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Research

Design and Evaluation of Bionanosuspension Loaded Cetirizine for Brain Targetting via Ocular Route

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ABSTRACT:

This thesis on the research topic ―Formulation and evaluation of bionanosuspension loaded with cetirizine for brain targeting via ocular route‖ using various biopolymers. All biopolymers (Santalum album, Glycin max, Phaseous vulgaris, Daucus Carota, Coriandrum sativum, Beta vulgaris) was separated by simplified process. The separated biopolymer were subjected for various physic chemical parameter like color, texture, particle size, solubility, chemical tests. Spectral analysis such as Raman, IR, NMR, DSC and UV spectroscopy was also done to check the polymeric nature of biopolymers. Drug- polymer interaction studies are also done to check the polymer safety. The eye can be used as site for drug administration for continuous drug infusion into the brain. For the delivery of drug into brain via eye nano sized Cetirizine loaded Nano suspension was formulated using Drug to polymer ratio was chosen at different levels for Santalum album polymer FS1(1:0.5), FS2(1:1), FS3(1:2), FS4(1:3), FS5(1:4), FS6(1:5), FS7(1:6), FS8(1:7), FS9(1:8), FS10(1:10) and ten level for Glycin max polymer FG1(1:0.5), FG2(1:1), FG3(1:2), FG4(1:3), FG5(1:4), FG6(1:5), FG7(1:6), FG8(1:7), FG9(1:8), FG10(1:10), and ten level for Vigna raidiata polymer FS1(1:0.5), FS2(1:1), FS3(1:2), FS4(1:3), FS5(1:4), FS6(1:5), FS7(1:6), FS8(1:7), FS9(1:8), FS10(1:10) and ten level for Phaseous vulgaris polymer FP1(1:0.5), FP2(1:1), FP3(1:2), FP4(1:3), FP5(1:4), FP6(1:5), FP7(1:6), FP8(1:7), FP9(1:8), FP10(1:10) and ten level for Daucus carota polymer FD1(1:0.5), FD2(1:1), FD3(1:2), FD4(1:3), FD5(1:4), FD6(1:5), FD7(1:6), FD8(1:7), FD9(1:8), FD10(1:10) and ten level for Beta vulgaris polymer FB1(1:0.5), FB2(1:1), FB3(1:2), FB4(1:3), FB5(1:4), FB6(1:5), FB7(1:6), FB8(1:7), FB9(1:8), FB10(1:10) and ten level for Coriandrum sativum polymer FC1(1:0.5), FC2(1:1), FC3(1:2), FC4(1:3), FC5(1:4), FC6(1:5), FC7(1:6), FC8(1:7), FC9(1:8), FC10(1:10) by weight. Nanosuspension os different batch were evaluated for various parameter such as ph, conductivity, drug uniformity, in-vitro drug study of above formulation. Based on in-vitro study a smart conclusion was drawn that all isolated biopolymer can serve as apromising biopolymer for formulation of nanoparticle loaded Cetirizine Nanosuspension for ocular to brain delivery.

KEYWORDS: Cetirizine, Nanoparticle, Brain, Biopolymer, Bio nanosuspension, Brain targeting, Ocular drug delivery

INTRODUCTION :

Ocular drug delivery has remained one of the challenging task for pharmaceutical scientists. In ocular drug delivery system, there is a main problem of rapid and extensive elimination of conventional eye drops from the eye . This problem results in extensive loss of drug. Only a few amount of drug penetrates the corneal layer and

reached to internal tissue of eye . The main region of drug loss includes lachrymal drainage and drug dilution by tears . This superfluity reduces the ocular bioavailability and lead to unwanted toxicity and side effect.[1,2]

The anterior and posterior segment of eye is affected by various vision threatening diseases. Diseases affecting anterior segment include, but not limited to glaucoma, allergic conjunctivitis, anterior uveitis and cataract. While, age-related macular degeneration (AMD) and diabetic retinopathy are the most prevalent diseases affecting posterior segment of the eye. Topical instillation is the most widely preferred non-invasive route of drug administration to treat diseases affecting the anterior segment. Conventional dosage forms such as eye drops account for 90% of the marketed ophthalmic formulations. The reason may be attributed to ease of administration and patient compliance.[3,4]

Nonetheless, the ocular bioavailability is very low with topical drop administration. Numerous anatomical and physiological constraints such as tear turnover, nasolacrimal drainage, reflex blinking, and ocular static and dynamic barriers pose a challenge and impede deeper ocular drug permeation [5]. Hence, less than 5% of topically applied dose reaches to deeper ocular tissues.[6] Also, it is difficult to achieve therapeutic drug concentration into posterior segment ocular tissues following topical eye drops instillation because of the above mentioned barriers. The drug can be delivered to the posterior segment ocular tissues by different mode of administrations such as intravitreal injections, periocular injections, and systemic administration.

Intravitreal injection is the most common and widely recommended route of drug administration to treat posterior ocular diseases. Though, the need of repeated eye puncture with intravitreal injections causes several side effects such as endophthalmitis, hemorrhage, retinal detachment and poor patient tolerance[5] The trans scleral drug delivery with periocular administration route is evolved as an alternative mode of drug delivery to the posterior ocular tissues. Although trans scleral delivery is comparatively easy, less invasive and patient compliant, drug permeation is compromised by ocular static and dynamic barriers. Ocular barriers to transscleral drug delivery include: static barriers i.e., sclera, choroid and retinal pigment epithelium (RPE), and dynamic barriers, i.e., lymphatic flow in the conjunctiva and episclera, and the blood flow in conjunctiva and choroid [7,8]

To overcome the ocular drug delivery barriers and improve ocular bioavailability, various conventional and novel drug delivery systems have been developed such as emulsion, ointments, suspensions, aqueous gels, nano micelles, nanoparticles, liposomes, dendrimers, implants, contact lenses, nanosuspensions, micro needles, and in situ thermo sensitive gels for the earlier mention ocular diseases.[4]

ROUTES OF OCULAR DRUG DELIVERY

The main administration routes for ocular drug delivery include topical, periocular, intraocular, and systemic. Topical administration is the most common route for treating diseases of the anterior segment of the eye, due to ease of application, drug localization and adequate efficacy, and low cost. [2]

The following characteristics are required to optimize ocular drug delivery systems:-

- A good corneal penetration.
- A prolonged contact time of drug with corneal tissue.
- Simplicity of installation and removal for the patient.
- A non-irritative and at ease form (the viscous solution should not irritate lachrymation and reflex flashing).
- Appropriate rheological properties and concentration of viscolyzer.[1]

Advantages of Ocular Drug Delivery System

1) Provides sustained and controlled drug delivery.
2) Increase the ocular bioavailability of drug by increasing the corneal contact time.
3) Increased accurate dosing to overcome the sides effects of pulsed dosing produced by conventional system.

Limitation of Ocular Drug Delivery System

1) Dosage form cannot be terminated during emergency.
2) Loss of drug during sleep or while rubbing eye just after during administration.[1]

Ocular to Brain Drug Delivery System

Eye is connected to the brain via Optic nerve. The optic nerve is second of twelve paired cranial nerves but is considered to be part of the central nervous system, as it is a derived from an out pouching of the diencephalon during embryonic development. As a consequence, the fibres are covered with myelin produced by oligodendrocytes, rather than Schwann cells, which are found in peripheral nervous system, and are

encased within the meninges. The name ―optic nerve‖ is, in the technical sense, amisnometer, as the optic system lies within the central nervous system and therefore should be named the ―optic tract‖.[4]

The optic nerve is composed of retina ganglion cell axons and support cells. The optic nerve components length are 1mm in the globe,24 mm in the orbit, 9 mm in the optic canal, and 16mm in the cranial space before joining the optic chiasm. There, partial decussation occurs, and about 53% of the fibres cross to form the optic tracts.[4]

NOVEL OCULAR DRUG DELIVERY SYSTEMS:-

Nanotechnology based ocular drug delivery

In a last few decades, many approaches have been utilized for the treatment of ocular diseases. Nanotechnology based ophthalmic formulations are one of the approaches which is currently being pursued for both anterior, as well as posterior segment drug delivery. Nanotechnology based systems with an appropriate particle size can be designed to ensure low irritation, adequate bioavailability, and ocular tissue compatibility. Several nanocarriers, such as nanoparticles, nanosuspensions, liposomes, nanomicelles and dendrimers have been developed for ocular drug delivery. Some of them have shown promising results for improving ocular bioavailability.[7]

NANOSUSPENSION

Nanosuspensions are the minute, colloidal, biphasic, solid drug particles dispersed in aqueous vehicle with a size not more than 1µm and have no matrix materials. These particles are stabilized by polymers and surfactants prepared by suitable methods for delivery by mean of various route of administration like topical, parenteral, oral, etc. the pharmaceutical Nano suspension has solved the problems of the poor solubility and bioavailability of the drugs. These particles have also altered the pharmacokinetics of the drug. There is a distinct difference between the pharmaceutical Nano suspension and the nanoparticles. Nano suspensions are the polymeric colloidal carriers to form solid lipid nanoparticles are also known as SLN. The nanotechnology has been used to formulate the insoluble and poor water soluble drugs to a Nano suspension formation to improve the deficiency associated with this class of drugs.[7]

Nano suspensions do have some advantages that is they are effective for those molecules insoluble in oil, secondly the high drug loading can be achieved as a drug exists in the form of pure solids, and can significantly reduce the administration volume of high dose and lastly, the Nano suspension can increase the physical and chemical stability of the drugs as they are actually in the solid state.

Approaches for Nano Suspensions:-

- Preparing nano suspensions is preferred for the compounds that are insoluble in water (but are soluble in oil) with high log P value.
- Conventionally the drugs that are insoluble in water but soluble in oil phase system are formulated in liposome, emulsion systems but these lipidic formulation approaches are not applicable to all drugs. In these cases nano suspensions are preferred.
- In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems Nano suspensions are used as a formulation approach.
- Nano suspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.

PREPARATION METHODS OF NANOSUSPENSIONS

The principle techniques used in recent years for preparing Nano suspensions can be classified into four basic methods:

A. Homogenization

B. Wet milling

C. Emulsification-solvent evaporation and

D. Precipitation or micro precipitation method

Preparation of Nano suspensions were reported to be a more cost effective and technically more simple alternative, particularly for poorly soluble drugs and yield a physically more stable product than liposomes; conventional colloidal drug carriers .

For the Nano suspensions manufacture, there are two converse methods -‗bottom-up‘ and the top down‘ technologies. The bottom-up technology is an assembling method from molecules to Nano sized particles, including micro precipitation, micro emulsion, melt emulsification method and so on. The top-down technology is a disintegration approach from large particles, micro particles to nanoparticles, such as high-pressure homogenization and media milling method.[7]

HOMOGENIZATION

The process can be summarized into three steps: firstly, drug powders are dispersed in a stabilizer solution to form pre-suspension; then pre-suspension was homogenized by the high-pressure

homogenizer at a low pressure for several times as a kind of pre milling, and finally was homogenized at a high pressure for 10 25 cycles until the nanosuspensions with the desired size were prepared.[7] MILLING

Recently, nanosuspensions can be obtained by dry milling techniques. Nanosuspensions are produced by using high-shear media mills or pearl mills. The mill consists of a milling chamber, milling shaft and a recirculation chamber. An aqueous suspension of the drug is then fed into the mill containing small grinding balls/pearls. As these balls rotate at a very high shear rate under controlled temperature, they fly through the grinding jar interior and impact against the sample on the opposite grinding jar wall. The combined forces of friction and impact produce a high degree of particle size reduction.[7] The milling media or balls are made of ceramic-sintered aluminum oxide or zirconium oxide or highly cross-linked polystyrene resin with high abrasion resistance. Planetary ball mills is one example of an equipment that can be used to achieve a grind size below 0.1 µm. A nanosuspension of ZnInsulin with a mean particle size of 150 nm was prepared using the wet milling technique . Media milling is a further technique used to prepare nanosuspensions. Nanocrystal is a patent protected technology developed by Liversidge. In this technique, the drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer.[7]

PRECIPITATION

Precipitation has been applied for years to prepare submicron particles within the last decade especially for the poorly soluble drugs. Typically, the drug is firstly dissolve in a solvent. Then this solution is mixed with a miscible antisolvent in the presence of surfactants. Rapid addition of a drug solution to the antisolvent (usually water) leads to sudden supersaturation of drug in the mixed solution, and generation of ultrafine crystalline or amorphous drug solids. This process involves two phases: nuclei formation and crystal growth. When preparing a stable suspension with the minimum particle size, a high nucleation rate but low growth rate is necessary. Both rates are dependent on temperature: the optimum temperature for nucleation might lie below that for crystal growth, which permits temperature optimization.[7]

LIPID EMULSION/SOLVENT EVAPORATION

Another way to produce nano suspensions is to use an emulsion which is formed by the conventional method using a partially water miscible solvent as the dispersed phase. Nano suspensions are obtained by just diluting the emulsion. Moreover, micro emulsions as templates can produce nano suspensions. Micro emulsions are thermodynamically stable and iso tropically clear dispersions of two immiscible liquids such as oil and water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase or the preformed micro emulsion can be saturated with the drug by intimate mixing. Suitable dilution of the micro emulsion yields the drug nano suspension. [7]

EVALUATION TECHNIQUES

- MEAN PARTICLE SIZE AND PARTICLE SIZE DISTRIBUTION: -

The mean particle size and the span of particle size distribution (polydispersity index, PI) are two important characteristic parameters because they affect the saturation solubility, dissolution rate, physical stability, even in-vivo behaviour of Nano suspensions. Particle size distribution determines the physiochemical behaviour of the formulation, such as saturation solubility, dissolution velocity, physical stability, etc. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and coulter counter multisizer. PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1– 0.25 indicates a fairly narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution .The coulter-counter gives the absolute number of particles per volume unit for the different size classes, and it is a more efficient and appropriate technique than LD for quantifying the contamination of nanosuspensions by micro particulate drugs.[7]

- SURFACE CHARGE (ZETA POTENTIAL)

Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanosuspensions. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself 31.For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient. [7]

- CRYSTALLINE STATE AND PARTICLE MORPHOLOGY

The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing .Nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high-pressure homogenization 2 .The changes in the solid state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis 33 and supplemented by differential scanning calorimetry .In order to get an actual idea of particle morphology, scanning electron microscopy is preferred. [7]

- FTIR SPECTROSCOPY

FTIR spectroscopy studies were carried out for identification of pure drug and compatibility study of physical mixture of nanosuspension. FTIR spectroscopy was conducted using a shimadzu FTIR 8400 spectrophotometer (shimadzu, Japan) and spectrum was recorded in the wavelength region of 4000-400cm-1. The procedure consisted of dispersing sample in KBr and compressing into discs by applying pressure. The pellet was placed in the light path and spectrum was recorded. [7]

- DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC studies were carried out for identification of pure drug and compatibility study of physical mixture of nanosuspention. DSC study was performed using DSC-60 C (Shimadzu, Tokyo, Japan) calorimeter to study the thermal behaviour of drug. The instrument comprised of calorimeter (DSC-60), flow controller (FCL60), thermal analyser (TA 60WS) and operating software (TA 60). The Diacerein drug sample was heated in hermetically sealed aluminium pans under air atmosphere at a scanning rate of 10°C/min from 30 to 300°C in an air atmosphere. Empty aluminium pan was used as a reference. [7]

- POLY DESPERSITY INDEX (PDI)

Mean particle size and Polydispersity index (PDI) of prepared Nanosuspension were obtained using Zetatrac. After suitable dilution, prepared nanosuspension was added to the sample cell and determination was carried out. PDI values give idea about uniformity of size distribution.

- SATURATION SOLUBILITY STUDIES

The saturation solubility studies were carried out for both unprocessed pure drug and different batches of Nanosuspension. Nanosuspension and suspension of bulk drug was stirred for 24 hours on magnetic stirrer at 100rpm and room temperature to have saturation. Then the sample was filled in centrifugation tube and centrifuge for 10 min at 10,000 rpm in Cooling Centrifuge. Clear supernant was collected using 0.22µm syringe filter and analysed using UV spectrophotometer at 258 nm. The results were analysed and noted.

- IN-VITRO DISSOLUTION STUDIES

In-vitro dissolution of Diacerein nanosuspension was studied for 60 min using USP apparatus type 2 (paddle type) at 75 rpm (Electrolab Dissolution Tester TDT-06P, USP) using 900 ml citrate buffer pH 6.0 as dissolution medium maintained at 37°±5°C. Diacerein nanosuspension (5ml) was accurately inserted in the medium and sample aliquots of 5ml sample was withdrawn from the vessel and filtered through 0.22µm syringe filter at predetermined time intervals (2, 4, 6, 8, 10,15, 20, 30, 45 and 60 min) and replaced with 5ml fresh dissolution media. The withdrawn samples were analysed using UV visible spectrophotometer at 258nm by the regression equation of standard curve developed in the same range in the linearity range of 2–8 µg/ml. The experimental data obtained were validated by ANOVA combined with the F test. The determination coefficient (R2, agreement between the experimental results and predicted values obtained from the model) and the model F value (Fisher variation ratio, the ratio of mean square for regression to mean square for residual) were applied for statistical evaluation.

- SHORT TERM ACCELERATED STABILITY STUDY

Optimized batch of Diacerein nanosuspension was subjected to short term stability study for a period of 1 month as per ICH guidelines. In the present study, stability study was carried out at 40 °C ± 2°C and 75% ± 5% relative humidity (RH).

Nanosuspension was evaluated for particle size, viscosity, sedimentation rate and cumulative percentage drug release.

Application of Bio Nanosuspensions :-

Nanosuspensions are used as oral, parenteral, ocular, and pulmonary drug delivery systems.

Oral Administration:-

Oral administration is the first patient choice because of painless and noninvasive administration [27]. In addition, oral formulations have several advantages for the pharmaceutical industry such as easy manufacturing, short production time, and reasonable production cost. Oleanolic acid, which has many applications such as hepatoprotective, antitumour, antibacterial, anti-inflammatory, and antiulcer effects, has low aqueous solubility which results in erratic pharmacokinetics after oral administration. Applying oleanolic acid in the form of nanosuspension increases dissolution rate to about 90% in the first 20 min compared to just 15% for micronized drug powder [28]

Parenteral Administration:-

In emergency cases such as cardiac arrest and anaphylactic shock parenteral administration is the first choice [30]. Parenteral administration includes administration of dosage forms by subcutaneous, i.v., intramuscular, and intra-arterial methods . Advantages of this type of administration include avoidance of first-pass metabolism, reliable doses, and higher bioavailability. Control over the dose and rate allows more predictable pharmacodynamic and pharmacokinetic profiles after i.v. administration compared to oral administration . Administered drug particles are required to be smaller than 5 μm to prevent blockage of capillaries . A study on mice investigated tumour growth inhibition rate and showed that oridonin in the form of nanosuspension decreased considerably the volume and weight of the tumour. [31]

Pulmonary Drug Delivery:-

Pulmonary drug delivery aims at treating several respiratory conditions such as asthma and chronic obstructive pulmonary diseases. Advantages of pulmonary drug delivery over oral and parenteral drug administration include direct delivery to the site of action which leads to decreased dosage and side effects . Conventional pulmonary delivery systems provide only rapid drug release, poor residence time, and lack of selectivity . Nanosuspensions can solve problems of poor drug solubility in pulmonary secretions and lack of selectivity through direct delivery to target pulmonary cells. Adhesiveness of nanosuspensions to mucosal surfaces leads to improved selectivity because of minimal drug loss and prolonged residence time at target site . Pulmonary nanosuspensions improve drug diffusion and dissolution rate and consequently increase bioavailability and prevent undesirable drug deposition in the mouth and pharynx. [32]

Ocular Administration:-

Major problems in ocular therapy include

(i) Poor drug solubility in lachrymal fluids,
(ii) Repeated instillation of conventional eye drops due to drainage through the nasolacrimal duct,
(iii) Repeated instillation and systematic drug absorption often causing side effects.

Nanosuspensions as ocular drug delivery systems offer several advantages.

(i) Nanoparticle modified surface by appropriate bioerodible polymer causes prolonged residual time in cul-de-sac desired for effective treatment. Commonly reported polymers in ocular nanosuspensions are poly(alkyl cyanoacrylates), polycaprolactone, and poly(lactic acid)/poly(lactic-co-glycolic acid) . Employing polymers in ocular drug delivery significantly prolongs drug ocular residence time and improves bioavailability.
(ii) Positively charged nanoparticles have strong adhesion to negatively charged mucin which extends the drug release. For example, polymer Eudragit RS 100 was used in ibuprofen nanosuspensions to increase drug residence time by creating positively charged surface which resulted in improved corneal adhesion . Flurbiprofen nanosuspensions covered by Eudragit polymers RS 100 and RL 100 exhibited prolonged drug release . Chitosan is another mucoadhesive cationic polymer used in ocular drug delivery to bond with negatively charged mucin and enhance drug residence time .
(iii) Reduced drug loss because of the natural adhesiveness of drug nanoparticles .

(iv) Enhanced rate and extent of drug absorption[37]

INSOMNIA

Insomnia, also known as sleeplessness, is a sleep disorder where people have trouble sleeping. They may have difficulty falling asleep, or staying asleep as long as desired. Insomnia is typically followed by daytime sleepiness, low energy, irritability, and a depressed mood. It may result in an increased risk of motor vehicle collisions, as well as problems focusing and learning. Insomnia can be short term, lasting for days or weeks, or long term, lasting more than a month.[38]

Symptoms of insomnia:

- difficulty falling asleep, including difficulty finding a comfortable sleeping position.
- waking during the night and being unable to return to sleep.
- feeling unrefreshed upon waking.
- daytime sleepiness, irritability or anxiety.

Risk factors Insomnia affects people of all age groups but people in the following groups have a higher chance of acquiring insomnia.

- Individuals older than 60
- History of mental health disorder including depression, etc.
- Emotional stress
- Working late night shifts
- Traveling through different time zones

ROLE OF H1 RECEPTOR IN SLEEP

Administration of histamine or H1 receptor agonists induced wakefulness, whereas administration of H1receptor antagonists promoted sleep.The H1 receptor is a typical G protein-coupled metabotropic receptor (~490 amino acids) with seven putative transmembrane domains . Encoded from the intronless region on human chromosome 3, the H1 receptor is coupled to phospholipase C through a pertussis toxin insensitive (Gq/11) G protein. The H1 receptor is widely distributed throughout the brain with high to moderate levels found in sleep-wakefulness regulatory regions including the basal forebrain, locus coeruleus, raphe nuclei, mesopontine tegmentum and the thalamus.

Systemic intraperitoneal(ip) administration of the first generation H1 receptor antagonists pyrilamine and diphenhydramine decreased W and increased NREM sleep in rats. Central administration of the H1 receptor agonist 2-thiazolylethylamine, dose-dependently, increased wakefulness and decreased both NREM and REM(rapid eye moment) sleep. Furthermore, the H1 receptor antagonists pyrilamine blocked the wakefulness inducing effects of 2-thiazolylethylamine. [39]

Need of the antiinsomnic drug in ocular drug delievery

Insomnia is a common sleep disorder that can make it hard to fall asleep, hard to stay asleep, or cause you to wake up too early and not be able to get back to sleep. You may still feel tired when you wake up. Insomnia can sap not only your energy level and mood but also your health, work performance and quality of life. At some point, many adults experience short-term (acute) insomnia, which lasts for days or weeks. It's usually the result of stress or a traumatic event. But some people have long-term (chronic) insomnia that lasts for a month or more. Insomnia in patient can cause chronic pain, cancer, diabetes, heart disease, asthma, gastroesophageal reflux disease (GERD), overactive thyroid, Parkinson's disease and Alzheimer's disease.

High dose of insomnic drugs cause problem with memory and attention. On high dose of insomnic drugs patient become additive of the drug.Over dose of insomnic drugs may cause itching in adults with severe chronic uticaria refractory. High dose may also causes minor central nervous system adverse effects including somnolence, fatigue, dizziness and dry mouth. So to prevent patient from all the above effects the antinsomnic drug is administered through ocular route. Antiinsomnic drugs can be administered by oral route, parental route, ocular route.[17]

MATERIALS AND METHODS:

Materials

Cetirizine was received from market. All other chemicals were of analytical grade purchased from local suppliers. Polymers used extracted by naturally by following:

List of bio polymer	Botanical name	Source
Sandalwood	Santalum album	From Local Market
Groundnut	Arachis hypogaea	From Local Market
Soyabean	Glycin max	From Local Market

Carrot	Daucus carota	From Local Market
Beetroot	Beta vulgaris	From Local Market
Corriander	Coriandrum sativum	From Local Market
Red/White Rajma	Phaseolus Vulgaris	From Local Market
Moong Dal	Vigna Radiata	From Local Marke

CETIRIZINE

CHEMICAL FORMULA: - C21H25ClN2O3

IUPAC NAME:- 2-(2-(4-((4-chlorophenyl) (phenyl)methyl) piperazin-1-yl) 1ethoxy)acetic

ROUTE OF ADMINISTRATION: - By mouth

BIOAVAILABILITY: - Well absorbed (>70%)

ONSET OF ACTION: - 20-42 minutes

BIOLOGICAL HALF-LIFE: - Mean 8.3hrs

DURATION OF ACTION: - ≥24HRS

CHEMICAL AND PHYSICAL PROPERTIES: - ¬

Experimental properties:-

Computed properties:-

Physical description: Solid

Molecular weight:-388.892g/mol

Color:- Crystal from ethanol

Hydrogen bond donar:- 1

Melting point:- 112.5ºC

Hydrogen bond acceptor: 5 Solubility:- Water

Biological half life:- 8.3hrs solubility(10mg/L)

PHARMACODYNAMICS:

Cetirizine, the active metabolite of the piperazine H1-receptor antagonist hydroxyzine, is used to treat chronic idiopathic urticaria, perennial allergic rhinitis, seasonal allergic rhinitis, allergic asthma, physical urticaria, and atopic dermatitis.

ABSORPTION:- Mean peak plasma concentration (Cmax) of 114ng/ml at a time (Tmax) of 2.2 hours postdose was observed for cetirizine.

PROTEIN BINDING:- Very high 93% approx.

HALF LIFE:- 8.3 hrs.

CLEARANCE: - 53 mL/min [healthy]

TOXICITY: - Semnolence (sleepiness or unusual drowsiness), restlessness, irritability.

ROUTE OF ELEMINATION: -

Cetirizine is eliminated approximately 70 to 85% in the urine and 10 to 13% in the feces. ... The elimination half-life of cetirizine is increased in the elderly (to 12 hours), in hepatic impairment (to 14 hours), and in renal impairment (to 20 hours)

Pharmacology:

Cetirizine, the active metabolite of the piperazine H1-receptor antagonist hydroxyzine, is used to treat chronic idiopathic urticaria, perennial allergic rhinitis, seasonal allergic rhinitis, allergic asthma, physical urticaria, and atopic dermatitis. Cetirizine is a metabolite of hydroxyzine and a selective peripheral histamine H1-receptor antagonist. It is used for symptomatic treatment of seasonal and perennial allergic rhinitis and for chronic urticaria. Cetirizine Hydrochloride is a synthetic phenylmethyl-piperazinyl derivative, antihistaminic Cetirizine is a metabolite of hydroxyzine and a selective peripheral histamine H1-receptor antagonist. It is used for symptomatic treatment of seasonal and perennial allergic rhinitis and for chronic urticaria.[8]

MECHANISM OF ACTION: -

Cetirizine competes with histamine for binding at H1-receptor sites on the effector cell surface, resulting in suppression of histaminic edema, flare, and pruritus. The low incidence of sedation can be attributed to reduced penetration of cetirizine into the CNS as a result of the less lipophilic carboxyl group on the ethylamine side chain.

Cetirizine, a human metabolite of hydroxyzine, is an antihistamine; its principal effects are mediated via selective inhibition of peripheral H1 receptors. The antihistaminic activity of cetirizine has been clearly documented in a variety of animal and human models. In vivo and ex vivo animal models have shown negligible anticholinergic and ant serotonergic activity. In clinical studies, however, dry mouth was more common with cetirizine than with placebo. In vitro receptor binding studies have shown no measurable affinity for other than H1 receptors. Auto radiographic studies with radiolabeled cetirizine in the rat have shown negligible penetration into the brain. Ex vivo experiments in the mouse have shown that systemically administered cetirizine does not significantly occupy cerebral H1 receptors.[10]

USES

Allergies

Cetirizine's primary indication is for hay fever and other allergies. Because the symptoms of itching and redness in these conditions are caused by histamine acting on the H1 receptor, blocking those receptors temporarily relieves those symptoms.

Rhinovirus infection

Interleukin 6 and interleukin 8 have been shown to be elevated in acute respiratory distress syndrome. One recent study of airway epithelial cells showed that levocetirizine, the active enantiomer of

cetirizine, may have beneficial effects on the pathophysiologic changes related to human rhinovirus (HRV) infection.[8]

SIDEEFFECTS

Cetirizine is a newer, second-generation antihistamine. Unlike first-generation antihistamines, cetirizine is less likely to cause side effects such as dangerous drowsiness, dry mouth, blurred vision, and overheating.

Cetirizine can cause adverse effects, such as:

- Drowsiness
- Excessive tiredness
- Dry mouth
- Stomach pain
- Diarrhea
- Vomiting[8]

Methods of Preparation of biomaterial:

- Isolation of biomaterial from Daucus carota:

500gm of daucus carota was procured from market and washed with distilled water. The upper layer

was peeled off using with help of peeler and it was cut into small pieces, later 500ml of water was

added and mixture was minced using mixture grinder. Then mixture was filtered through muslin cloth.

Then filtrate was collected. Few ml of Sodium benzoate was added and kept in refrigerator for 4hrs.

After 4 hrs mixture was further subjected for centrifugation at 4000 rpm for 15 min. Then supernatant

liquid was poured by discarding residue. To supernatant liquid optimize quantity of methanol was

added in 1:2 and mixture was further subject to refrigeration in order to collect biopolymer. After 24hrs

mixture was recovered by centrifugation at 4000rpm for half hour. Later supernatant was discarded and

biopolymer was collected and dried naturally for period of 24hrs. The collected dried biomaterial was

passed through sieve 120 and stored in well closed container. The same procedure was repeated 6

times. The %yield was calculated and reported. Then the collected biomaterial are subjected for various

physiochemical and drug-excipient interaction.

- Isolation procedure of biomaterial from Phaseous vulgaris

500gm of red/white rajma was procured from market and washed with distilled water. The upper layer

was peeled off. Later 500ml of water was added and mixture was minced using mixture grinder. Then

mixture was filtered through muslin cloth. Then filtrate was collected. Few ml of Sodium benzoate was

added and kept in refrigerator for 4hrs. After 4 hrs mixture was further subjected for centrifugation at

4000 rpm for 15 min. Then supernatant liquid was poured by discarding residue. To supernatant liquid

optimize quantity of methanol was added in 1:2 and mixture was further subject to refrigeration in

order to collect biopolymer. After 24hrs mixture was recovered by centrifugation at 4000rpm for half

hour. Later supernatant was discarded and biopolymer was collected and dried naturally for period of

24hrs. The collected dried biomaterial was passed through sieve 120 and stored in well closed

container. The same procedure was repeated 6 times. The %yield was calculated and reported. Then the

collected biomaterial are subjected for various physiochemical and drug-excipient interaction.

- Isolation procedure of biomaterial from Glycin max

500gm of Soyabean was procured from market and washed with distilled water. The upper layer was

peeled off and later 500ml of water was added and mixture was minced using mixture grinder. Then

mixture was filtered through muslin cloth. Then filtrate was collected. Few ml of Sodium benzoate

was added and kept in refrigerator for 4hrs. After 4 hrs mixture was further subjected for centrifugation

at 4000 rpm for 15 min. Then supernatant liquid was poured by discarding residue. To supernatant

liquid optimize quantity of methanol was added in 1:2 and mixture was further subject to refrigeration

in order to collect biopolymer. After 24hrs mixture was recovered by centrifugation at 4000rpm for

half hour. Later supernatant was discarded and biopolymer was collected and dried naturally for period

of 24hrs. The collected dried biomaterial was passed through sieve 120 and stored in well closed

container. The same procedure was repeated 6 times. The %yield was calculated and reported. Then

the collected biomaterial are subjected for various physiochemical and drug-excipient interaction.

- Isolation procedure of biomaterial from Beta vulgaris

500gm of beetroot was procured from market and washed with distilled water. The upper layer was

peeled off using with help of peeler and it was cut into small pieces, later 500ml of water was added

and mixture was minced using mixture grinder. Then mixture was filtered through muslin cloth. Then

filtrate was collected. Few ml of Sodium benzoate was added and kept in refrigerator for 4hrs. After 4

hrs mixture was further subjected for centrifugation at 4000 rpm for 15 min. Then supernatant liquid

was poured by discarding residue. To supernatant liquid optimize quantity of methanol was added in

1:2 and mixture was further subject to refrigeration in order to collect biopolymer. After 24hrs mixture

was recovered by centrifugation at 4000rpm for half hour. Later supernatant was discarded and biopolymer was collected and dried

naturally for period of 24hrs. The collected dried biomaterial was

passed through sieve 120 and stored in well closed container. The same procedure was repeated 6

times. The %yield was calculated and reported. Then the collected biomaterial are subjected for various

physiochemical and drug-excipient interaction.

- Isolation procedure of biomaterial from Santalum album

500gm of red sandalwood powder was procured from market. Later 500ml of water was added. Then

mixture was filtered through muslin cloth. Then filtrate was collected. Few ml of Sodium benzoate was

added and kept in refrigerator for 4hrs. After 4 hrs mixture was further subjected for centrifugation at

4000 rpm for 15 min. Then supernatant liquid was poured by discarding residue. To supernatant liquid

optimize quantity of methanol was added in 1:2 and mixture was further subject to refrigeration in

order to collect biopolymer. After 24hrs mixture was recovered by centrifugation at 4000 rpm for half

hour. Later supernatant was discarded and biopolymer was collected and dried naturally for period of

24hrs. The collected dried biomaterial was passed through sieve 120 and stored in well closed

container. The same procedure was repeated 6 times. The %yield was calculated and reported. Then the

collected biomaterial are subjected for various physiochemical and drug-excipient interaction.

- Isolation procedure of biomaterial from Arachis hypogaea

500gm of groundnut was procured from market and washed with distilled water. The upper layer was

peeled off Later 500ml of water was added and mixture was minced using mixture grinder. Then

mixture was filtered through muslin cloth. Then filtrate was collected. Few ml of Sodium benzoate was

added and kept in refrigerator for 4hrs. After 4 hrs mixture was further subjected for centrifugation at

4000 rpm for 15 min. Then supernatant liquid was poured by discarding residue. To supernatant liquid

optimize quantity of methanol was added in 1:2 and mixture was further subject to refrigeration in order

to collect biopolymer. After 24hrs mixture was recovered by centrifugation at 4000rpm for half hour.

Later supernatant was discarded and biopolymer was collected and dried naturally for period of 24hrs.

The collected dried biomaterial was passed through sieve 120 and stored in well closed container. The

same procedure was repeated 6 times. The %yield was calculated and reported. Then the collected

biomaterial are subjected for various physiochemical and drug-excipient interaction.

- Nanosizing:-

Nanosizing of Cetirizine by standard method (Solvent evaporation Method)

Drug was taken in mortar pestle; methanol was added to it. It was then triturated. Water was added in the solution and sonicated six times (one cycle for 3 minutes). The solution was then micro centrifuged. Residue was collected and dried. Nanosizing of Cetirizine by novel method Drug, dextron was added in a mortar pestle, and triturated well. Distilled water added to it gradually and sonicated for 5 cycles (one cycle for 3 minutes).residue was collected and dried . Physiochemical properties of isolated biopolymer. The physiochemical properties such as color, odor, taste, grittiness of the isolated biopolymer was determined by means of the physical examination. The biopolymer was subjected to various solubility studies with different solvents such as: Distilled water, chloroform, methanol, and ethanol.

Formulation of Bio-Nanosuspension: -

The various formulations of Nano suspension were prepared. Biomaterial of different ratios for different formulation were taken in 50ml beaker and add 20 mg maltose, then it was dispersed uniformly using solvent water by sonication, after that drug (Cetirizine) 10mg was dissolved in methanol by continuous stirring in a sonicator. After that it was poured in the polymeric solution and sonicate for 3 cycles. Then this same procedure was followed up for the remaining all formulations.

FORMULATION CONTENT	FS1 (1:0.5)	FS2 (1:1)	FS3 (1:2)	FS4 (1:3)	FS5 (1:4)	FS6 (1:5)	FS7 (1:6)	FS8 (1:7)	FS9 (1:8)	FS10 (1:10)
Cetirizine (mg)	10	10	10	10	10	10	10	10	10	10
Santalum album Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose (mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water	10	10	10	10	10	10	10	10	10	10

TABLE 1:- Formulation of Cetirizine using Sandalwood (*Santalum album)* polymer

FORMULATION CONTENT	FG1 (1:0.5)	FG2 (1:1)	FG3 (1:2)	FG4 (1:3)	FG5 (1:4)	FG6 (1:5)	FG7 (1:6)	FG8 (1:7)	FG9 (1:8)	FG10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Glycine max Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose	20	20	20	20	20	20	20	20	20	20
Sterlized	10	10	10	10	10	10	10	10	10	10

wate(ml)										

TABLE 2 Formulation of Cetirizine using Soyabean (*Glycine max)* polymer

ORMULATION CONTENT	FV1 (1:0.5)	FV2 (1:1)	FV3 (1:2)	FV4 (1:3)	FV5 (1:4)	FV6 (1:5)	FV7 (1:6)	FV8 (1:7)	FV9 (1:8)	FV10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Vigna radiate Biopolymer(mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE 3 Formulation of Cetirizine using Moong Dal (*Vigna radiate)* polymer

FORMULATION CONTENT	FP1 (1:0.5)	FP2 (1:1)	FP3 (1:2)	FP4 (1:3)	FP5 (1:4)	FP6 (1:5)	FP7 (1:6)	FP8 (1:7)	FP9 (1:8)	FP10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Phaseolus Vulgaris Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE 4 Formulation of Cetirizine using Red/White Rajma (*Phaseolus Vulgaris*) polymer

FORMULATION CONTENT	FA1 (1:0.5)	FA2 (1:1)	FA3 (1:2)	FA4 (1:3)	FA5 (1:4)	FA6 (1:5)	FA7 (1:6)	FA8 (1:7)	FA9 (1:8)	FA10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Arachis Hypogaea Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE 5 Formulation of Cetirizine using Groundnut (*Arachis Hypogaea)* polymer

FORMULATION CONTENT	FD1 (1:0.5)	FD2 (1:1)	FD3 (1:2)	FD4 (1:3)	FD5 (1:4)	FD6 (1:5)	FD7 (1:6)	FD8 (1:7)	FD9 (1:8)	FD10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Daucus Carota Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE 6 Formulation of Cetirizine using Carrot(*Daucus Carota)* polymer

FORMUL	FB1	FB2	FB3	FB4	FB5	FB6	FB7	FB8	FB9	FB10

ATION CONTENT	(1:0.5)	(1:1)	(1:2)	(1:3)	(1:4)	(1:5)	(1:6)	(1:7)	(1:8)	(1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Beta vulgaris Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE7 Formulation of Cetirizine using Beet Root (*Beta vulgaris)* polymer

FORMULATION CONTENT	FC1 (1:0.5)	FC2 (1:1)	FC3 (1:2)	FC4 (1:3)	FC5 (1:4)	FC6 (1:5)	FC7 (1:6)	FC8 (1:7)	FC9 (1:8)	FC10 (1:10)
Cetirizine(mg)	10	10	10	10	10	10	10	10	10	10
Coriandrum Sativum Biopolymer (mg)	0.5	10	20	30	40	50	60	70	80	100
Maltose(mg)	20	20	20	20	20	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10	10	10	10	10	10

TABLE 8 Formulation of Cetirizine using Corriander (*Coriandrum Sativum)* polymer

FORMULATION CONTENT	FH1 (1:0.5)	FH2 (1:1)	FH3 (1:2)	FH4 (1:8)	FH5 (1:10)
Cetirizine (mg)	10	10	10	10	10
Hydroxy propyl methyl cellulose(HPMC) (mg)	0.5	10	20	80	100
Maltose(mg)	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10

TABLE 9 Formulation of Cetirizine using HPMC as standard polymer

FORMULATION CONTENT	FS1 (1:0.5)	FS2 (1:1)	FS3 (1:2)	FS4 (1:8)	FS5 (1:10)
Cetirizine (mg)	10	10	10	10	10
Sodium Alginate(mg)	0.5	10	20	80	100
Maltose(mg)	20	20	20	20	20
Sterlized Water (ml)	10	10	10	10	10

TABLE10 Formulation of Cetirizine using Sodium Alginate as standard polymer

FORMULATION CONTENT	FG1 (1:0.5)	FG2 (1:1)	FG3 (1:2)	FG4 (1:8)	FG5 (1:10)
Cetirizine (mg)	10	10	10	10	10
Guargum (mg)	0.5	10	20	80	100
Maltose(mg)	20	20	20	20	20

Sterlized Water (ml)	10	10	10	10	10

TABLE11 Formulation of Cetirizine using Guargum as standard polymer

Physiochemical properties of isolated biopolymer

- PHYSICAL TESTS:

The isolated biopolymer was subjected to various physical parameters like color, odor, smell, solubility and melting point and the observations were recorded. The melting point was determined by ―Open Capillary Method‖, the biopolymer was taken in a glass capillary whose one end was sealed by flame. The capillary containing the biomaterial was dipped inside the melting point apparatus. Melting point is a good first indication of purity of the sample since the presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. The biomaterial was subjected for solubility studies with different solvents like Distilled water, Methanol, Ethanol Chloroform, Acetone etc.

- CHEMICAL TESTS:

The biopolymer was subjected to chemical tests.

1. Carbohydrates:

a) Fehling‘s test: Addition of Fehling‘s reagent (A+B) to polymer solution and a red precipitate indicates the presence of carbohydrates.

b) Molisch‘s test: 2ml of the biomaterial solution was taken in a test tube and 2-3 drops of alcoholic α naphthol was added, Conc. Sulphuric acid (2-3 drops) was added from the sides of the test tube and observed for the formation of purple to violet color ring at the junction.

c) Benedict‘s test: 2ml of biomaterial solution and 2-3 drops of Benedicts solution was taken in a test tube and was subjected for heating and noticed for reddish color change.

2. PROTEINS: Ninhydrin‘s test: 2 ml of the biomaterial solution was taken in a test tube and 2-3ml of ninhydrin solution was added and was subjected for heating and observed for formation of blue color.

3. STARCH TEST: 2 ml of biomaterial solution was taken in a test tube and 1-2ml of iodine solution was added, noticed for the formation of blue or purple color, which disappears on heating.

SPECTRAL STUDIES OF ISOLATED BIOPOLYMER:-

- IR SPECTROSCOPY:-

Fourier transform infrared spectroscopy of the adsorbent was done by using an FT-IR spectrophotometer. Samples were grounded with potassium bromide and compressed under very high pressure (at least 25,000 p sig) to form a small pellet about 1-2mm thick and 1cm in diameter. The powder KBr and sample was introduced in a device to gets its I.R spectroscopy. Potassium bromide pellet of identical thickness was used as a blank in the path of reference beam. Spectra obtained in the range of 3500-400 cm-1were analyzed and reported.

- Ultravoilet- visible spectroscopy:-

Optical absorbance and difference absorbance spectra were recorded at room temperature by a double beam Schimadzu -1800 UV/visible spectrophotometer by using 1-cm path length cuvettes and 2-nm bandwidth. The investigated spectral range was 200–800 nm.10mg of the biomaterial was dispersed in 5ml of distilled water and sonicated for 25min, the it was subjected for U.V analysis. Absorbance spectra were collected using doubled distilled water as reference. For the acquisition of difference absorbance spectra, the cuvette containing the biomaterial solution was placed on the sample beam path, while a cuvette filled with double distilled water was on the reference path, the two solutions (sample and reference) being at the same concentration. The absorbance of the sample was recorded as a function of the wavelength.

- NMR Spectroscopy

The NMR spectra were recorded spectrometer (Jamia hamdard university) operating at 400.13MHz for proton in D2O solution. The sample under investigation (biomaterial) was taken in a glass tube which was placed between the pole faces of a magnet. A radiofrequency source (ύ = 60 mega cycles sec-1) was made to fall on the sample. It was done by feeding energy (Radio- frequency source) into a coil wound round the sample tube. A signal was detected when the nuclei in the sample resonates with the source. Energy was transferred from the source via nuclei to the detector coil. The output from the detector was fed to a cathode ray oscillograph or to a strip chart recorder after aplication etc.

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Evalation of isolated polymers:

- Study of surface morphology of isolated biopolymer:-

  Surface morphology of isolated biopolymer was determined by two methods:
- Optical Microscopy:-

  For this method ocular and stage micrometer was adjusted in the microscope for determining particle shape and size of biomaterial. Small quantity of biomaterial was applied on slide which was covered with coverslip and was observed under microscope.
- Scanning Electron Microscope :-

  A small amount of biomaterial was fixed on an alluminium studs and coated with gold using a sputter coater under vaccum(0.1 mmHg). The biomaterial was then analyzed under SEM at magnification of 1000x and picture was taken and surface morphology was compared with standards.
- The % yield of biopolymer :-

  The biopolymer were isolated from various edible natural sources and by simple economic process and the %yield was calculated after optimization process. The %yield of biopolymer was found from in the range of 23.21±0.06 to 0.4±0.06.
- Drug excipient interaction by chemical test: -

  Drug excipient interaction with various polymer formed by administration drug and polymer in equal proportion. Then the mixture was treated with various reagent and observed for color changed.
- Drug-Excepient Interaction Study:

  Drug- Polymer interaction study of biopolymer was done by TLC/UV techniques. The drug interaction study was performed by using wet and dry method.

  Wet Method: -

  Drug and polymer was mixed in the ratio 1:1, 1:3, 3:1(i.e., 1:1 ratio -10mg drug and 10mg biopolymer is used) respectively in a petri plate with a drop of water. It was then dried in oven. After drying it was scratched and dissolve 10ml methanol. It was then observed at λ max 230nm, compared and reported. Dry Method: -

  Drug and polymer was mixed in the ratio of 1:1, 1:3, 3:1(i.e., 1:1 ratio -10mg drug and 10mg biopolymer is used) respectively in a petri plate. It was then kept in oven for 20min at 55ºC. After that it was scratched and dissolved in 10ml of methanol. It was then observed at λ max 230nm, compared and reported.
- Preparation of Standard Curve of Cetirizine:

  The Standard curve of Cetirizine prepared using buffer of ph 7.2, ph 7.0, and Methanol.

  1. Preparation of stock solution:

     10 mg of accurately weigh Cetirizine was taken in a 100 ml volumetric flask and was first

     dissolved in 50 ml of distilled water, it was then subjected to sonication for complete solubility of

     drug in water, after that 50 ml distilled water was added to make a solution containing 100 µg/ml.

  Scanning of Cetirizine by UV Visible Spectroscopy in pH 7.2

  From the standard stock solution, 1 ml was transferred to 10 ml of volumetric flask. The volume

  was made upto 10 ml with pH 7.2. The resulting solution containing 10 was scanned between 200

  to 400 nm and the spectrum was obtained. The found to be 230 nm and was used as analytical

  wavelength throughout the study.

  2. Preparation of calibration curve: From the stock solution (100), appropriate aliquots of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9ml was taken into different volumetric flask and made upto 10ml with pH 7.2, so as to get the drug concentration from 0-9 The absorbance of these drug solutions were estimated at 230 nm. This procedure was performed in triplicate to validate the calibration curve. The data is given in the table and a calibration curve was constructed using the data.

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Evaluation Of The Formulated Cetirizine Bio-Nanosuspension:

The formulated Cetirizine bio-nanosuspension were evaluated for drug content, entrapment efficacy,

particle size, release studies, viscosity, stability, ph, conductivity.

1. Drug Content Uniformity:-

Drug content was determined by weighing equal amount of nanoparticle and suspended into 10 ml

of methanol, it was left over for orbital shaking for hrs. The 1ml solution was taken and diluted

with methanol up to 10 ml, the resulting solution was taken and diluted with methanol up to 10 ml,

the solution was filtered through Whastman filter paper. This solution was assayed for drug

content by U.V spectroscopy at λ max at 230nm.

2. pH studies:- In order to know the presence of relative amount of free hydrogen and hydroxyl ions, pH is measured by using pH meter.
3. Parts per trillion and Parts per million (ppt & ppm) In order to measure the mass of a chemical or contaminate per unit volume of water ppt &ppm by using Total Dissolved Solids (TDS) meter.
4. Conductivity:- In order to know the presence of charge particles & nature of nanosuspension, conductivity is measured by using Conductivity meter.
5. IN-VITRO DRUG RELEASE OF CETIRIZINE LOADED NANOSUSPENSION:-

In-vitro drug release was carried out by following apparatus:-

By Modified M.S Diffusion Apparatus(Static Method)

In-vitro drug Release of Cetirizine Loaded Nanosuspension by Modified M.S Apparatus(Static

Method):-

The In vitro drug diffusion was carried out in M.S diffusion apparatus. This was static method and

employed complete replacement of the sample. 1ml of nanosuspension was kept in the donor

compartment and the receiver compartment was filled with 10 ml of buffer. The complete sample

was withdrawn after 60 mins. And the receiver compartment was refilled with 10 ml of fresh

buffer. The samples were withdrawn at regular time intervals for 24 hours. The amount of drug

release was assessed by measuring the absorbance at 230 nm using UV spectrophotometer.

6. Stability Studies:-

Drug loading

Stability studies were conducted as per ICH guidelines. Stability testing of pharmaceutical product

is done to ensure the efficacy, safety and quality of active drug substance and dosage forms and

shelf life expiration period. The stability studies of the formulation were conducted at 40ºC±2ºC

and ±45±5%RH, 25±2ºC AND 60± 5% RH AND 2±5ºC temperature and RH values respectively.

After every 15 days, the aggregation, nature, color change, dispersability and in vitro drug release

of formulations was determined.

RESULT:

PHYSIOCHEMICAL PROPERTIES OF ISOLATED BIOMATERIAL

- The biomaterial obtained from Santalum Album(Sandalwood) was obtained in fine to lightly coarse powder that was light orangish color and odor: characteristic sandalwood aroma. The color changing point of Sandalwood was found to be 290ºC. The biopolymer confirmed the chemical test of carbohydrate, protein, KMnO4, methyl red and iodine solution. The isolated biopolymer was soluble in water, methanol.

- The biomaterial obtained from Glycin Max(Soyabean) was obtained in fine to lightly coarse powder that was ceamish in color and odorless in nature. The color

changing point of Soyabean was found to be 260ºC. The biopolymer confirmed the chemical test of carbohydrate, protein, KMnO4, methyl red and iodine solution. The isolated biopolymer was soluble in water, methanol.

- The biomaterial obtained from Vigna Radiata (Moong dal) was obtained in fine powder that was light ceamish in color and odorless in nature. The color changing point of Moong dal was found to be 270ºC. The biopolymer confirmed the chemical test of carbohydrate, protein,KMnO4, methyl red and iodine solution. The isolated biopolymer was slightly soluble in water and soluble in methanol.

- The biomaterial obtained from Phaseolus Vulgaris (Red/White Rajma) was obtained in fine powder that was light brownish in color and odorless in nature. The color changing point of red/white Rajma was found to be 190ºC. The biopolymer confirmed the chemical test of carbohydrate, protein,KMnO4, methyl red and iodine solution. The isolated biopolymer was slightly soluble in water and soluble in methanol.

- The biomaterial obtained from Arachis Hypogaea (Groundnut) was obtained in coarse powder that was ceamish in color and odorless in nature. The color changing point of Groundnut was found to be 290ºC. The biopolymer confirmed the chemical test of carbohydrate, protein, KMnO4, methyl red and iodine solution. The isolated biopolymer was slightly soluble in water and soluble in methanol.

- The biomaterial obtained from Beta Vulgaris (Beet root) was obtained in fine powder that was dark red in color and odorless in nature. The color changing point of beetroot was found to be 240ºC. The biopolymer confirmed the chemical test of carbohydrate, protein, KMnO4, methyl red and iodine solution. The isolated biopolymer was insoluble water and soluble in methanol.

- The biomaterial obtained from Daucus Carota (Carrot) was obtained in fine powder that was light red in color and odorless in nature. The color changing point of carrot was found to be 270ºC. The biopolymer confirmed the chemical test of carbohydrate, protein,KMnO4, methyl red and iodine solution. The isolated biopolymer was insoluble in water and soluble in methanol.
- The biomaterial obtained from Coriandrum Sativum (Coriander) was obtained in fine powder that was light brownish in color and odorless in nature. The color changing point of Coriander was found to be 230ºC. The biopolymer confirmed the chemical test of carbohydrate, protein,KMnO4, methyl red and iodine solution. The isolated biopolymer was slightly soluble in water and soluble in methanol.

1. **% yield of polymer:-**
   The biopolymer isolated by simplified economic procedure as mention in 5.1 and it showed 0.4±0.06 to 23.21±0.06 and details were shown in table below.

Name of Biopolymer	**Percentage Yield(%)**
Santalum Album (Sandalwood)	2.05±0.06
Glycin Max (Soyabean)	3.01±0.06
Vigna Radiata (Moong dal)	9.45±0.06
Phaseous vulgaris (White rajma)	3.44±0.06
Arachis hypogaea (Groundnut)	13.68±0.06
Phaseous vulgaris (Red rajma	23.21±0.06
Daucus Carota (Carrot)	7.68±0.06
Beta vulgaris (Beetroot)	0.15±0.06
Coriandrum Sativum (Corriander)	0.4±0.06

TABLE12 % Yield of Biopolymer

2. **Drug excipient interaction by chemical test:-**
   Drug exceipient interaction performed by chemical test using 1:1 ratio with all isolated biomaterial and results reveled. Iodine treat with drug and polymer shows

change in black color. Iodine treat with drug shows change in black color and same color change with polymer. This clearly indicate drug is nonreactive and polymer is nonreactive with drug and contain no functional group. So these polymers are selected for further formulations.

Iodine solution	**KMNO4**	**Methyl red**	**Picric acid**	**Test for carbohydrate (fehlings solution)**	**Test for protein (ninhydrin)**	**Solubility**	**Drug**
No Color change	Light color change	Light color Change	No Color change	Light color change	Light cream	No color change	**Red Rajma**
Black	Light color change	Light color change	Light yellow	Light Gray	Transparnt	Partially soluble	**Soyabean**
Light Brown	Light Pink	Light Pink	Light yellow	Light Gray	Light cream	Partially soluble	**Groundnut**
Black	Light color change	Light color change	Dark yellow	Light Gray	Light brown	Partially soluble	**White Rajma**
Black	Light color change	Light color change	Light yellow	Dark gray	Light brown	Partially soluble	**Moong dal**
Black	Light cream	Light cream	Light yellow	Light gray	Light cream	Partially soluble	**Red Sandalwood**
Light Brown	Light brown	Light brown	Light Yellow	Light gray	Transparent	Partially soluble	**Beetroot**
Light Brown	Light brown	Light brown	Light yellow	Light cream	Transparent	Partially soluble	**Carrot**

TABLE13 Drug-Excipient Interaction by Chemical Test

3. Drug-Excipient Interaction Study:

The drug excipient studies was conducted as per both methods and result show that there is no significant shift in λmax of API molecule upon preferred test which clearly indicate that the biopolymer are non reactive and no biopolymer contain any reactive group.Hence this biopolymer used for design bio nanosuspension in various proportion.

1) Wet Method

RATIO DRUG:POLYMER	1:1	1:3	3:1
λmax after 1hr	271	274	276
λmax after 2hr	273	275	278

TABLE14 Drug excipient Interaction studies by Wet Method

2) Dry Method:

RATIO DRUG:POLYMER	1:1	1:3	3:1
λmax after 1hr	262	266	269
λmax after 2hr	264	267	270

TABLE15 Drug excipient Interaction studies by Dry Method

4. Preparation of Standard Curve of Cetirizine:

The Standard curve of Cetirizine prepared using buffer of ph 7.2, ph 7.0, and Methanol.

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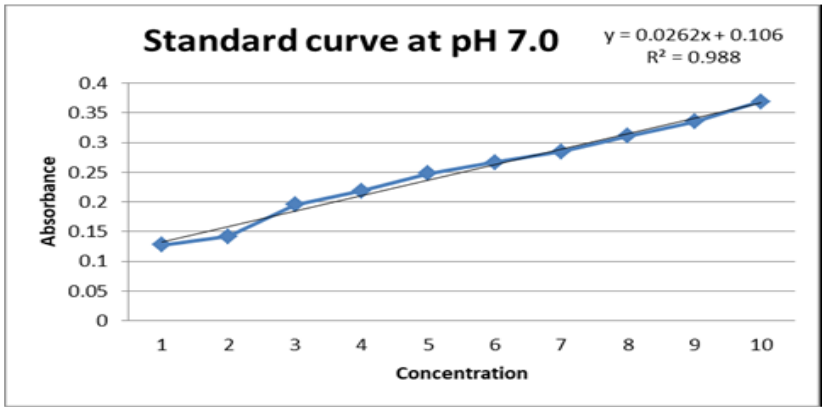

FIGURE NO 1 Standard curve of cetirizine at pH 7.0

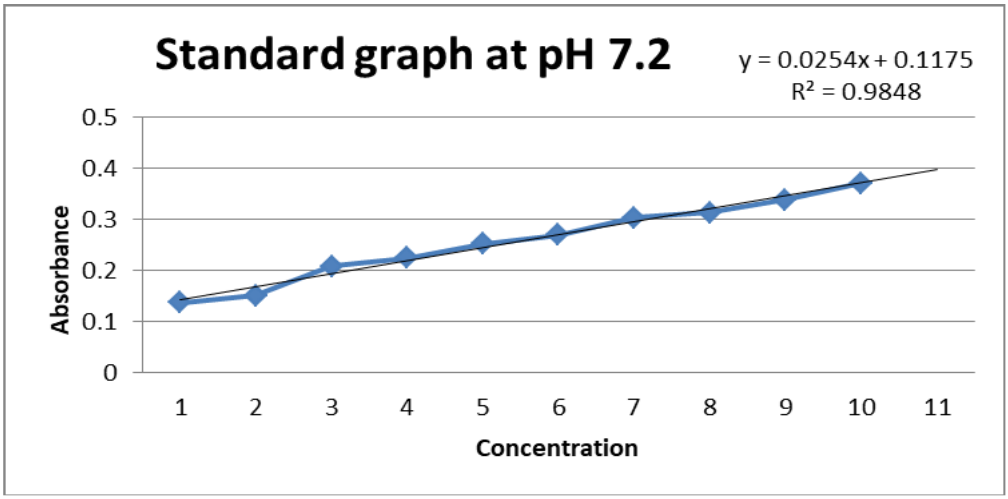

FIGURE NO 2 Standard curve of cetirizine at pH 7.2

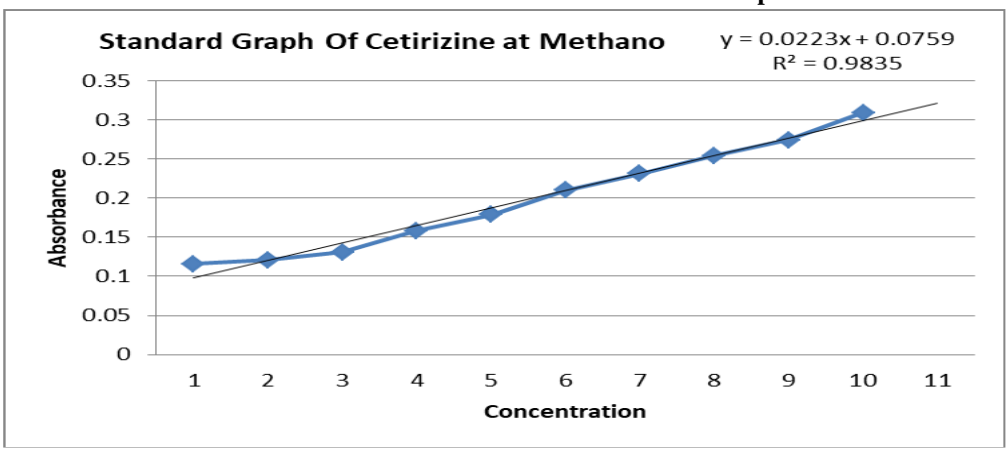

FIGURE NO 3 Standard curve of cetirizine using Methanol

EVALUATION OF FORMULATED CETIRIZINE BIO-NANOSUSPENSION

1. **PH Measurement**:

The bionanosuspension prepared from various biopolymer showed the pH in the range of 7.6 to 7.8.

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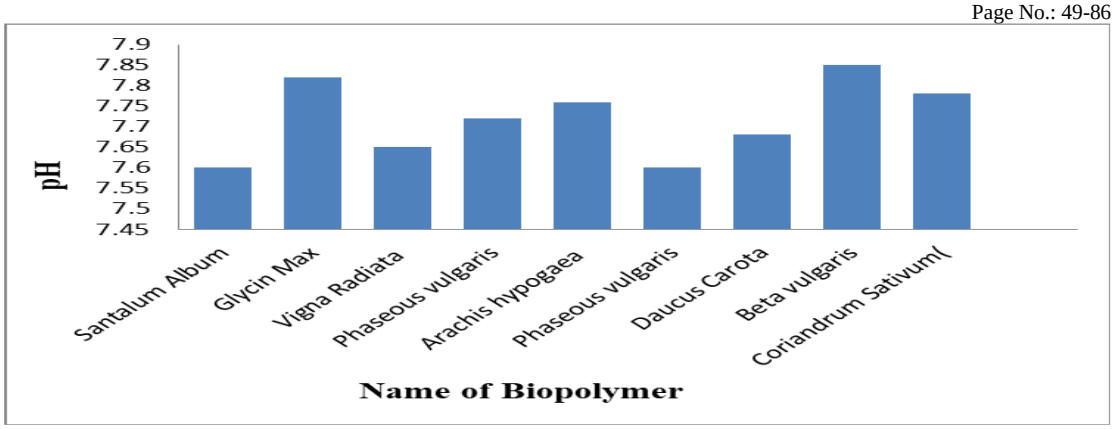

FIGURE NO 4 pH Measurement Of Different Bio nanosuspension

2. Parts per trillion and parts per million:-

The bionanosuspension prepared from various biopolymer showed the ppt and ppm solution starting from low and high and bionanosuspension showed results in form of bargraph

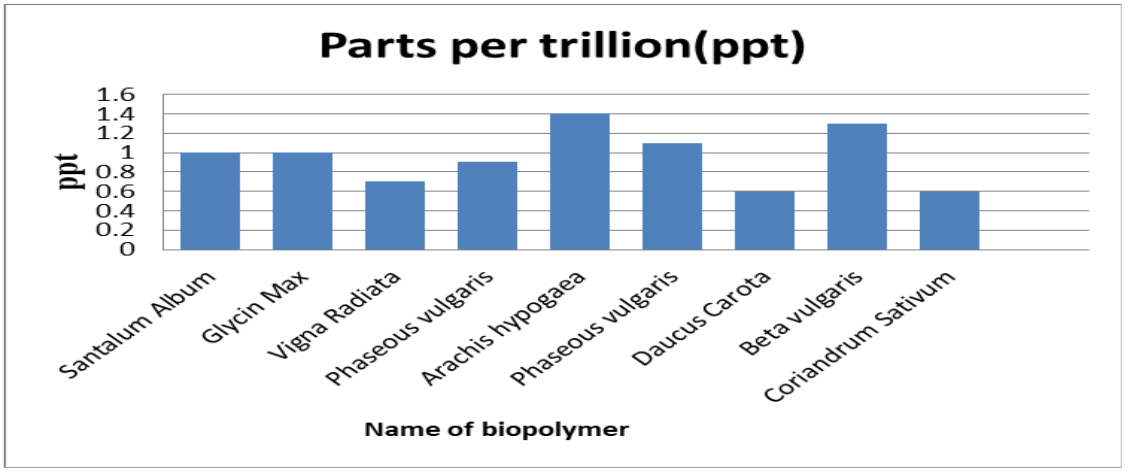

FIGURE NO 5 Parts per trillion (ppt) of different bionanosuspension

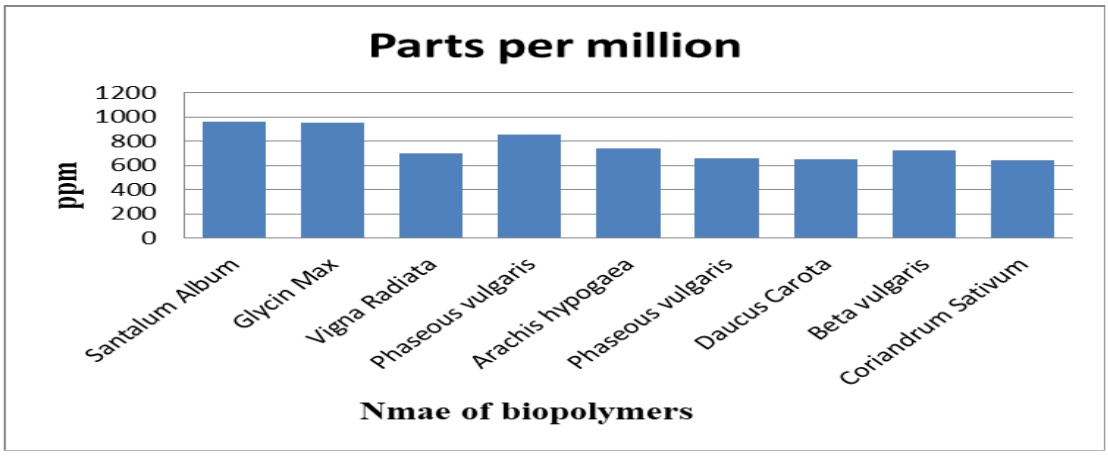

FIGURE NO 6 Parts per million(ppm) of different bionanosuspension

3) Conductivity:-

The bionanosuspension prepared from various biopolymer showed the conductivity in the range of 2.0 to 7.76.

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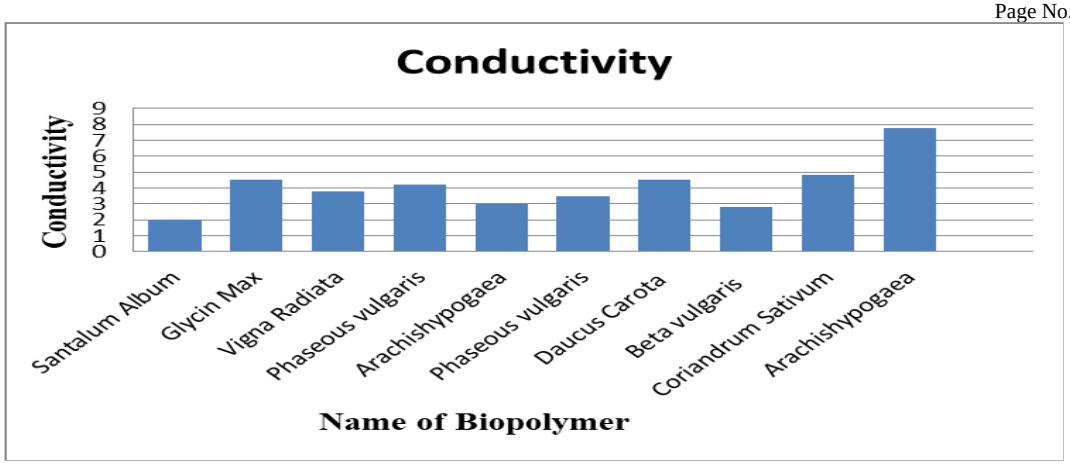

FIGURE NO 7 Conductivity of different bionanosuspension

4) IN-VITRO DRUG RELEASE OF CETIRIZINE LOADED NANOSUSPENSION:-

Cetirizine loaded nanosuspension was prepared by various biopolymers was subjected to Ex Vivo

diffusion studies for 24 hours. The % drug release was calculated and was plotted against time. From

this t50 and t80 was calculated using BITS software along with release kinetics in order to select best-

fit model and to propose release kinetic model. All the formulations were compared for t50; t80 and the

best formulations are arranged in the ascending order.

For formulation by GLYCIN MAX

The drug release pattern for GM1-GM6 bsed on T80 was found to be

GM1>GM6>GM5>GM2>GM4>GM3. In vitro drug relaease was performed for all the formulations

and the data indicate that drug loaded formulation show sustained release behavior. Graph was plotted

between Cpr and time, the R2 values and T50% and T80% was calculated from graph, the GM1

formulation was found to be the best formulation showing R2 value 0.9863. According to release

kinetics the best fit model was found to be zero order with Anomalous Transport as the mechanism of

drug release.

For formulation by VIGNA RADIATA

The drug release pattern for VR1-VR6 bsed on T80 was found to be

VR2>VR3>VR6>VR4>VR5>VR1. In vitro drug relaease was performed for all the formulations and

the data indicate that drug loaded formulation show sustained release behavior. Graph was plotted

between Cpr and time, the R2 values and T50% and T80% was calculated from graph, the VR2

formulation was found to be the best formulation showing R2 value 0.9599. According to release

kinetics the best fit model was found to be zero order with Anomalous Transport as the mechanism of

drug release.

For formulation by DAUCUS CAROTA

The drug release pattern for DC1-DC6 bsed on T80 was found to be

DC2>DC5>DC4>DC3>DC6>DC1. In vitro drug relaease was performed for all the formulations and

the data indicate that drug loaded formulation show sustained release behavior. Graph was plotted

between Cpr and time, the R2 values and T50% and T80% was calculated from graph, the DC2

formulation was found to be the best formulation showing R2 value 0.9697. According to release

kinetics the best fit model was found to be zero order with Fickian Diffusion as the mechanism of drug

release.

For formulation by PHASEOUS VULGARIS

The drug release pattern for PV1-PV6 bsed on T80 was found to be

PV5>PV4>PV2≥PV6>PV1>PV3. In vitro drug relaease was performed for all the formulations and the

data indicate that drug loaded formulation show sustained release behavior. Graph was plotted between

Cpr and time, the R2 values and T50% and T80% was calculated from graph, the PV5 formulation was

found to be the best formulation showing R2 value 0.9515. According to release kinetics the best fit

model was found to be zero order with Anomalous Transport as the mechanism of drug release.

For formulation by HPMC

The drug release pattern for FH1-FH5 bsed on T80 was found to be

FH2>FH3>PV2≥FH1>FH4>FH5. In vitro drug relaease was performed for all the formulations and the

data indicate that drug loaded formulation show sustained release behavior. Graph was plotted between

Cpr and time, the R2 values and T50% and T80% was calculated from graph, the FH2 formulation was

found to be the best formulation showing R2 value 0.9192. According to release kinetics the best fit

model was found to be Higuchi Matrix with Anomalous Transport as the mechanism of drug release.

For formulation by Sodium Alginate

The drug release pattern for FS1-FS5 bsed on T80 was found to be

FS2>FS3>FS1≥FS4>FS5. In vitro drug relaease was performed for all the formulations and the data

indicate that drug loaded formulation show sustained release behavior. Graph was plotted between Cpr

and time, the R2 values and T50% and T80% was calculated from graph, the FS2 formulation was found

to be the best formulation showing R2 value 0.9217. According to release kinetics the best fit model

was found to be Higuchi Matrix with Anomalous Transport as the mechanism of drug release.

For formulation by Guargum

The drug release pattern for FG1-FG5 bsed on T80 was found to be

FG2>FG4>FG1≥FG3>FG5. In vitro drug relaease was performed for all the formulations and the data

indicate that drug loaded formulation show sustained release behavior. Graph was plotted between Cpr

and time, the R2 values and T50% and T80%was calculated from graph, the FG2 formulation was

found to be the best formulation showing R2 value 0.9239. According to release kinetics the best fit

model was found to be Higuchi Matrix with Anomalous Transport as the mechanism of drug release.

On the basis of In vitro/ Ex Vivo drug release profile of nanosuspension loaded with Cetirizine with

different biopolymer, the best formulation ratios were found to be GM1 (1:1) for Glycin Max, VR2

(1:2) for Vigna Radiata, DC2 (1:2) for Dacus Carota , PV5 (1:5) for Phaseous Vulgaris, FH2 (1:2) for

HPMC, FS2 (1:2) for Sodium Alginate, FG2 (1:2) for Gaurgum.

FOURIER TRANSFORM INFRARED SPECTROSCOPY(FTIR)

Instrument: Bruker, Model: Alpha

Scans: 16

Scan range: 400 to 4000 cm-1

Mode: Transmittance

I. Glycin Max

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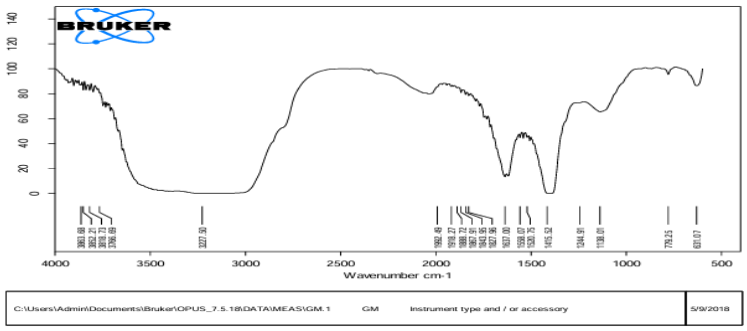

FIGURE NO 8 FTIR Of Glycin Max

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Glycin Max is supposed to have the following groups such Alkenes (3- ring), Amides (RCONH2), Aromatics (1,2,3-trisub) and Alcohols.

II. Vigna Radiata

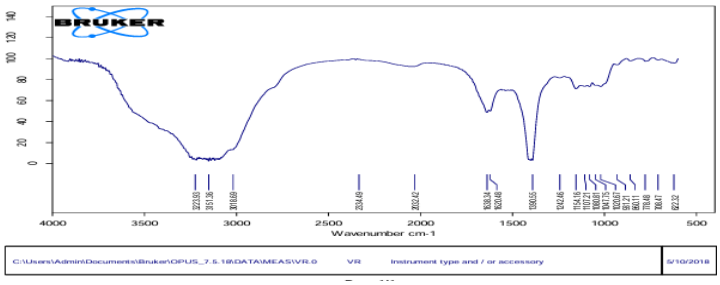

FIGURE NO 9 FTIR of Vigna Radiata

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Vigna Radiata is supposed to have the following groups such Carboxylic acid (RCO-OH), Alkenes (3- ring), Amides (RCONH2), and Aromatics (1,2,3- trisub).

III. Arachis Hypogaea

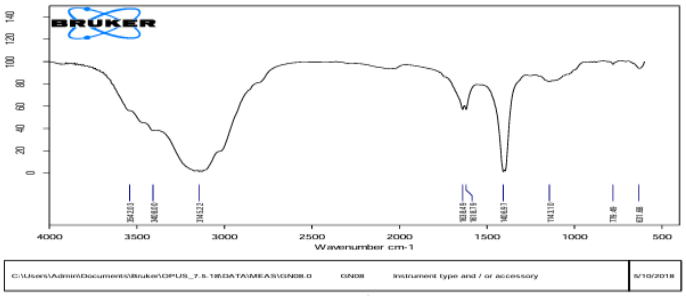

FIGURE NO 10 FTIR of Arachis Hypogaea

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Arachis Hypogaea is supposed to have the following groups such as Ethers (R-O-R), Alkenes (R2C=CH2), Amides (RCONH2), Alkyl halides (CH2X), Aromatics (1,2,3- trisub) and Alkynes**.**

IV Beta Vulgaris

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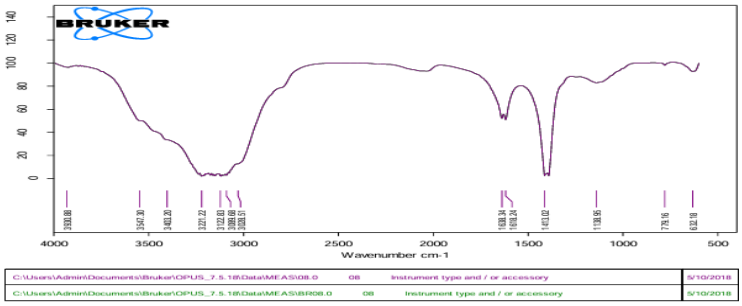


FIGURE NO 11 FTIR of Beta Vulgaris

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Beta Vulgaris is supposed to have the following groups such as Alkenes (R2C=CH2), Amides (RCONH2), Alkyl halides (CH2X), and Alkynes.

V. Phaseous Vulgaris

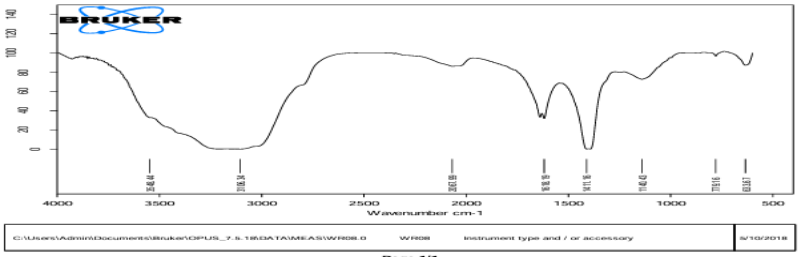


FIGURE NO 12 FTIR of Phaseous Vulgaris

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Phseous Vulgaris is supposed to have the following groups such as Esters (RCOOR), Alkenes (R2C=CH2), Amides (RCONH2), Alkyl halides (R- Br), Aromatics (1,2,3- trisub) and Amines (R2NH).

VI. Santalum Album

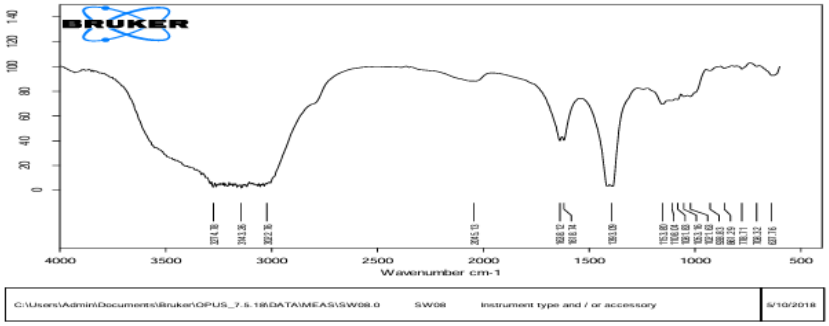
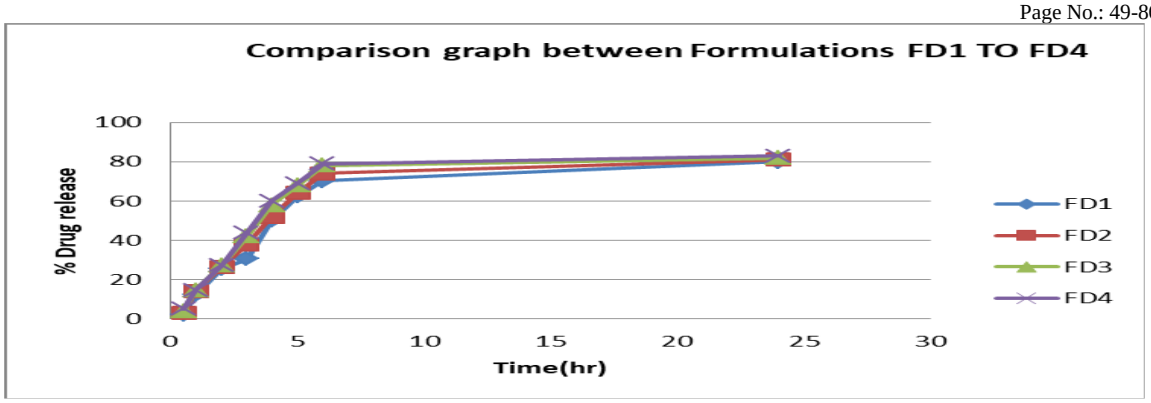


FIGURE NO 13 FTIR of Santalum Album

According to the data obtained from the IR Spectral studies, the biomaterial obtained from Santalum Album is supposed to have the following groups such as Esters (RCOOR), Alkenes (R2C=CH2), Amides (RCONH2), Alkyl halides (R- Br), Aromatics (1,2,3- trisub) and Amines (R2NH).

In-vitro drug release (MS Diffusion Cell)

- Daucus Carota



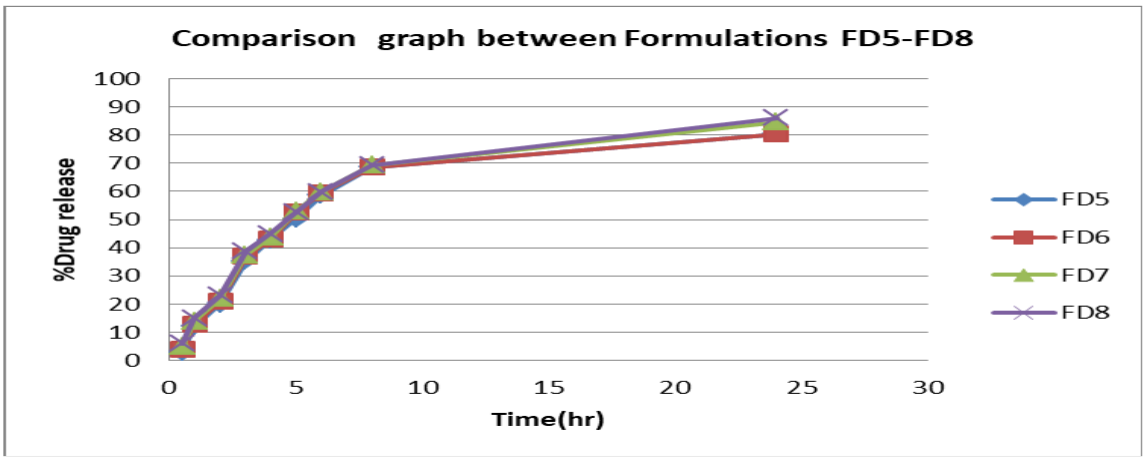
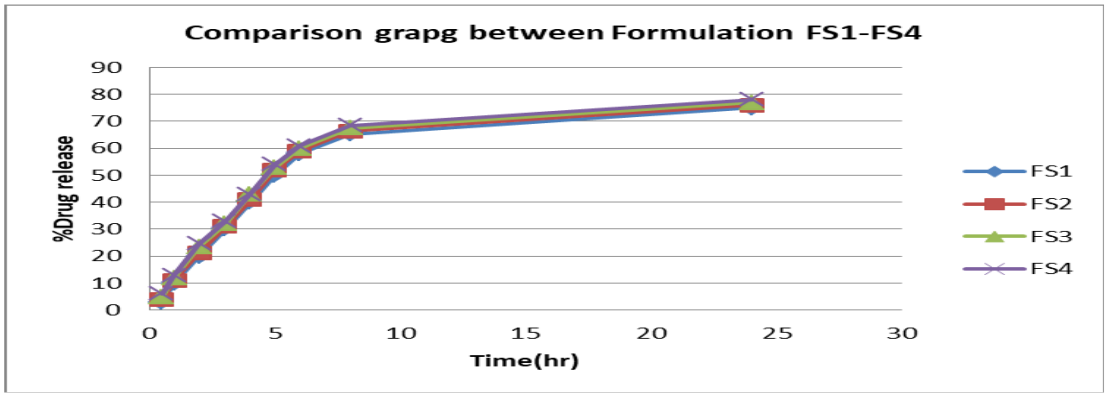


FIGURE NO 14 Invitro release of Cetirizine Bionanosuspension using Daucus Carota

- Santalum Album



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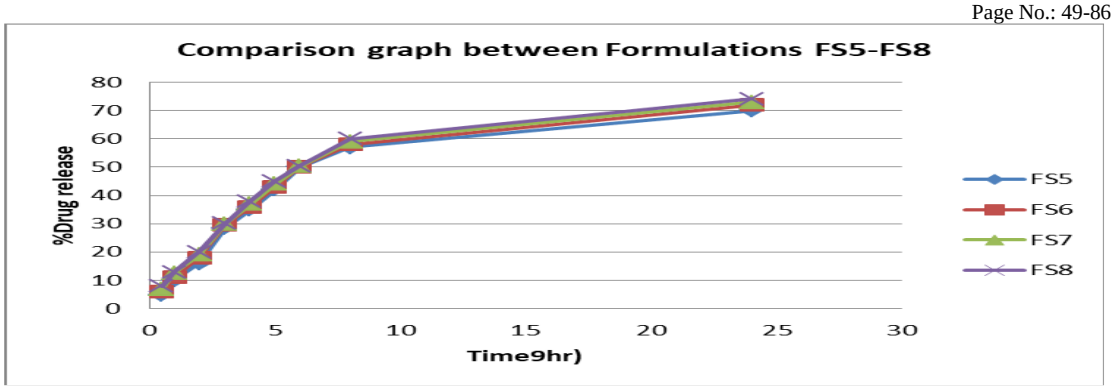
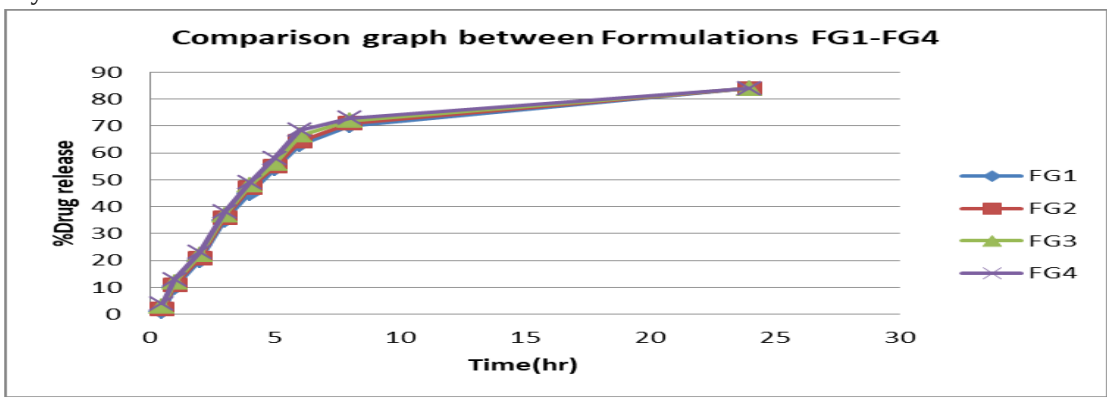


FIGURE NO 15 Invitro release of Cetirizine Bionanosuspension using Santalum Album

- Glycin Max



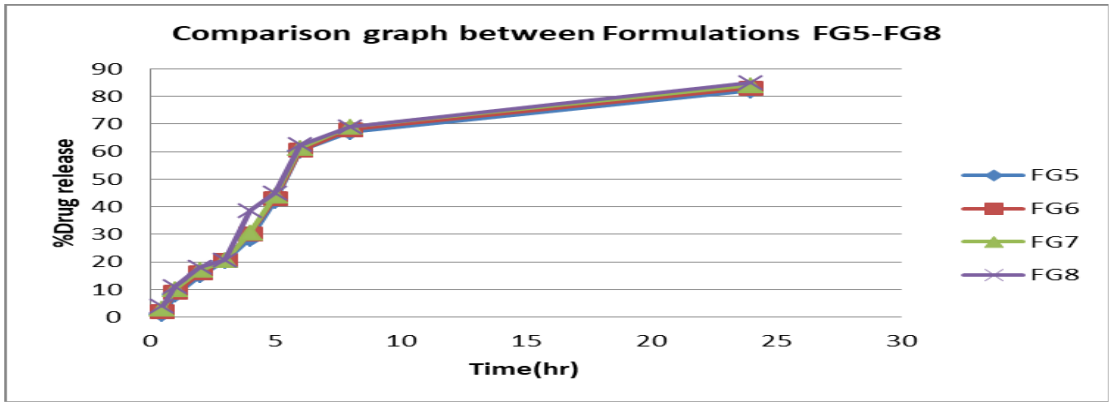
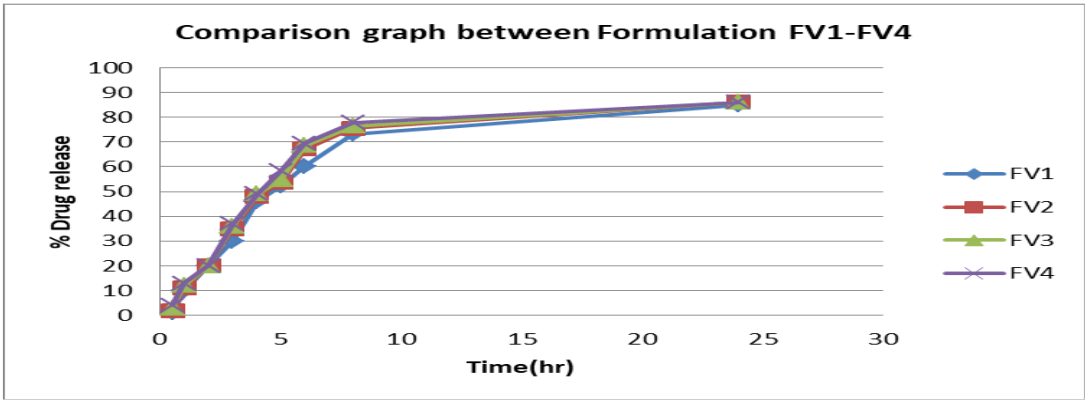


FIGURE NO 16 Invitro release of Cetirizine Bionanosuspension using Glycin Max

- Vigna Radaiata



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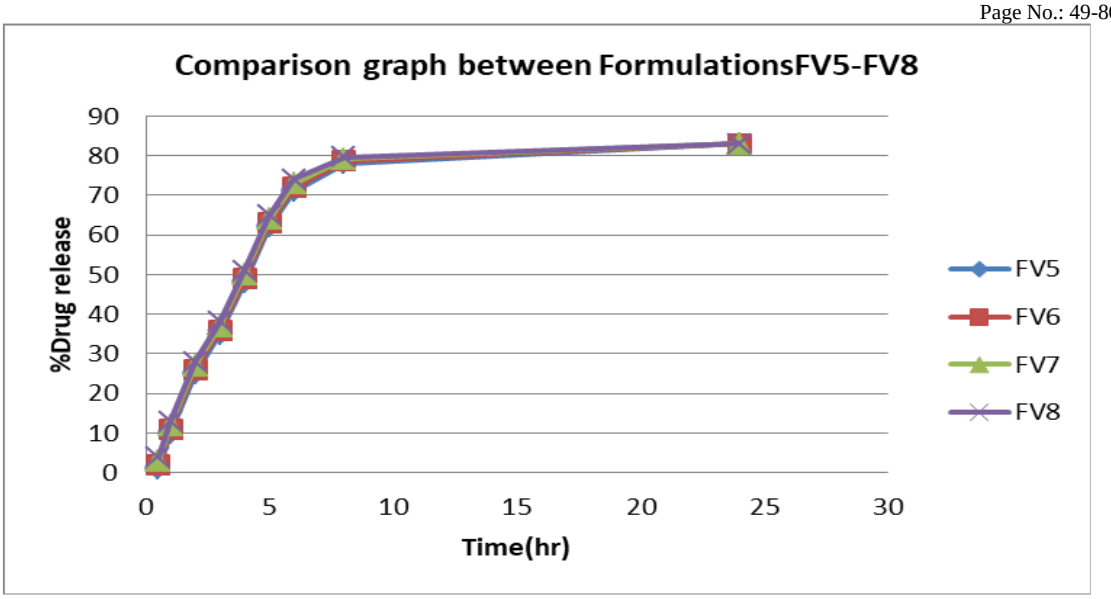

FIGURE NO 17 Invitro release of Cetirizine Bionanosuspension using Vigna Radiata

- Phaseous Vulgaris

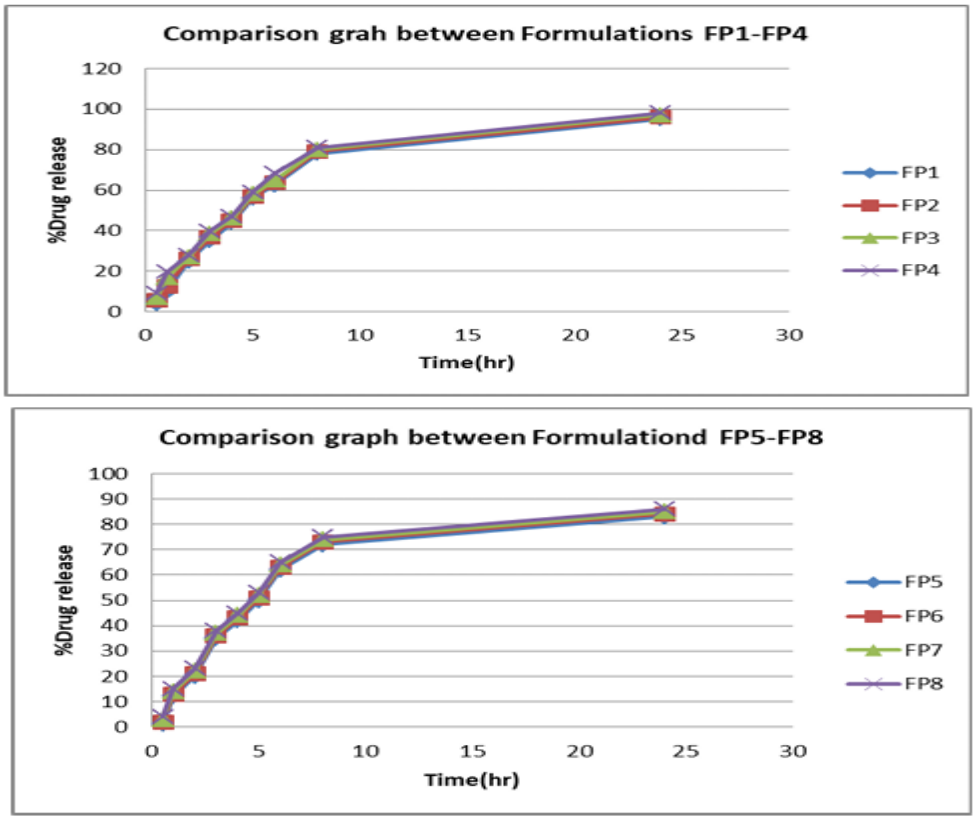

FIGURE NO 18 Invitro release of Cetirizine Bionanosuspension using Phaseous Vulgaris

- Arachis Hypogaea

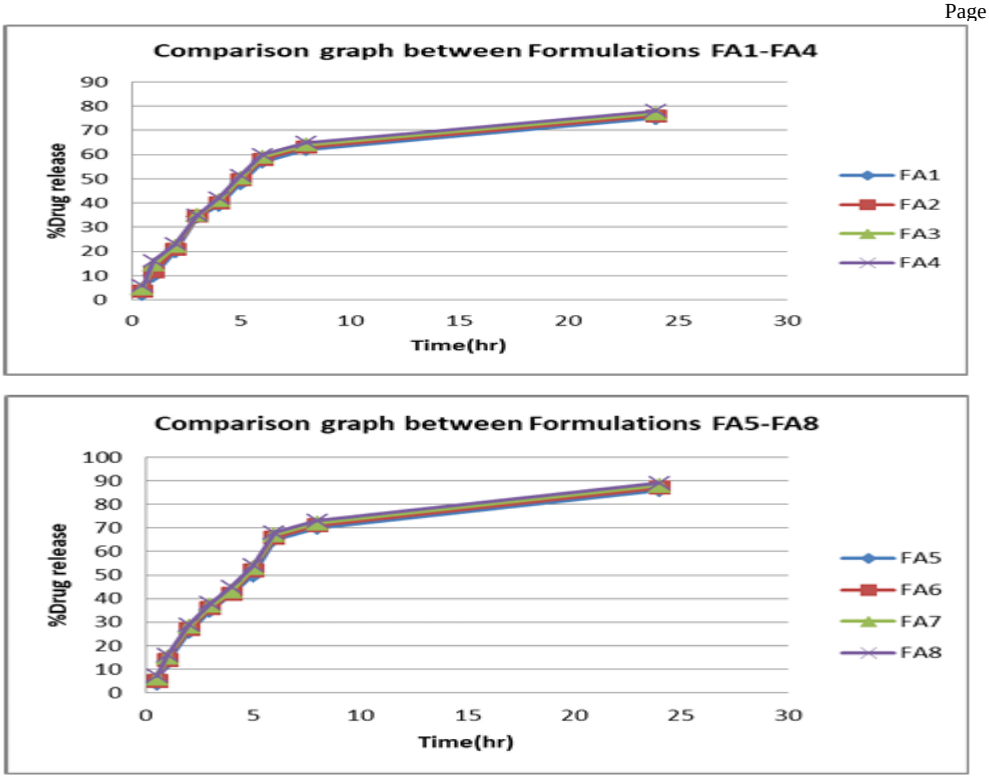

FIGURE NO 19 Invitro release of Cetirizine Bionanosuspension using Arachis Hypogaea

- Corriandrum Sativum

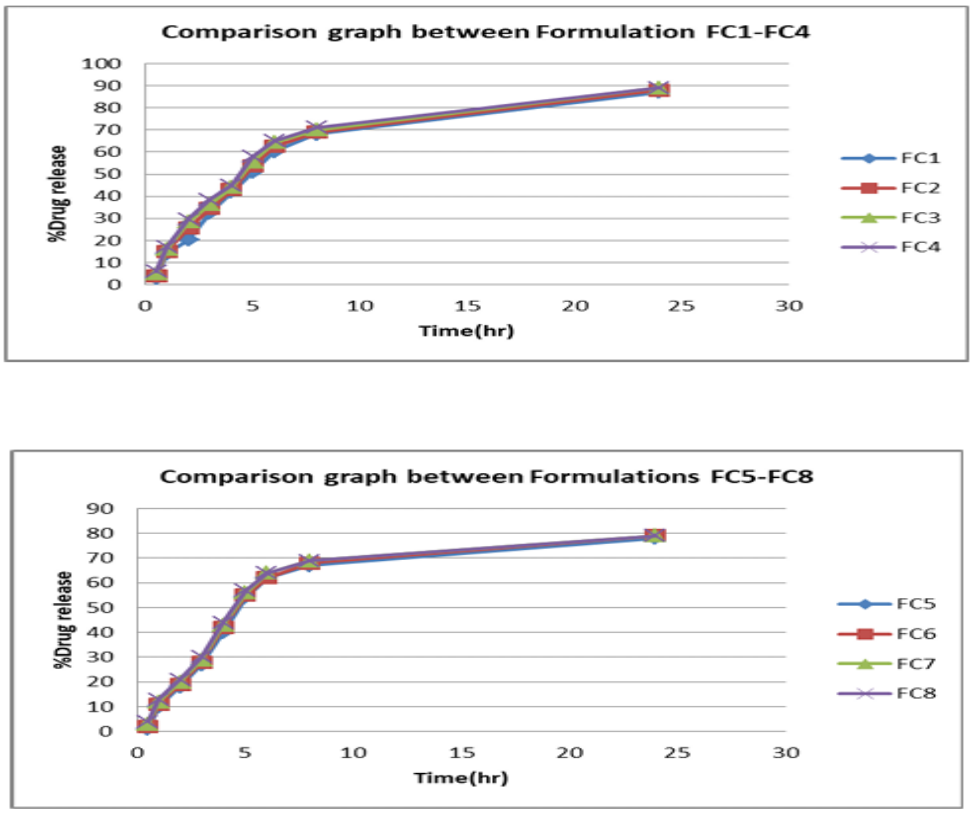

FIGURE NO 20 Invitro release of Cetirizine Bionanosuspension using Coriandrum Sativum

- Hydroxypropyl methyl cellulose (HPMC)

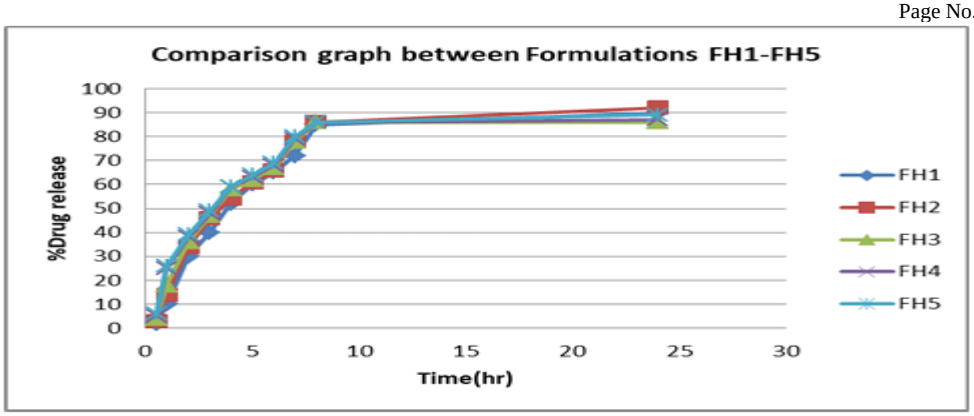

FIGURE NO 21 Invitro release of Cetirizine Bionanosuspension using HPMC

- Sodium Alginate

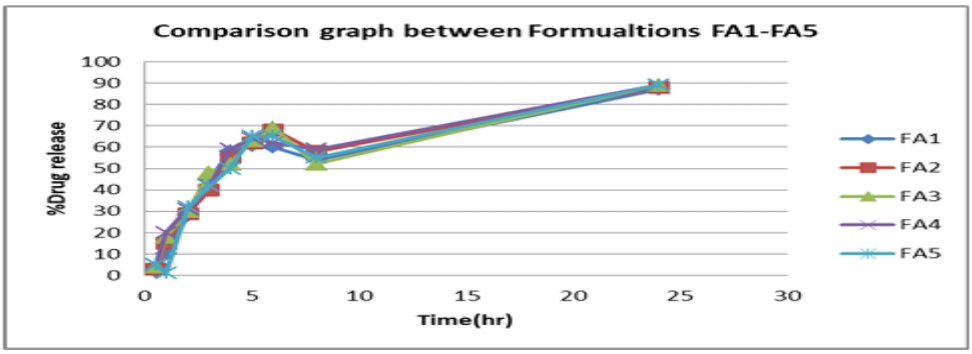

FIGURE NO 22 Invitro release of Cetirizine Bionanosuspension using Sodium Alginate

- Guargum

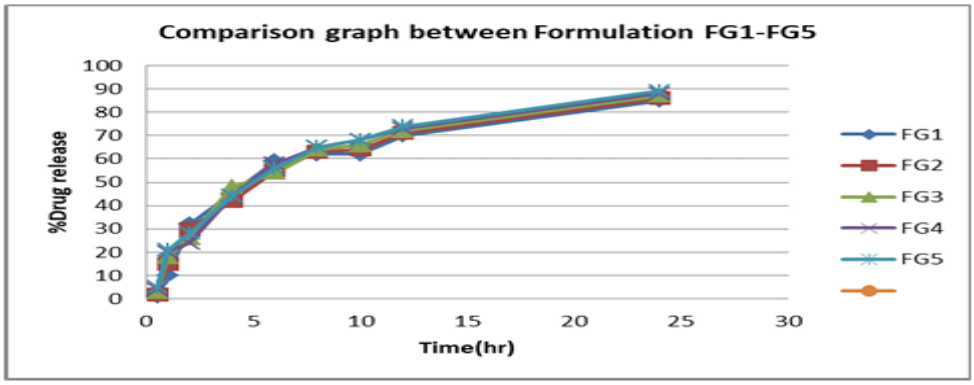

FIGURE NO 23 Invitro release of Cetirizine Bionanosuspension using Guargum

- Comparison of Best Formulation (FA2&FG4) with HPMC

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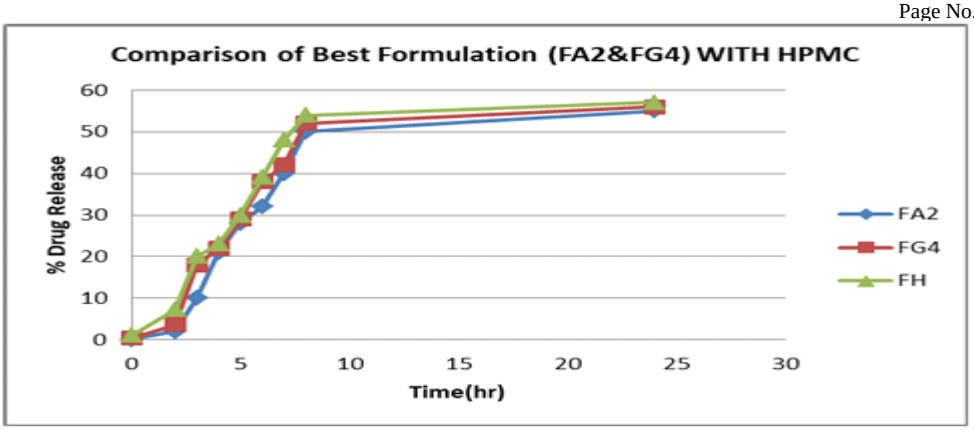

FIGURE NO 24 Invitro release of Cetirizine Bionanosuspension Between best formulation (FA2&FG4) with HPMC

- Comparison of graph between best formulation(FA2&FG4) and Sodium Alginate

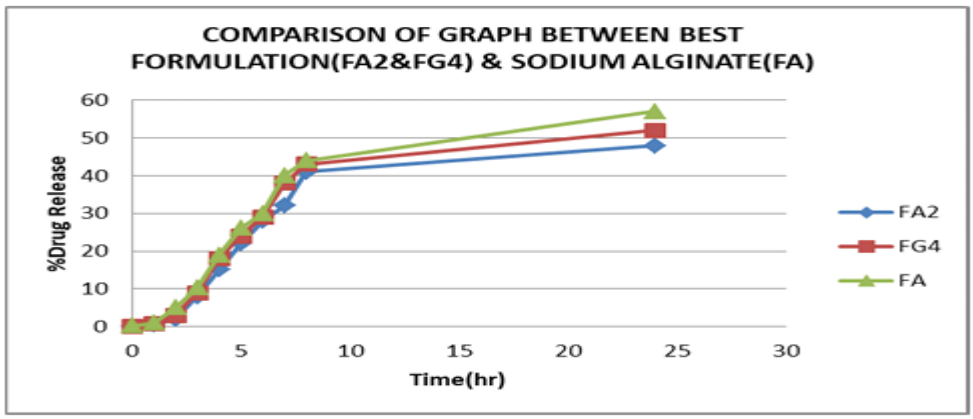

FIGURE NO 25 Invitro release of Cetirizine Bionanosuspension between best formulation (FA2&FG4) and Sodium Alginate

- T50 and T80 value of Cetrizine bio nanosuspension using Glycine max biopolymer

Formulation code	T_{50}	T_{80}
GM1	5	20
GM2	5.8	19.8
GM3	5.6	18.1
GM4	4.9	18.3
GM5	6	19.4
GM6	5.2	15.5

- T50 an0d T80 value of Cetirizine bio nanosuspension using Santalum Album biopolymer

Formulation code	T_{50}	T_{80}
SA1	4	12.3
SA2	5.1	14.4
SA3	5.3	9.3
SA4	5.8	14.6
SA5	4.8	10.8
SA6	5.2	18.5

- T50 and T80 value of Cetirizine bio nanosuspension using Vigna Radiata polymer

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Formulation code	T_{50}	T_{80}
VR1	4.1	14.3
VR2	4.5	20
VR3	4.3	17.3
VR4	5.8	21.6
VR5	4.6	19.8
VR6	5	19.5

- T50 and T80 value of Cetirizine bio nano suspension using Phaseolus Vulgaris biopolymer

Formulation code	T_{50}	T_{80}
PV1	3.9	21.3
PV2	6	23.4
PV3	5.2	19.3
PV4	4.8	22
PV5	4	20
PV6	5.1	18.2

- T50 and T80 value of Cetirizine bio nanosuspension using Arachis Hypogaea biopolymer

Formulation code	T_{50}	T_{80}
AH1	4	11.3
AH2	5.8	12
AH3	5.5	19.3
AH4	4.4	20
AH5	4.3	20
AH6	4.8	10.5

- T50 and T80 value of Cetirizine bionanosuspension using Daucus Carota biopolymer

Formulation code	T_{50}	T_{80}
DC1	4.5	15.3
DC2	5.8	12
DC3	4.8	10.9
DC4	4.6	17.2
DC5	5.5	19.2
DC6	5.2	18.5

Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
GM1	0.9988	0.9919	0.9441	0.9863	0.9946	ZERO ORDER	Anomalous Transport
GM2	0.9959	0.9883	0.9363	0.9984	0.9909	ZERO ORDER	Anomalous Transport
GM3	0.9956	0.9881	0.9363	0.9881	0.9907	ZERO ORDER	Anomalous Transport
GM4	0.9958	0.9888	0.9361	0.9880	0.9907	ZERO ORDER	Anomalous Transport
GM5	0.9964	0.9889	0.9371	0.9946	0.9917	ZERO ORDER	Anomalous Transport
GM6	0.9977	0.9901	0.9487	0.9834	0.9930	ZERO ORDER	Anomalous Transport

TABLE16 Release kinetics analysis method formulation of glycine max

Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
VR1	0.6892	0.8879	0.9211	0.9525	0.8251	ZERO ORDER	Anomalous Transport
VR2	0.8052	0.9876	0.9414	0.9599	0.9049	ZERO ORDER	Anomalous Transport
VR3	0.7776	0.9520	0.9244	0.9584	0.9050	ZERO ORDER	Anomalous Transport
VR4	0.7042	0.8883	0.9245	0.9573	0.8382	ZERO ORDER	Anomalous Transport
VR5	0.7037	0.8883	0.9228	0.9528	0.8359	ZERO ORDER	Anomalous Transport
VR6	0.7095	0.8987	0.9298	0.9586	0.8361	ZERO ORDER	Anomalous Transport

TABLE17 Release kinetics analysis method formulation of Vigna Radiata

Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
DC1	0.6859	0.8793	0.9151	0.8580	0.8174	ZERO ORDER	Anomalous Transport
DC2	0.8038	0.9728	0.9420	0.9697	0.9331	ZERO ORDER	Fickian Diffusion
DC3	0.7375	0.9069	0.9155	0.9490	0.8562	ZERO ORDER	Anomalous Transport
DC4	0.7360	0.9140	0.9176	0.9582	0.8611	ZERO ORDER	Anomalous Transport
DC5	0.7429	0.9279	0.9197	0.9694	0.8742	ZERO ORDER	Anomalous Transport
DC6	0.7142	0.8819	0.9154	0.8903	0.8290	ZERO ORDER	Anomalous Transport

TABLE18 Release kinetics analysis method formulation of Daucus Carota

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Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
PV1	0.6913	0.8785	0.9096	0.9127	0.7944	Higuchi Mtrix	Anomalous Transport
PV2	0.7306	0.9282	0.9190	0.9533	0.8641	ZERO ORDER	Anomalous Transport
PV3	0.6885	0.8218	0.9009	0.8416	0.7850	ZERO ORDER	Anomalous Transport
PV4	0.7810	0.9692	0.9192	0.9566	0.9232	Higuchi Mtrix	Anomalous Transport
PV5	0.7864	0.9274	0.9194	0.9515	0.9304	ZERO ORDER	Anomalous Transport
PV6	0.7129	0.8968	0.9022	0.9465	0.8419	ZERO ORDER	Anomalous Transport

TABLE19 Release kinetics analysis method formulation of Phaseous Vulgaris

Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
FS1	0.7384	0.9001	0.9318	0.9766	0.8494	Peppas Korsmeyer	Anomalous Transport
FS2	0.7530	0.9033	0.9217	0.8713	0.8569	Higuchi Matrix	Anomalous Transport
FS3	0.7295	0.8951	0.9252	0.9756	0.8433	Peppas Korsmeyer	Anomalous Transport
FS4	0.7296	0.9076	0.9239	0.9630	0.8528	Peppas Korsmeyer	Anomalous Transport
FS5	0.7150	0.8984	0.9206	0.9459	0.8416	Peppas Korsmeyer	Anomalous Transport

TABLE20 Release kinetics analysis method of Sodium Alginate Nanosuspension

Formulation code	R^2					BEST FIT MODEL	MECHANISM OF ACTION
	ZERO ORDER	1ST ORDER	HIGUCHI MATRIX	PEPPAS	HIXON CROWELL		
FG1	0.7304	0.8999	0.9300	0.8567	0.8469	Higuchi Matrix	Anomalous Transport
FG2	0.7425	0.9125	0.9139	0.8137	0.8605	Higuchi Matrix	Anomalous Transport
FG3	0.7719	0.9245	0.9195	0.9675	0.8795	Peppas Korsmeyer	Anomalous Transport
FG4	0.7903	0.9450	0.9198	0.9779	0.9018	Peppas Korsmeyer	Anomalous Transport
FG5	0.7272	0.9020	0.9265	0.9553	0.8475	Peppas Korsmeyer	Anomalous Transport

TABLE21 Release kinetics analysis method of Guargum Nanosuspension

5) **Stability Studies**

The best formulations GM1,VR2, DC2, PV5, FH2, FS2, FG2 were examined and showed no physical changes, related to the color, odor, taste etc. When samples were stored at 40°C and 45 ± 5% RH, at room temperature, 60°C and 60 ± 5% RH in hot air oven and 4-8°C for 3 months, a phase separation was observed. However, the phase separation disappeared with slight shaking in hand. The drug content, entrapment efficacy and in-vitro release

was found to be the same, no significant change was observed. Comparison of best formulations, GM1, VR2, DC2, PV5, FH2, FS2, FG2 showed 40% drug release within 3h of stability studies. So it can be concluded that the formulated nanoemulsion of Vitamin C is stable at different storage temperatures.

DISCUSSION:

The study on the design and evaluation of bionanosuspension loaded with cetirizine for brain targeting via the ocular route presents a novel and promising approach to enhancing drug delivery to the brain. The major challenge in ocular drug delivery lies in overcoming physiological barriers such as rapid drug elimination, limited corneal permeability, and the presence of static and dynamic ocular barriers. By leveraging nanosuspension technology, this study effectively addresses these limitations and demonstrates an innovative pathway for targeted brain drug delivery.

A key finding of this study is the role of biopolymers in stabilizing nanosuspensions and improving drug bioavailability. The selected biopolymers—Santalum album, Glycin max, Phaseolus vulgaris, Daucus carota, Coriandrum sativum, and Beta vulgaris—served as efficient stabilizing agents, ensuring nanosuspension stability while also contributing to their biocompatibility and biodegradability. These naturally derived polymers not only reduce the risk of toxicity but also offer an eco-friendly alternative to synthetic excipients.

The physicochemical characterization of the formulations, including spectral analysis (Raman, IR, NMR, DSC, UV spectroscopy) and drug-polymer interaction studies, confirmed that the nanosuspensions maintained their structural integrity while ensuring the safety and compatibility of cetirizine with the selected biopolymers. The optimized formulations exhibited a uniform particle size distribution, an essential parameter for ocular penetration and drug absorption. The nanosuspensions also demonstrated appropriate surface charge (zeta potential) values, which play a critical role in preventing particle aggregation and maintaining formulation stability over time.

One of the most significant advantages of this approach is the potential for ocular-to-brain drug transport via the optic nerve. The study highlights how this route can effectively bypass systemic circulation, reducing drug loss due to hepatic metabolism and systemic clearance. This direct delivery mechanism enhances therapeutic efficacy while minimizing potential systemic side effects, making it an attractive option for treating neurological conditions requiring targeted brain drug delivery.

The sustained drug release observed in the formulations is another noteworthy outcome. Controlled release profiles ensure prolonged therapeutic action, reducing the need for frequent administration and improving patient compliance. The in vitro drug release studies confirmed that the formulated nanosuspensions provided a gradual and sustained release of cetirizine, which is crucial for maintaining therapeutic drug levels over an extended period.

Moreover, the study provides insights into the importance of various formulation parameters such as pH, conductivity, and drug uniformity, all of which were optimized to ensure the effectiveness and stability of the nanosuspensions. The successful formulation and evaluation of these nanosuspensions indicate their potential applicability in treating neurological disorders, particularly those requiring sustained and localized drug action within the brain.

Despite the promising results, further research is required to validate the in vivo performance of these nanosuspensions. Future investigations should focus on in vivo pharmacokinetic and pharmacodynamic studies to confirm the efficacy and safety of the formulations. Additionally, exploring alternative biopolymers, optimizing formulation techniques, and developing patient-friendly ocular dosage forms such as eye drops or ocular inserts could further enhance the practical applicability of this drug delivery approach.

CONCLUSION:

In the present research work, the potential of Cetirizine loaded nano suspension for ocular delivery is investigated. Biopolymer is used to prepare nano suspension because of its biodegradability, biocompatibility, non-toxicity, non-irritant in nature and no reaction on ocular surface. The isolation procedure was carried out by economical method. The optimization was done by repeating the same procedure 3 times and %yield was calculated everytime to calculate the average %yield. The %yield of biopolymer Santalum album, Glycin mx, Vigna radiate, Phaseous

vulgaris, Arachis hypogaea, Daucus carota, Beta vulgaris, Coriandrum sativum found to be 2.05±0.06, 3.01±0.06., 9.45±0.06, 3.44±0.06, 13.68±0.06, 23.21±0.06, 7.68±0.06, 0.15±0.06, 0.04±0.06. Isolated biopolymer was screening for various phsio-chemical properties. The IR was performed to determine the functional group present in the biopolymer, NMR was done to determine the number and environment of the proton in the structure, SEM analysis was done to determine surface morphology of the biopolymer while particle size was used to determine using UV spectroscopy. The chemical test was performed for presence of carbohydrate and proteins. The standard curve of model drug Cetirizine were prepared in different medium like ph 6.9, ph 7.0, ph7.2, methanol to determine slope and R2 value. The drug-exceipient interactions studies were performed by both UV nd TLC method, using dry and wet samples and no significant interaction was found between drug and biopolymer. Nanosuspension ws prepared by using solvent evaporation technique which is the easiest and reproducible method to prepare nanoparticle without need of any sophisticated instruments. Drug to polymer ratio was choosen different levels Santalum album polymer FS1(1:0.5), FS2(1:1), FS3(1:2), FS4(1:3), FS5(1:4), FS6(1:5), FS7(1:6), FS8(1:7), FS9(1:8), FS10(1:10) and ten level for Glycin max polymer FG1(1:0.5), FG2(1:1), FG3(1:2), FG4(1:3), FG5(1:4), FG6(1:5), FG7(1:6), FG8(1:7), FG9(1:8), FG10(1:10), and ten level for Vigna raidiata polymer FS1(1:0.5), FS2(1:1), FS3(1:2), FS4(1:3), FS5(1:4), FS6(1:5), FS7(1:6), FS8(1:7), FS9(1:8), FS10(1:10) and ten level for Phaseous vulgaris polymer FP1(1:0.5), FP2(1:1), FP3(1:2), FP4(1:3), FP5(1:4), FP6(1:5), FP7(1:6), FP8(1:7), FP9(1:8), FP10(1:10) and ten level for Daucus carota polymer FD1(1:0.5), FD2(1:1), FD3(1:2), FD4(1:3), FD5(1:4), FD6(1:5), FD7(1:6), FD8(1:7), FD9(1:8), FD10(1:10) and ten level for Beta vulgaris polymer FB1(1:0.5), FB2(1:1), FB3(1:2), FB4(1:3), FB5(1:4), FB6(1:5), FB7(1:6), FB8(1:7), FB9(1:8), FB10(1:10) and ten level for Coriandrum sativum polymer FC1(1:0.5), FC2(1:1), FC3(1:2), FC4(1:3), FC5(1:4), FC6(1:5), FC7(1:6), FC8(1:7), FC9(1:8), FC10(1:10) by weight.ph was found in the range of 7 to 7.5 which is in the range of physiological ph, so prepared formulations suitable for ocular formulations.. Bioavailability of formulation VR1 showed maximum plasma concentration. Short term stability studies reveled stable nanosuspension with no significant change in physical appearance, drug content uniformity, ph, % entrapment efficacy and dispersability, so prepared nanosuspension was found stable. On the basis of In vitro/ Ex Vivo drug release profile of nanosuspension loaded with Cetirizine with different biopolymer, the best formulation ratios were found to be GM1 (1:1) for Glycin Max, VR2 (1:2) for Vigna Radiata, DC2 (1:2) for Dacus Carota , PV5 (1:5) for Phaseous Vulgaris, FH2 (1:2) for HPMC, FS2 (1:2) for Sodium Alginate, FG2 (1:2) for Gaurgum. The research work was focused to exploit a novelistic route for brain specifically via ocular route. As the ocular route is enriched of nerve supply like optic nerve, trigeminal nerve, which are directly connected to brain. When a active pharmaceutical ingredient suitable formulated and placed in ocular cavity, significant amount of drug which is reached to the brain via a neural pathway using optic, trigeminal neural pathway, simultaneously drug will also reach through the nerve supply. This novelistic work was scientifically proven by suitably nanosized active pharmaceutical ingredient and in pharmacological action, significant action was produce. Efficient efforts are made in this research work for approach this novel route by formulated nanosuspension loaded Cetirizine for meningitis likewise to reduce its effects. the brain via a neural pathway and retained for prolonged period of time. So concluded that formulation of Cetirizine loaded nanosuspension could be utilized as potential delivery system for brain targeting.

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