the different properties of material we are using in mixing with sludge to get good strength and mixing.

3.4 Addition of Cement, Quarry Dust and Sludge in Various Proportion: After analysis we have to decide the proportion of different material used with sludge. First we decide the set1-20% sludge, 20%cement and Quarry dust-60%, Set2- 30%sludge,10%cement,60%Quarry dust, Set3-20%sludge,30%cement and 50%Quarry dust, Set4- 50%sludge,20%cement and 30%quarry dust. These 4 sets for adding proportion we decide.

3.5 Moulding of bricks: In the moulding of brick the required size and shape is given to the prepared brick. There are two types of moulding by hand or by machines.The specimens used for the test includes cubes of (23x10x7)cm for compressive test,The test is conducted for 7 and 14 days.

3.6 Water Curing: After moulding of bricks the brick curing can be done for 7 to 10 days for proper strength and bonding the water curing can be done.

3.7 Air Drying: This process includes the removing of moisture from the surface and coating by using air.It prevents rusting and corrosion which may be caused by redundant moisture.

3.8 Testing: Following First testing done on cement that is fineness test, standard consistency test, The setting time test, specific gravity test is done for cement. Foe quarry dust specific gravity and sieve analysis test carried out and for sludge specific gravity and fineness test. For Sludge brick water absorption and compressive strength should be done.

3.9 Comparing Results: After the testing of brick obtained result should be compared.

IV. TESTING AND EVALUATION

4.1 Testing of Cement

4.1.1 Fineness Test:

The fineness of cement is calculated by passing the cement through 90mm sieve. The dry shrinkage cracks will easily form when the fineness increases.

Fineness of cement = (W1- W2) /W1 X 100

= 6 %

4.1.2 Standard Consistency Test

Co Standard consistency test is the amount of water required to prepare the plastic mix. It is used to find quantity of water for mixing with cement. The consistency of this cement is 33%

Sr. No.	% of water added to cement	Quantity of water added	Initial reading	Final reading
1	25	100	50	40
2	26	4	50	39
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4	28	4	50	36
5	29	4	50	29
6	30	4	50	20
7	31	4	50	16
8	32	4	50	10
9	33	4	50	6

4.1.3 The Setting Time Test

- Initial setting test
- Final setting test

4.1.4 Specific Gravity Test

With the apparatus density bottle and weighing balance, the following procedure was carried out to determine the specific gravity of cement. 100gm of cement is weighted 990 ml of kerosene is filled in a specific gravity bottle. sample of cement is placed on specific gravity bottle, till the level of kerosene reaches 100ml mark the quality of cement placed in the bottle is calculated. Then the specific gravity of cement is found out by using he following reaction specific gravity of cement =weight of cement (volume of 10ml by weight of kerosene of equal volume of cement.

Specific gravity of cement = 3.19

4.2 Testing of Sludge

4.2.1 Specific Gravity Test

The same procedure was followed to determine the specific gravity of sludge. Instead of cement we used sludge in that procedure.

Specific gravity of sludge = W5X (W3- W1)/ (W5+W3- W4) X (W2- W1)

by calculating using the formula we got specific gravity of sludge as 2.90

4.2.2 Fineness Test

The fineness test was done for the dry sample of sludge. 100gms of sludge was sieved horizontally for about 10 to 15 min and then residues in each sieve was weighed. From that we came to get the fineness value as 7%, which is higher than cement.

4.3 Testing of Quarry Dust

4.3.1 Specific Gravity Test

The specific gravity of quarry dust that we had got selected gives a value of 2.612. This value is slightly coincides with the specific gravity value of sand so that instead of sand we have used quarry dust.

4.3.2 Sieve analysis

The fineness value of quarry dust is calculated by sieve analysis process with the use of graph we have got the uniformity coefficient value as 2 and coefficient of curvature as 1.125

V. RESULT AND DISCUSSION

The results obtained are as discussed below

5.1 Compressive Strength Test

SET-1 Load Calculation's For SET 1 for 7day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2100	110	230x100mm^2	4.78N/mm^2

Load Calculation's For SET 1 for 14 day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2200	130	230x100mm^2	5.6N/mm^2

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SET-2 Load Calculation's For SET 2 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1570	65	230x100mm^2	2.82N/mm^2

Load Calculation's For SET 2 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1678	70	230x100mm^2	3.04N/mm^2

SET-3 Load Calculation's For SET 3 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1840	70	230x100mm^2	3.04N/mm^2

Load Calculation's For SET 3 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1978	75	230x100mm^2	3.26N/mm^2

SET- 4 Load Calculation's For SET 4 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1670	50	230x100mm^2	2.17N/mm^2

Load Calculation's For SET 4 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1780	55	230x100mm^2	2.39N/mm^2

The Following Compressive Strength Test determine by as per IS-3495(part1)1992

5.2 Water Absorption Test

Dry weight of sludge brick = 2.5 Kg
Wet weight of sludge brick = 2.92 Kg
Water absorption = (2.92 – 2.5/ 2.5) X 100 = 16.8 %

VI. CONCLUSION

The conclusion is based on different sets of proportion, material and experimental sets are used and its tests is as follows, The water treatment plant sludge (WTP) is a best replacement in conventional brick or cement brick with sludge waste with high chrome content.
The researched brick type will be a big competitor to the cement brick and clay brick type in the market.
The maximum value of compressive strength was obtained in the 20% of sludge replacement in bricks.
Set 1 (60% quarry dust, 20% cement and 20% sludge) is best suitable for the structural applications.
Set 2 (60% quarry dust, 10% cement and 30% sludge) is best suitable for non-structural applications.
Set 3 (50% quarry dust, 20% cement and 30% sludge) it is also suitable for non-structural applications.
Set 4 (20% quarry dust, 30% cement and 50% sludge) will not be suitable for both structural and non-structural applications.

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Specific gravity of cement = 3.19

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Load Calculation's For SET 1 for 14 day's

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Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
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Load Calculation's For SET 2 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1678	70	230x100mm^2	3.04N/mm^2

SET-3 Load Calculation's For SET 3 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1840	70	230x100mm^2	3.04N/mm^2

Load Calculation's For SET 3 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1978	75	230x100mm^2	3.26N/mm^2

SET- 4 Load Calculation's For SET 4 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1670	50	230x100mm^2	2.17N/mm^2

Load Calculation's For SET 4 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1780	55	230x100mm^2	2.39N/mm^2

The Following Compressive Strength Test determine by as per IS-3495(part1)1992

5.2 Water Absorption Test

Dry weight of sludge brick = 2.5 Kg
Wet weight of sludge brick = 2.92 Kg
Water absorption = (2.92 – 2.5/ 2.5) X 100 = 16.8 %

VI. CONCLUSION

The conclusion is based on different sets of proportion, material and experimental sets are used and its tests is as follows, The water treatment plant sludge (WTP) is a best replacement in conventional brick or cement brick with sludge waste with high chrome content.
The researched brick type will be a big competitor to the cement brick and clay brick type in the market.
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Set 2 (60% quarry dust, 10% cement and 30% sludge) is best suitable for non-structural applications.
Set 3 (50% quarry dust, 20% cement and 30% sludge) it is also suitable for non-structural applications.
Set 4 (20% quarry dust, 30% cement and 50% sludge) will not be suitable for both structural and non-structural applications.

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Analysis of G+4 building structure for Seismic Retrofitting using Cross Bracing

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Abstract
As the earthquakes are an inconsistent phenomenon they may or mostly may not occur in entire lifespan of building, Designing abuilding structure to sustain during an earthquake makes it very uneconomical, Hence a Structural model is going to be used for comparison in between building structure models with seismic retrofitting techniques such as Steel Bracings. In this paper a G+4 building structural model is analysed in zone III and zone IV by using Arduino Earthquake Detector Alarm with Seismic Graph using Accelerometer. Various characters like consistency, lateral displacement and storey drift will be studied. The main aim of this paper is to compare the differences in Structural model without Steel Bracing and Structural model with Steel Bracings with help of different levels applied to the model with increase in force applied from zone III to zone IV.
Keywords: *Structural Model, Arduino Earthquake Detector Alarm, Accelerometer, Seismic Graph.*

Date of Submission: 02-07-2022 Date of acceptance: 14-07-2022

I. INTRODUCTION

In the past thirty years, moderate to severe earthquakes occurs around the world every year. Such events lead to damage to the concrete structures as well as failures. Thus, the aim is to Focus on a few specific procedures which may improve the practice for the evaluation of seismic vulnerability of existing reinforced concrete buildings of more importance and for their seismic retrofitting by means of various innovative techniques such as base isolation and mass reduction. So Seismic Retrofitting is a collection of mitigation technique for Earthquake engineering. It is of utmost importance for historic monuments, areas prone to severe earthquakes and tall or expensive structures. The existing building stock poses a much more serious and complex seismic safety problem when compared to safe earthquake design of new construction. The vast majority of structures located in seismic areas exhibit deficiencies in their resistance to earthquake loads due to a number of reasons, highlighted below.

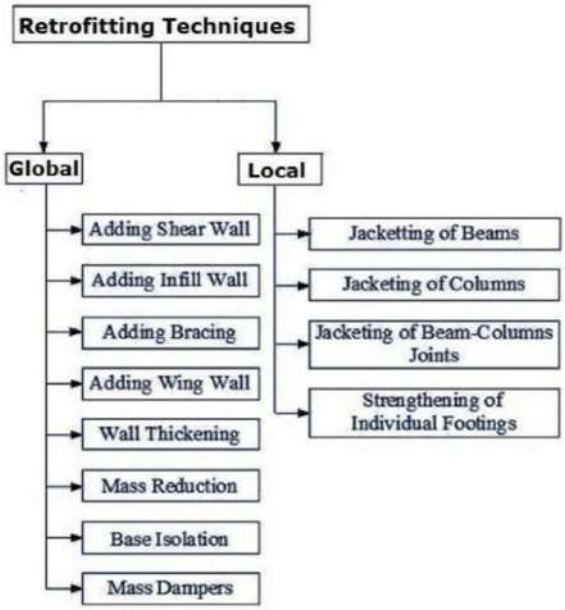
Older construction, designed according to earlier codes, may not comply with current seismic regulations since focus used to be primarily on warranting sufficient capacity for gravity loads alone. Moreover, the past thirty years have witnessed such a significant increase of knowledge in the field of earthquake engineering that even relatively modem structures may no longer meet the prerequisites of constantly-developing regulations. As a result, several shortcomings can be found in existing buildings such as irregular structural configuration, inappropriate member detailing for ductility and insufficient lateral stiffness, amongst others.All the above considered, it seems clear that repair and strengthening of both old structures designed according to outdated codes and new but defective earthquake-resistant construction, is urgently needed. This requirement also arises where existing structures must comply with more recent code stipulations, or when these structures are to be reassessed for higher loads.

1.1 Seismology

Seismology is the scientific study of earthquakes and the propagation of elastic waves through the Earth or through other planet-like bodies. The field also includes studies of earthquake environmental effects such as tsunamis as well as diverse seismic sources such as volcanic, tectonic, glacial, fluvial, oceanic, atmospheric, and artificial processes such as explosions. A related field that uses geology to infer information regarding past earthquakes is paleo-seismology. A recording of Earth motion as a function of time is called a seismogram. A seismologist is a scientist who does research in seismology.

National Centre for Seismology (NCS) is the nodal agency of the Government of India for monitoring earthquake activity in the country. NCS maintains the National Seismological Network of more than 150 stations each having state of art equipment and spreading all across the country NCS monitors earthquake activity all across the country through its 24x7 around-the-clock monitoring centre. NCS also monitors earthquake swarm and aftershock by deploying a temporary observatory close to the affected region

1.2 Classification



Above mentioned are various types of Seismic Retrofitting Techniques used both locally and on Global level. Seismic Retrofitting Techniques are required for concrete constructions which are vulnerable to damage and failures by seismic forces. In the past thirty years, moderate to severe earthquakes occurs around the world every year.Thus the aim is to Focus on a few specific procedures which may improve the practice for the evaluation of seismic vulnerability of existing reinforced concrete buildings of more importance and for their seismic retrofitting by means of various innovative techniques such as base isolation and mass reduction. It is of utmost importance for historic monuments, areas prone to severe earthquakes and tall or expensive structures. In this project we will be dealing with the most commonly used Seismic Retrofitting technique known as Cross Bracing.

1.3 Cross Bracing

Bracing is a very effective global upgrading strategy to enhance the global stiffness and strength of steel and composite frames (Fig 02). It can increase the energy absorption of structures and/or decrease the demand imposed by earthquake loads. Structures with augmented energy dissipation may safely resist forces and deformations caused by strong ground motions. Generally, global modifications to the structural system are conceived such that the design demands, often denoted by target displacement, on the existing structural and non-structural components, are less than their capacities (Fig 02). Lower demands may reduce the risk of brittle failures in the structure and/or avoid the interruption of its functionality. The attainment of global structural ductility is achieved within the design capacity by forcing inelasticity to occur within dissipative zones and ensuring that all other members and connections behave linearly.

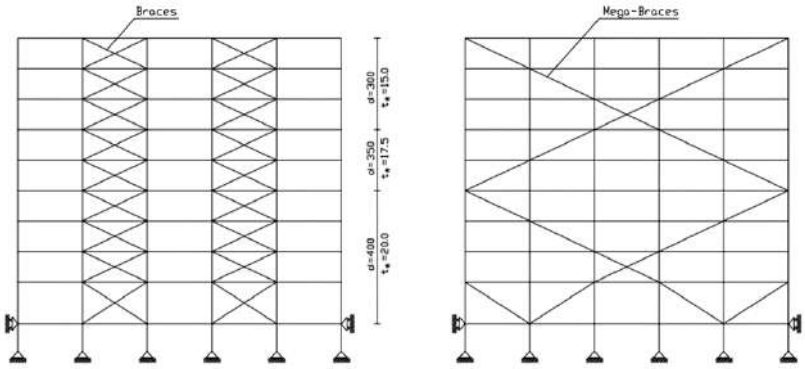


Figure 1:Layout of braced frames: concentrically- (left) and mega-braced (right) frames.

There are different types of Cross Bracing Techniques which are as follows:

1) Concentric based frames (CBFs)
2) Eccentric based frames (EBFs)
3) The novel knee-base frames (KBFs)

Concentric based frames are further classified in following types:

(i) V – Type Cross Bracing
(ii) X – Type Cross Bracing
(iii) K – Type Cross Bracing
(iv) Opposite V – Type Bracing
(v) Diagonal Bracing
(vi) 2 Storey X – Bracing.

In this project we will be dealing with V – Type and X – Type Cross Bracing.

1.4 Seismic Zones

Different Seismic Zones in India

(i) Seismic Zone II: Zone II is classified as the low-damage risk zone.
(ii) Seismic Zone III: Seismic Zone 3/III is classified as the moderate-damage risk zone.
(iii) Seismic Zone IV: Zone IV is considered the high-damage risk zone.
(iv) Seismic Zone V: Zone V has the highest risk of damaging earthquakes.

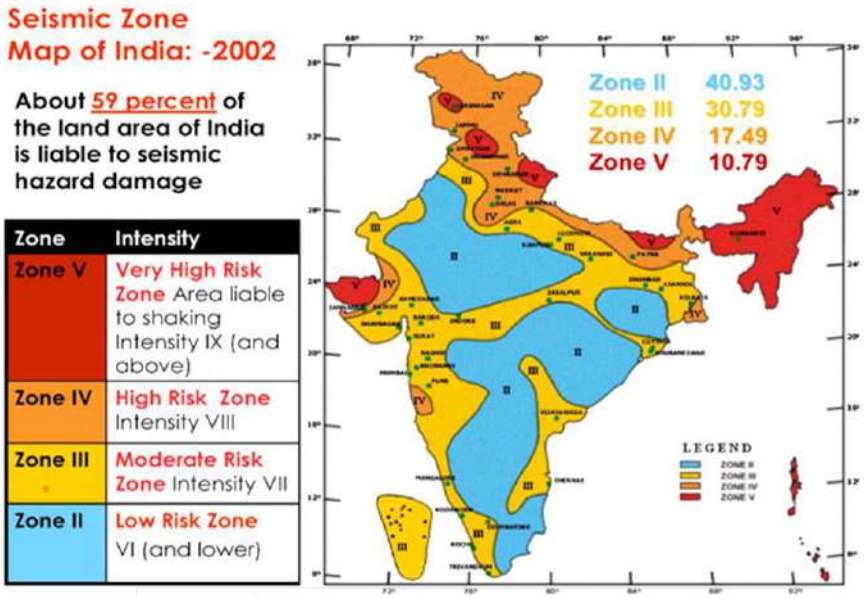


Figure 2: Seismic Zone Map of India

According to the seismic zoning map of the country, India is divided into four seismic zones. Also known as earthquake zones, these seismic zones are formed on the basis of scientific inputs related to the following:

(i) The Seismicity or the Frequency of Earthquakes in a Region
(ii) Earthquakes That Have Hit the Country in the Past The four zones of earthquake in India, as discussed below:
(iii) Seismic Zone II: Zone II is classified as the low-damage risk zone. This is the least seismically active zone, meaning the areas that fall under these zones in India have a low chance of having an earthquake. Zone II covers earthquake-prone areas, which are 41% of India. Here, the Indian Standard (IS) Code allots a zone factor of 0.10.
(iv) Seismic Zone III: Seismic Zone 3/III is classified as the moderate-damage risk zone. Here, the IS Code allots 0.16 to this zone. Zone III, or moderate earthquake zone, covers 30% of India.
(v) Seismic Zone IV: Zone IV is considered the high-damage risk zone. The IS Code allots 0.24 to this zone. Moreover, 18% of the total area of the country belongs to Zone IV.
(vi) Seismic Zone V: Zone V has the highest risk of damaging earthquakes. The IS Code has assigned a factor of 0.36 for this very high-risk damage zone. Around 11% of India falls under Zone V.
Note: There are no cities in India which fall under Seismic Zone I

The above-mentioned list of earthquake zones in India gives a comprehensive knowledge of the different zones and total areas they cover. Let us now take a look at the top 10 cities prone to an earthquake.

Table 1: Magnitudes & Intensity of Earthquake Globally

Magnitude	Description	Intensity (Mercalli)	Average Frequency of Occurrence Globally
1.0 – 1.9	Micro	I	Several Million Per year
2.0 – 2.9	Minor	I to II	Over one million per year
3.0 – 3.9		III to IV	Over 1,00,000 per year
4.0 – 4.9	Light	IV to VI	10,000 to 15,000 per year
5.0 – 5.9	Moderate	VI to VII	1,000 to 1,500 per year
6.0 – 6.9	Strong	VIII to X	100 to 150 per year
7.0 – 7.9	Major	X or greater	10 to 20 per year
8.0 – 8.9	Great	X or greater	One per year
9.0 & greater		X or greater	One per 10 to 50 years

1.5 Satara District Seismic Zones

(i) As Satara District comes under earthquake prone areas it mainly gets divided into two different zones.
(ii) It gets divided into Zone III and Zone IV respectively.
(iii) As Zone III is also called as Very strong intensity zone its intensity on MMI scale is around "VII".
(iv) As Zone IV is called as Severe Intensity zone its intensity on the MMI scale is around "VIII".
(v) Hence, as for Satara District region the typical MMI (Modified Mercalli Intensity) is around VII & VIII.
(vi) Which then comes under the Magnitude of Earthquake as in 6.0 to 7.0.

Table 2: Magnitude vs. MMI Scale

Magnitude	Typical MMI
1.0 – 2.9	I
3.0 – 3.9	II – III
4.0 – 4.9	IV – V
5.0 – 5.9	VI – VII
6.0 – 6.9	VII – IX
7.0 & higher	VIII or higher

III. OBJECTIVES

2.1 To Develop G+4 Building structure model with Cross Bracing.
2.2 To apply different levels of magnitudes to all models.
2.3 To compare between Building Structure models of both buildings with Cross Bracing and without Cross Bracing.
2.4 To Obtain result and conclusion based on testing, that demonstrates the validity of the designed technique.

IV. METHODOLOGY

As the earthquakes are an inconsistent phenomenon they may or mostly may not occur in entire lifespan of building, Designing a building structure to sustain during an earthquake makes it very uneconomical, Hence a Structural model is going to be used for comparison in between building structure models with seismic retrofitting techniques such as Steel Bracings. In this paper a G+4 building structural model is analysed in zone III and zone IV by using Arduino Earthquake Detector Alarm with Seismic Graph using Accelerometer. Various characters like consistency, lateral displacement and storey drift will be studied. The main aim of this paper is to compare the differences in Structural model without Steel Bracing and Structural model with Steel Bracings with help of different levels applied to the model with increase in force applied from zone III to zone IV.

Bracing play important role in keeping structure stable. Earthquake produces inertial forces in structure. These inertial forces act in the form of base shear on structure. Base shear is distributed to different floor along the height of the building. This force produces later displaces in structure. For high rise building, lateral displacements are common due towing loading. But if the earthquake is of high intensity, it can be disastrous. Bracings play important role in distributing this force in columns and beams. In this project we have analysed unbraced structure with structures having different bracings.

X-bracing system has shown good results when it comes to reducing lateral displacements. Base shear values are same in both directions. Since number of bracings along X-directions were more, bracings shown good performance in lateral displacements along X-axes. Diagonal bracing shows overall good performance considering maximum bending moment. V-bracing has shown good performance considering Maximum support Reactions. Weight of the structure remains almost same. Not more than 2 percent change in weights of structure. Since base shear is dependent on weight, base shear also remain similar.

V. TESTING AND CONVERGENCE

1. As for Amplitudes regarding in the application in Project Model.
2. There will be levels used for change in amplitude of earthquake.
3. i. 1 Hz to 20 Hz in 30 seconds amplitude 0.50 as for the Shake in Platform Level 1, we will be using Total Shake.
   ii. Level 2 - 0.75 amplitude change in every 30 seconds from 20 Hz to 40 Hz.
   iii. Level 3 – 1.00 amplitude change in every 30 sec.
4. We will also be adding pulse motions for sudden jerks in the structural model.
5. Then we will be increasing the amplitudes on the shake table till the building with no bracing collapse.
6. After the collapse of the building with no bracing, we will be taking results from the other two building structures.
7. We will take different results for both building structure models with X-Type and V-Type bracing to check its durability with increase in the amplitudes simultaneously.
8. As the project model is based on the Satara district seismic zone, the earthquake zones will be Zone III and Zone IV.
9. Hence, we will need to take results up to the magnitude of amplitude level 7.5 to 8.0 as per the severe intensity zone in the MMI scale.

VI. CONCLUSION

Lateral forces are distributed to beams and columns by bracings. In this project a comparative analysis of unbraced structure with structures having different bracings. With parameters such as Bending Moments, Lateral displacements, support reactions. X-bracing system has shown good results when it comes to reducing lateral displacements.
On the basis of the present study, following conclusions are made:

- As per displacement criteria, bracings are good to reduce the displacement and in case of X and V-bracing, the displacement is higher than without bracing because of irregularity in shape of the structure.
- The reactions and weight of the structure are more in different types of bracing structures when compared to un braced structure with same configuration of the structure.
- It is also seen that as there are different bracing systems employed the displacement and storey drifts, may increase or decrease for the braced buildings with the same configurations.

- The braced buildings of the storey drift either increases or decreases, as compared to un braced building with the same configuration for the different bracing system.

VII. FUTURE SCOPE

This project primarily focused on concentric bracings. There are so many different types of concentric bracings. In this project only four of them are utilized. There are various types of eccentric bracings too. Eccentric bracings can useful when lateral loads are of know directions. In future works this analysis can be utilized as a source of data for further analysis. There could be multiple arrangements. Here we have only focused on only one type of arrangements. This work can be further carried out with different arrangements. Bracing types can be compared by using many more parameter

This project can also be tested for dynamic loading, wind loads. Work is done on static coefficient method. It can be redone using Response spectra method, Time history analysis. This is a symmetrical structure. Further projects can be done on irregular structures. Irregularity can induce unexpected forces in structure.

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Comparative Study of Behavior of Framed Structure Under Seismic Zone III & IV Using STAAD Pro

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Abstract
Designing a structure to sustain during an earthquake makes it very uneconomical, as the earthquakes may or mostly may not occur in entire lifespan of building since it is inconsistent phenomena. In this paper a G+4 RCC building is designed in zone III and zone IV by using STAAD Pro software. Various characters like lateral displacement and storey drift will be studied. The main aim of this paper is to think on variations in RCC members, most extreme shear power, greatest redirection all these factors shows increase from zone III to zone IV.

Keywords: *Seismic zones, STAAD PRO, Lateral Displacement, .*

Date of Submission: 05-06-2022 Date of acceptance: 20-06-2022

I. INTRODUCTION

Designing structure with the help of STAAD Pro V8i which is referenced to IS 1893(PART 1): 2002 "Criteria for Earthquake Resistant Design of Structure" ensures that building has minimum strength to withstand minor earthquake occurring frequently and resisting moderate earthquakes without significant structural damage. This document is presented to improve the productivity of sustained earthquake mitigation strategies and the capacity to secure structures, frameworks, to Investigate a multiplex RCC operating for open shaking strength to think about the effects of different seismic zones, Knowing the relationship between different procedures for seismic inspection and their seismic response, gain useful learning in basic inspection, seismic assessment, drafting and identification of auxiliary parts using earthquake resistant design norms. We are also configuring the G+4 custom build, it means that if the zone changes from zone III to zone IV, the structure planned by us at that point will be fixed. Also, by calculating this we will perceive the amount spent putting together such a structure.

Seismic tremor shaking is irregular and varies with time. Be that as it may, most plan codes speak of inertia forces caused by jolting as the net effect of arbitrary jolts, such as static parallel power proportional to the structure. This strength is called the seismic design base shear VB and remains the base quantity associated with the strength-based earthquake resistant structure of structures. This strength is based on the seismic hazard in the area of the structure spoken by the seismic zone factor z. The codes reflect this by presenting a flexibility factor sa/g. This way of thinking is presented with the help of the response reduction factor r, which is larger for flexible structures and smaller for weak structures. Therefore, the seismic shake claim plan is evaluated solely on the basis of probabilistic ideas and the earthquake effects plan is called a seismic shake safe structure against reasonable estimate of interest. The design base shear VB was taken according to the Indian seismic code is 1893(part 1)-2007.

1.1 Basic Design Codes

Design should be carried so as to confirm to to the following:
1. IS 456: 2000- Plain and reinforced concrete- code of practice (fourth revision)
2. National Building Code 2005
3. Loading Standards IS 875 (Part 1-5): 1987- code of practice for design loads (other than earthquake) for buildings and structures (second revision)
Part 1: Dead Loads
Part 2: Live Loads
Part 3: Wind Loads
Part 4: Snow Loads

Part 5: Special Loads and load combinations

1.2 Design Handbooks

SP 16: 1980- Design Aids (For RCC) to IS 456: 1978
SP 24: 1983- Explanatory handbook on IS 456:1978
SP 34: 1987- Handbooks on concrete Reinforced and Detailing.

1.3 STAAD Pro. V8i

Structural Analysis & Design is used to create the model which would then be able to investigated, analysed & designed. After examination and configuration is finished, the GUI can likewise be utilised to see outcomes graphically. It is a general useful census for auxiliary inspection and combines of Steel, concrete, Timber and aluminum construction. Its adaptability for different codes of design makes it versatile.

II. OBJECTIVES

2.1 To design G+ 4 structure for zone III & IV on STAAD Pro.
2.2 To compare the behavior of framed structure in seismic zone III & IV.
2.3 To make a total plan of the main auxiliary components of a specific structure & find out steel increment.

III. METHODOLOGY

3.1 Creation of node foci: Considering the centreline layout of the plan, we entered the hub documents into the STAAD document.
3.2 Representation of bars and segments: Using the inclusion bar layout, we plotted between beams & columns.
3.3 3D perspective on the building: Here we used the transition repetitive pattern in the Y header to get a 3D perspective on the structure.
3.4 Supports and property: After the formation of the structure, the supports at the base of the structure are specified as fixed. Likewise, the Materials were determined and the cross segments were distributed to the individuals.
4 3D render view: After feature clustering, a 3D rendering perspective can be viewed on the structure.
5 Assignment of seismic loads: We have defined the seismic loads specified in the IS1893:2002 code with appropriate ground loads in order to disable seismic loads instantly. Loads are included load case subtleties in +X, -X, +Z, -Z headings with determined seismic factor.
6 Assignment of wind loads: Wind loads are characterized according to IS 875 Part 3, depending on the determined power and input factor.
7 Assignment of dead loads: For external dividers, internal dividers, parapet dividers, constant loads including the self-weight of the structure are determined in accordance with IS 875 part 1.
8 Assignment of live loads: Live loads are relegated for each floor as 3 KN/M^2 dependent on IS 875 PART 2.
9 Adding of load combination: After all batches have been dropped, batch mixes are given with the appropriate factor of safety in accordance with IS 875 Part 5.
10 Analysis: After all the above progress paid off, we played out examination and checked for errors.
11 Design: Finally, the solid plan proceeds according to IS 456:2000, characterizing the appropriate plan orders for the various key segments. After the allocation of orders, we investigated whether there were errors again concrete design.
12 Report: After no error found the reports are downloaded and same procedure is repeated but this time with different Seismic Zone.
After following the above specifications the structure is designed for the Seismic zone III. Since, the same structure can be designed for Zone IV only with minor alterations in the Seismic Load case and reports can be compared.

IV. SIMULATION

The input data is as follow,
1.START CONCRETE DESIGN
2.CODE INDIAN
3.CLEAR 0.025 MEMB 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 - 228. 281 TO 344 360 TO 424 440 TO 504 520 TO 584 229.
3.CLEAR 0.04 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 264 - 230. 265 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 231.

4.FYMAIN 415000 ALL
5.FYSEC 415000 ALL
6.MAXMAIN 32 ALL
7.MAXSEC 16 ALL
8.MINMAIN 8 ALL
9.MINSEC 8 ALL
10.RATIO 4 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 - 238. 264 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 239.
11.DESIGN BEAM 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 TO 344 - 240. 360 TO 424 440 TO 504 520 TO 584

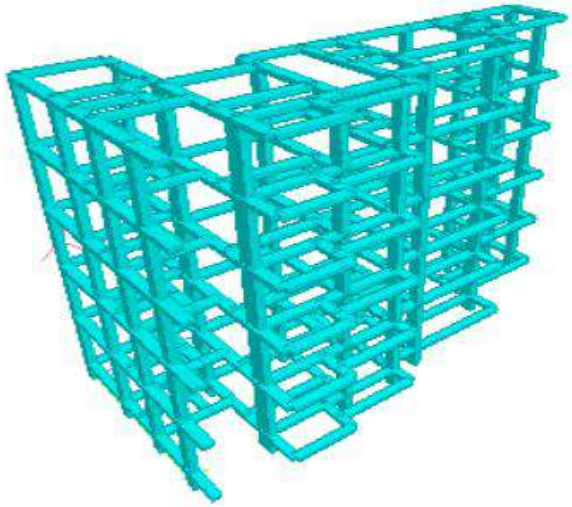

Figure1: 3-D Rendered View

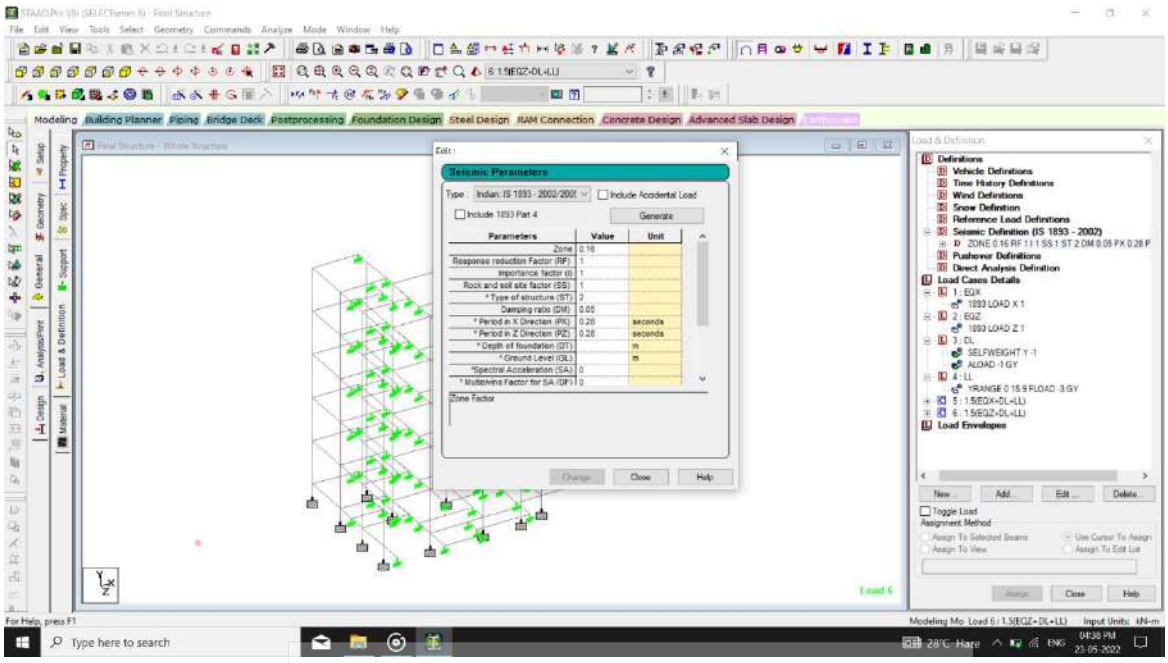

Figure2: Seismic Parameters

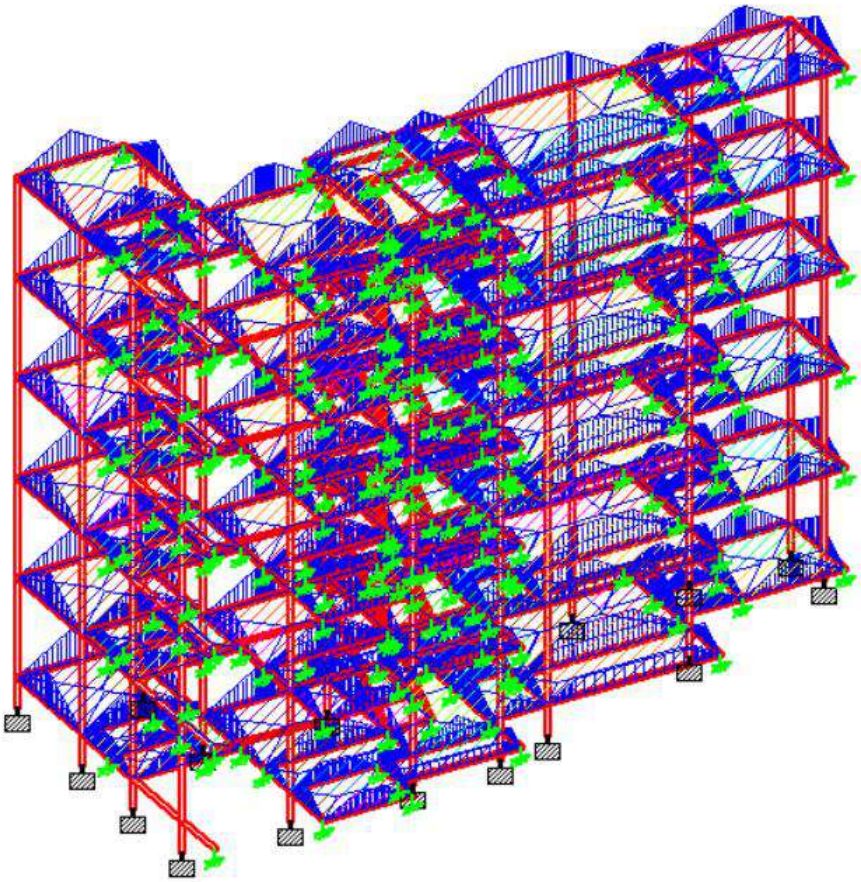

Figure3: Dead Load & Live Loads

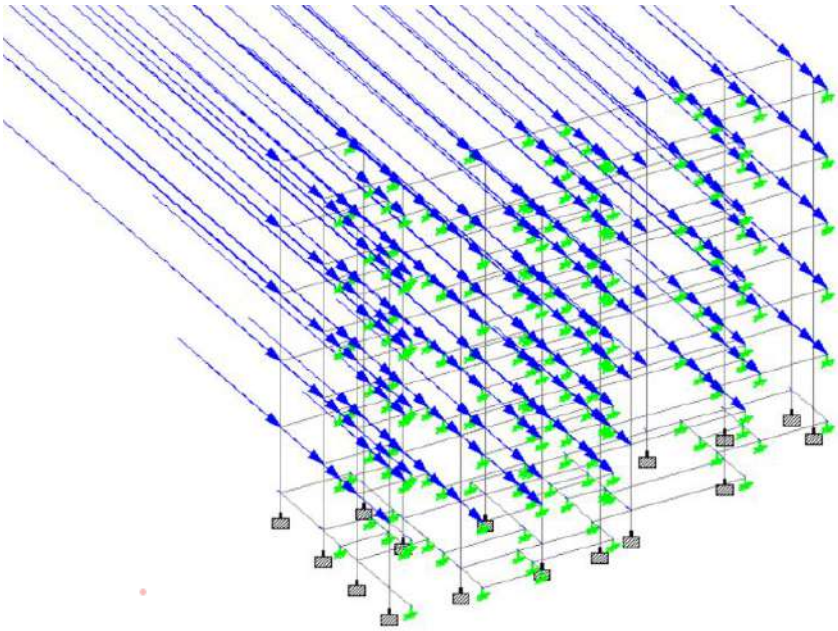

Figure4: Seismic Forces in X- Direction (maximum)

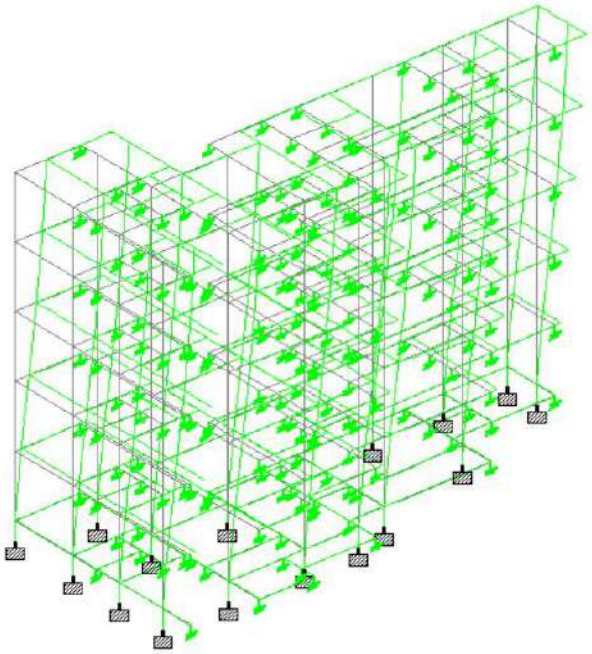

Figure5: Deflection Of Members

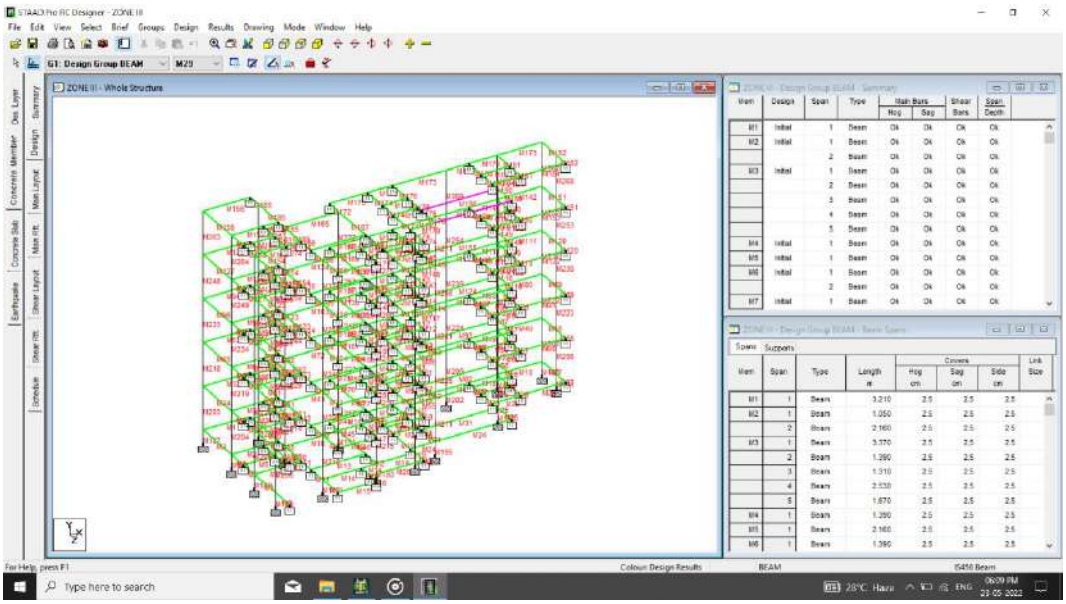

Figure6: Beam Check

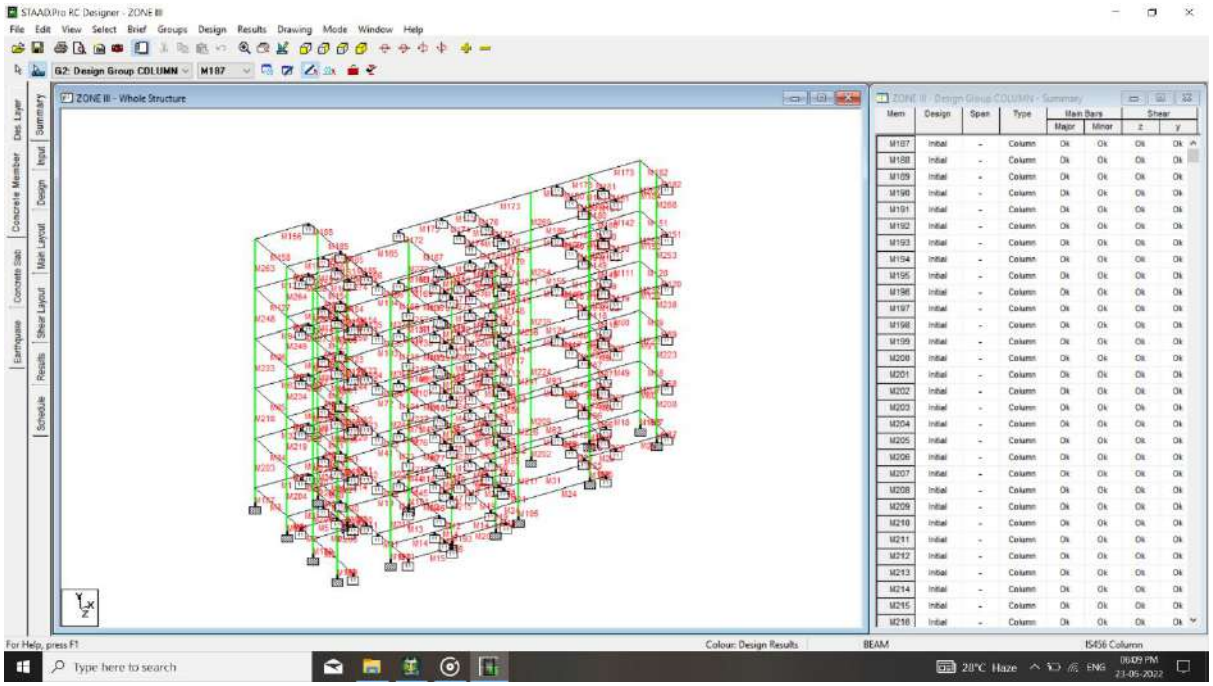

Figure7: Column Check

V. CONCLUSION

5.1 Total 2.47% more steel is require to design the structure from Zone III to Zone IV.
5.2 Maximum nodal displacement is increased by 8.33mm showing more horizontal forces in higher zone.
5.3 Maximum bending moment is increased by 30.84 kNm results in more steel in beam section.
5.4 Maximum shear forces increased by 15.32 kN resulting in additional 1.3% shear reinforcement in zone IV.
5.5 After analyzing the G+4 storey building structure, it was concluded that the building is safe under dead load, wind load and seismic loads in both zones if additional 2.5% reinforcement is provided.
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S j sv mnnwR / Nj v j nmj fi[j,k,*] - L /b/ Nj nmqj [l]

[j] j t to rwl rw o : Ut o t umtrw v : l wy o yn y t y : e wo : c syo
[k] R o owtp Uo vt T tvt ua vy tt y v o s mtrw v : R wo: c syo
[l] j o o o po c y t umtrw v : c o : c syo

A R T I C L E I N F O

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A B S T R A C T

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Sss v r

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Z_{VT}	V 0z nq z
ZMT	M zz0z nq z
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n_{TPVL}	Tvflr r z
L_{Ss}	J s Od zvflr r yfi
L_{Ns}	J s qv vo v fl nfl s fi r
L_{Ns}	J s Kd
n_{KdJ}	Z r z vfl qv vo v fl nfl s fi r
$n_{a\ Nr}$	d nz z vfl Tf Kc vu pn npv
a	Td zrflt u
$n_{S\ Nr}$	d nz rq p v fl vfl z r q r pn npv vfl nzzn v fl
n_o	d nz rq p v fl vfl z r q r Of Kc
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L_{as}	J s Td flr y
L_{Td}	J s Td zvflr r S fi
f	V fi or s Kd fl ur srrqr
L_{Ss}	J s ur Od flr y

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