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FORMULATION AND EVALUATION OF POLYHERBAL *IN-SITU* GEL FOR GLAUCOMA

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Keywords:

Glaucoma, Intraocular pressure, *Camellia sinensis*, *Areca catechu*, Herbal drug, Rabbit eye

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ABSTRACT: Worldwide, glaucoma is the second most important cause of blindness and irreversible vision loss. Raised IOP (Intraocular Pressure) is not always a mark of glaucoma; it is a key risk factor and a cause of glaucomatous optic neuropathy. Herbal preparations have various active and inactive compounds which are not standardized likewise, *Camellia sinensis* and Areca catechu having various active constituents such as polyphenolic compounds, epigallocatechin-3-gallate, quercetin, *etc* which are having few therapeutic responses that are not yet reported. Aim is to study the pharmacological responses of drugs for Glaucoma. Drugs were authenticated by botanist of SUK. *Camellia sinensis* was extracted with ethanol, and Areca catechu was extracted using hydroalcohol by Soxhlet method. The extracted powder was analyzed by UV, FTIR, and phytochemical screening were carried out. Phytochemical test confirms the presence of phytoconstituents. The extract was administered by formulating an ocular film using polymers such as chitosan and HPMC. The film was evaluated for folding endurance, thickness, drug content uniformity, pH, eye irritancy test, sterility and In vitro drug release. The Animal experimentation protocol in prescribed proforma Form B was approved by IAEC BVCP Kolhapur. Toxicity study was performed like eye irritation test, sensitivity test and Draize test on Rabbit eye as per OECD guidelines. Glaucoma was induced by dexamethasone 0.5% eye drop, effect of this drug was observed, then the increased IOP was measured by the tonometer after that extract was administered and significant IOP of 18.5% was observed. Hence the proposed work may be extended for further clinical trials.

INTRODUCTION: Globally, glaucoma is the second-leading cause of blindness and irreversible vision loss. Elevated IOP (Intraocular Pressure) is not always a sign of glaucoma; it is a major risk factor and a cause of glaucomatous optic neuropathy. The IOP was significantly decreased by combining prostaglandins with timolol in fixed combinations. Since, 2011, more than 1200 original articles and 130 reviews have been published addressing latanoprost's clinical efficacy in managing glaucoma.

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Glaucoma is the foremost reason of blindness in the United States and other industrialized nations. Glaucoma affects more than 2 million people in the United States, with 80,000 of them legally blind. By 2025, over 5 million Americans will have glaucoma. Glaucoma is expected to become the second most common disease in India as the population becomes grayer. Almost 11 million Indians are estimated to have glaucoma.

Open-angle glaucoma and closed-angle glaucoma are two types of glaucoma. Open-angle glaucoma occurs when the drainage angle formed by the iris and cornea remains open. However, other parts of the drainage system do not drain properly. This may result in a slow, gradual increase in eye pressure. Glaucoma with closed angle occurs when the eye fluid cannot escape suddenly.

This causes a rapid and severe increase in eye pressure. Dilating eye drops and certain medications can cause an acute glaucoma attack. Two routes are available for drug absorption in the eye: 1) Corneal and 2) noncorneal. Topically applied drugs are absorbed through the cornea. Passive permeability in the cornea is influenced by drug properties such as lipophilicity, molecular weight, charge, and degree of ionization. Conventional drugs administered *via* the cornea do not reach adequate concentrations in the vitreous and retina. The non-corneal route avoids the cornea by using the conjunctiva and sclera.

This route is important, predominantly for large, hydrophilic molecules like peptides, proteins, and siRNA. There are some synthetic drugs used in the treatment of glaucoma such as Latanoprost, Timolol, Brimonidine, Travoprost, Bimaprost, Pilocarpine *etc*. which can decrease aqueous production in the ciliary body or enhance aqueous humor outflow through the trabecular meshwork or uveoscleral pathway but on long term use these drugs cause toxic effects like bradycardia, tachyphylaxis, blurred vision, pigmentation, *etc*., apart from their high rate of treatment. Some plants have been reported in the earliest literature for ocular use but there are no technical data on them. Few herbal formulations are available in the market for the treatment of glaucoma. Herbal drugs such as *Camellia sinensis* and *Areca catechu* have been essential elements of therapeutic systems for ocular ailments in many ancient civilizations.

Camellia sinensis is considered to be the least processed type of tea and therefore contains the maximum antioxidants and valuable polyphenols. One of the main groups of polyphenols found in green tea is catechins, of which epigallocatechin gallate (EGCG) is the most abundant (about 50% of all catechins) and the most active. Recently, experiments have shown that catechins found in *Camellia sinensis* can penetrate the tissue of the rabbit eye. The *Areca catechu* contains various chief phytoconstituents including polyphenolic compounds (Catechin, epicatechin, leucocyanidin), fats, fiber, protein, polysaccharides, alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, *etc*. In current data found that the plant possesses alkaloids (arecoline) which are effective and responsible for the miotic effect of the plant.

It is useful for the preparation of eye drops, and eye lotion. *In-situ* gelling system was developed first time in 1980s. In situ gel system is a liquid aqueous solution before administration and change to gel under physiological conditions. In this system, the formulation is in solution form and converted to gel when it contacts with an eye. In this system gel forms due to cross-linking of polymer chains resulting formation of a covalent bond or noncovalent bond. In situ gel formation rate is important because a solution or weak gel is produced by the fluid mechanism.

Review of Literature: Gasiunas & Galgauskas, (2022) [19] studied that People, who have elevated IOP or risk factors for glaucoma development, could help from drinking green tea or its concentrated extracts in reasonable doses. Considering that catechins can enter the tissue of human eye in a similar way as the tissue of the rat eye, it can be speculated that catechins could have beneficial antioxidant effects in the human eye as well.

Ansari *et al.*, (2021) [20] developed summary of many specific characteristics of areca nut and their therapeutic effect of phytochemicals on numerous disease conditions. Biochemical compounds of areca-nut have been currently documented as functionally active molecules, possessing antioxidant, hypoglycemic activity, antifungal, antimigraine, anti-allergic, and additional beneficial properties, as well as they exert protective effects against cardiovascular and other diseases.

Ahmad, (2020) [16] studied about the Prevalence and Associated Risk Factors of Glaucoma in India. With increasing life expectancy, the prevalence of glaucoma is expected to rise. The study is to find the prevalence of glaucoma among the middle-aged and elderly population and to find out its associated factors.

Conlon *et al.*, (2017) [14] described about Glaucoma is one of the most common causes of blindness worldwide, and its prevalence is increasing. The aim of the present review is to describe the current medical and surgical treatment trends in the management of open-angle glaucoma. There has been an increase in the availability of glaucoma medications and the use of laser trabeculoplasty

over the past decade, with a subsequent decrease in invasive incisional surgery. Weinreb *et al.,* (2014) [17] studied that Glaucoma can be classified into 2 wide categories: open-angle glaucoma and angle-closure glaucoma. In the United States, more than 80% of cases are open-angle glaucoma; however, angle-closure glaucoma is responsible for anunequal number of patients with severe vision loss. Both open-angle and angle-closure glaucoma can be primary diseases. Secondary glaucoma can result from shock, certain medications such as corticosteroids, inflammation, tumor, or conditions such as pigment dispersion or pseudo-exfoliation.

Zarikar *et. al.,* (2013) studied that the sol-gel transition arises as a result of a chemical/physical change tempted by the physiological situation. This type of gel combines the advantage of a solution (accurate and reproducible administration of the drug) and gels (prolong residence time) for improving ocular bioavailability.

Objectives:

- Extraction and formulation of the *Camellia sinensis* and Areca catechu *in-situ* gel
- Evaluation of *Camellia sinensis* and Areca catechu *in-situ* gel
- Preclinical study of *in-situ* gel on Albino Rabbit

Need of Work: There are various formulations available in the market for the treatment of glaucoma as well as other eye diseases but are having certain limitations such as having poor bioavailability and permeability, the very short time the solution stays at the eye surface, instability of the dissolved drug as well as the necessity of using preservatives. Conventional ophthalmic solutions rapidly eliminate after administration. The use of viscous formulations such as gel and ointments has been widely used to increase the retention time of the drugs on the ocular surface by limiting drug elimination via nasal lacrimal drainage. The main objective of the formulation is to achieve an effective drug concentration at the site of administration for a longer period of time and without loss of formulation. In situ gelling system rises the viscosity by changing the pH or temperature due to the presence of certain electrolytes in the tear film and leads to an enhance in drug bioavailability by retardation of drainage. For the formulation of In situ gel different polymers are used such as Pluronic F 127, Carbopol, Xanthan gum, Poloxamer, *etc*. There are various marketed formulations for glaucoma such as eye drops, eye ointments, nanotechnology-based formulations, *etc* which contains synthetic drug leading to various side effects like redness, irritations, blurred vision, watery or dry eyes, burning or itching eyes.

To avoid such side effects herbal drugs are used. *Camellia sinensis* and *Areca catechu* are aimed at healing and tend to be much gentler in effect. Both drugs have less toxicity or fewer side effects in contrast to synthetic drugs. The ultimate norm for any medication is non-toxicity, effectiveness, specificity, and potency. Due to the use of herbal drugs, it is a cost-effective formulation. The producers of herbal drugs do not have to follow regulated procedures and batches.

MATERIAL AND METHOD:

Procurement of Materials: *Camellia sinensis* and Areca catechu was procured from local market of Kolhapur, Pluronic F127 was procured from BASF, Mumbai, India, Hydroxy propyl methyl cellulose was procured from Loba Chem PVT. LTD., Mumbai, Benzalkonium chloride was procured from Finar chemicals Ltd., Mumbai 400008, India, Sodium chloride was procured from Himedia Lab PVT. LTD. Mumbai.

Year of experimentation: 2022-2023

Method:

Plant Collection and Authentication: The *Camellia sinensis* plant was collected from local market of Kolhapur and *Areca catechu* plant was collected from nursery, Kolhapur and both plants were authenticated from department of botany, New college, Kolhapur.

Extraction: Dried leaves of *Camellia sinensis* were collected from the local market and the ethanolic extraction were carried out by maceration method. The seeds of *Areca catechu* were also procured from the local market and the aqueous ethanolic extraction was carried out by maceration method. The solvent was evaporated using Rotary evaporator and dried powdered extract was obtained.

Preliminary Phytochemical Screening: Phytochemical tests of extract for alkaloid, glycoside, saponin, polyphenolic compounds and tannin etc were performed as per official monographs.

Melting Point: The melting point of dried extract was determined by capillary method.

Molecular Docking: Molecular docking was performed to check the Binding affinity of drug with protein (PDB ID: 1I90) with the help of PyRx software at B.V. C. P. Kolhapur.

PXRD: Powder XRD of the extracted powder was done to observe the crystallinity of the drug i.e drug is either crystalline or amorphous (2ɵ = 1.54060).

Solubility Study: Solubility of extracted powder of both the drugs was determined in five different solvents like ethanol, methanol, distilled water, acetone, and chloroform. Take 10ml of volumetric flasks and dissolve 1 mg drug in 10 ml solvent. Put it in orbital shaker for 24 hrs after 24 hrs centrifugation was done for 15 min at 1000 rpm. Undissolved Particles were settle at the bottom and the supernatant fluid was analyzed by UV spectrophotometer.

Spectrophotometric Analysis of *Camellia sinensis* and *Areca catechu:*

Preparation of Standard Drug Solution: Standard stock solution containing extracts of *Camellia sinensis* and *Areca catechu* was prepared by dissolving 10 mg of extract in 100 ml of respective solvent to get stock solution.

Preparation of Working Standard Solutions: The standard stock solution of *Camellia sinensis* and *Areca catechu* extract was used for the preparation of working standard solutions. From this stock solution 1, 2, 3, 4 and 5ml aliquots were withdrawn and diluted with solvent to 10 ml.

The absorbance of this solution was measured using UV spectrophotometer (SHIMADZU) 81220 against solvent (ethanol) as blank solution at specific 279 nm and 280 nm respectively.

Preparation of Calibration Curve: The above five working standard solutions for each extract were scanned at the selected wavelength and the calibration curves were constructed. The calibration curve for *Camellia sinensis* and *Areca catechu* was plotted by taking absorbance at 279 nm and 280 nm. By using quantitative modes of instrument slope, intercept and correlation coefficient values for calibration curve were obtained for each extract.

Fourier - Transform Infrared Spectroscopy (FTIR) of *Camellia sinensis* and *Areca catechu*: FTIR was used to determined the functional groups by Bruker alpha 100508 instrument and compared with each other to ascertain the presence of possible functional groups and hence active constituents present in different solvent extracts. About 2mg of each of the extract was ground thoroughly with KBr; uniformly mixed sample kept in sample holder and spectra was recorded over the wave number 400-4000 cm^{-1} on spectrophotometer.

Utilization of DoE: For the optimization of Ocular In situ gel CCD was for the evaluation of the effect of the two independent variables on primary and secondary responses using Design-Expert® software. In this design, 2 factors were studied each at three levels and a number of experimental runs was find using the formula $N=2^k + 2k + Cp$. Here, for 2 factor designs, a total of 10 experiments were run which involves 4 axial, 4 factorial, and 2 replicates of central points.

Amount of Pluronic F-27 and amount of HPMC K4m were choose as independent variables (Table No. 1) pH, Viscosity, and Gelation temperature were considered as dependent variables to evaluate the responses. An optimized batch was provided by the software to evaluate the desirability.

Independent Variables: Low (-1), Middle (0), High (+1)

TABLE 1: INDEPENDENT VARIABLES

X1= Amount of Pluronic F-127 (gm)	3	4	5
X2= Amount of HPMC K 4m (mg)	200	300	400

Dependent Variables:

1. pH
2. Viscosity
3. Gelation temperature

Method of Preparation:

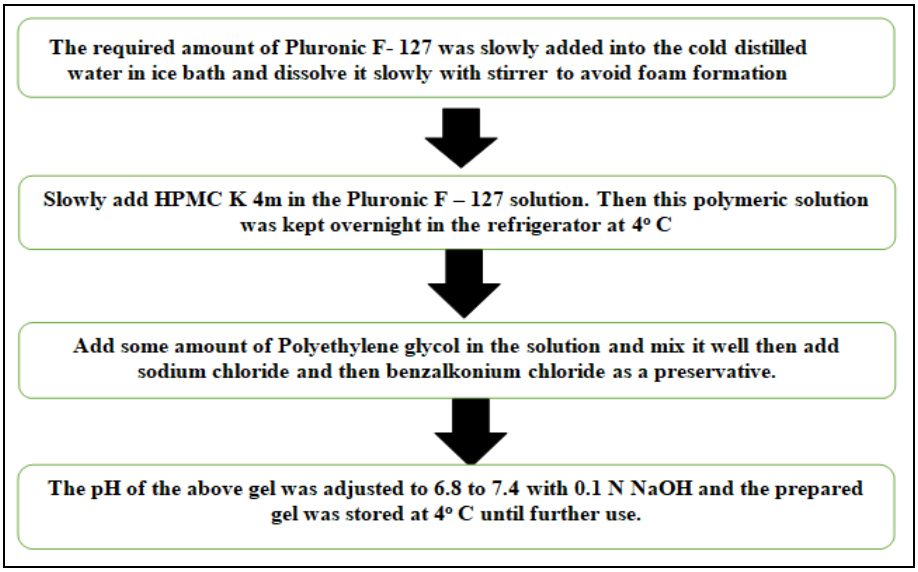

FIG. 1: METHOD OF PREPARATION

Evaluation of Gel:

Appearance: Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

pH: The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 6.8 to 7.4. The developed formulations were evaluated for pH by using digital pH meter.

***In-vitro* Gelation Study:** *In-vitro* gelation study The gelling capacity was determined by placing a 2 ml of the formulation in a vial containing 2 ml of artificial tear fluid and equilibrated at 34ºC and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to redissolve.

The composition of artificial tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 0.008 g, purified water Q.S. 100.0 g.

Isotonicity Study: Isotonicity is the most important characteristic of the ophthalmic formulation. Isotonicity has to be maintained to prevent the tissue damage or the irritation of the eye. Formulations were mixed with few drops of blood and observed under motic microscope and compared with standard marketed ophthalmic formulation.

Viscosity: The developed gel formulations were analysed for viscosity by using Brookfield R/S-CPS+ Rheometer at room temperature. A small amount of sample was kept on the lower plate of the rheometer and spindle was immersed into a sample fluid. For rotating that spindle at constant speed torque is required. Applied torque is proportional to the viscosity of sample. Thus, viscosity in Pa-s was detected from the instrument reading.

Rheological Behavior: Formulations were evaluated for rheological behavior by using Brookfield R/S-CPS+ Rheometer at room temperature. The viscosity (η), shear stress (τ), and shear rate (γ), and were determined. Rheological behavior was elucidated from the rheogram.

Gelation Temperature: Tsol-gel of gel formulations was measured according to the method described by Yun *et al.* In brief, 10 ml of gel formulation was taken in a beaker containing a magnetic bead. The beaker was placed on a magnetic stirrer at 80rpm and heated at an increasing rate of 1°C. The temperature at which movement of the magnetic bead stopped due to gelation was recorded as gelation temperature.

SEM Analysis: The surface structure of the optimized gel and the plain gel was obtained and studied using SEM (JEOL JSM-6360, Japan). The gel samples were freeze-dried using an instrument Freezone 12 labconco by applying pressure

0.016mbar, temperature of 50℃ for 72 hours. The lyophilized powder of optimized gel and the plain gel was scanned to evaluate and confirm the shape and surface characteristics.

TEM Analysis: The structure and shape of micelles formed in the optimized gel and plain gel were studied using the transmission electron microscope. The gel samples were freeze-dried using an instrument Freezone 12 labconco by applying pressure 0.016mbar, temperature of 50℃ for 72 hours. The freeze-dried samples of gel were visualized using TEM (Philips Ltd., Tokyo, Japan) at different magnifications. Finally, images were obtained by high-resolution TEM.

Stability Study: Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the formulations are sealed with aluminium foil and were subjected to stability studies according to ICH guidelines at temperature 40±1°C and RH 75%fora period of one month.

Clarity:

pH:

Gelling Sapacity:

Eyeirritation Study: Eye irritation of institute was tested on albino rabbits. The irritation test was performed according to the Organization for Economic Cooperation and Development test guideline. Prepared In situ gels (50 µl) were placed in the conjunctival sac of the eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about 1 s to limit loss of the material. The other eye, which was untreated, served as a control. The eyes were examined at 1, 3, 6, 9 and 12 h. for redness, swelling, inflammation, and eye blinking. There was no evidence of irritation at 12 h, the study was terminated. Experiments were done in triplicate.

***In-vivo* Study:** Albino rabbits, ranging in weight from 2.0 to 2.5 kg, were obtained from the animal house of Global Bioresearch solution Pvt. Ltd, Shirval, Maharashtra, India and were treated as prescribed in the publication guide for the care and the use of Laboratory Animals. The *in-vivo* experimental protocol was approved by the IAEC under CPCSEA with approval number BVCPK/CPCSEA/IAEC/2022-2023. The animals were housed in standard cages, in a light-controlled room (dark/light cycle) at 19±1°C with no restriction of food or water. During experiments, the rabbits were placed in restraining boxes in a way that they could move their heads and eye freely. Induction of Glaucoma was done with 0.5% dexamet has one eye drop instilled into the eye. Timolol male ate eye drop is used as standard formulation to compare with the test. The IOP changes can be recorded at 0, 30, 60, 90, and 120 min. IOP WAS recorded at every day for 10 days. Reduction of IOP was recorded as shown in **Fig. 2.**

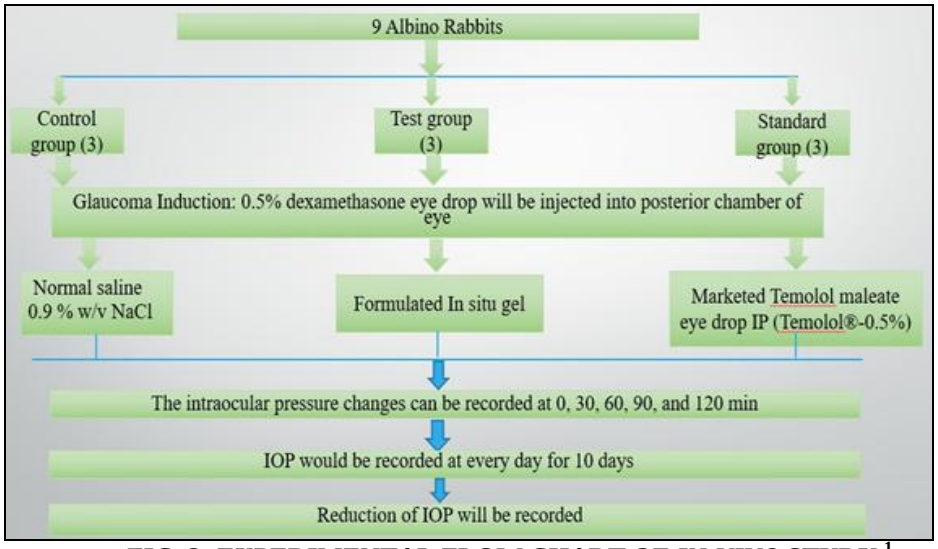

FIG. 2: EXPERIMENTAL FLOW CHART OF *IN-VIVO* STUDY [1]

RESULT AND DISCUSSION:

Method of Extraction and Evaluation of Extracts: Ethanolic extraction of *Camellia sinensis* was carried out by maceration method and with the help of Rotary evaporator the solvent get evaporated and powder form of extract was obtained **Fig. 3**. Hydroalcoholic extraction of Areca catechu was carried out by maceration method and with the help of Rotary evaporator the solvent get evaporated and powder form of extract was obtained **Fig. 4.**

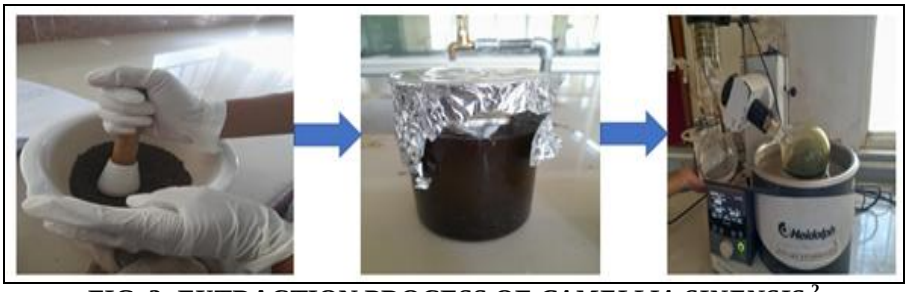

FIG. 3: EXTRACTION PROCESS OF *CAMELLIA SINENSIS* [2]

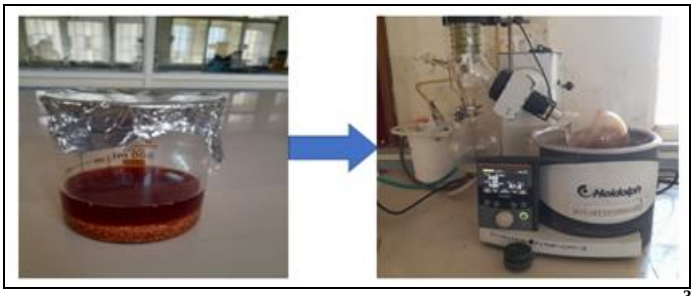

FIG. 4: EXTRACTION PROCESS OF *ARECA CATECHU* [3]

Melting Point: The melting point of both drugs was determined by the capillary method with Thiel's tube. The average reading of mp of *Camellia sinensis* was observed at 200-240ºC and that of Areca catechu was 160-180ºC.

Molecular Docking: Molecular docking was done with the help of PyRx software and drug discovery studio software. Target was selected as the enzyme *i.e* Carbonic anhydrase which is responsible to cause Glaucoma. The protein use to inhibit carbonic anhydrase enzyme having (PDB ID: 1I90) **Fig. 5.**

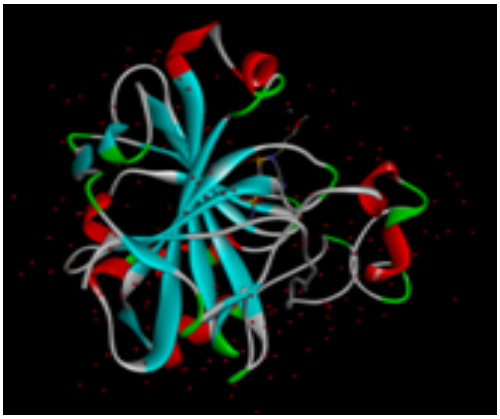

FIG. 5: STRUCTURE OF PROTEIN (PDB ID: 1I90) [4]

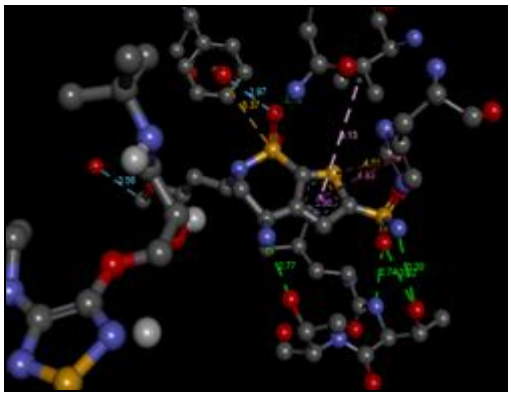

FIG. 6: 3D STRUCTURE OF TIMOLOL BIND WITH 1I90 AS STANDARD

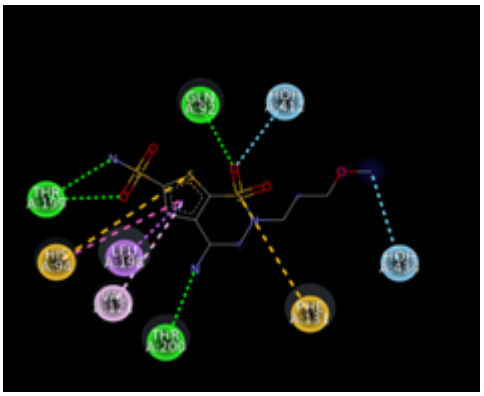

FIG. 7: 3D STRUCTURE OF TIMOLOL BIND WITH 1I90 AS STANDARD

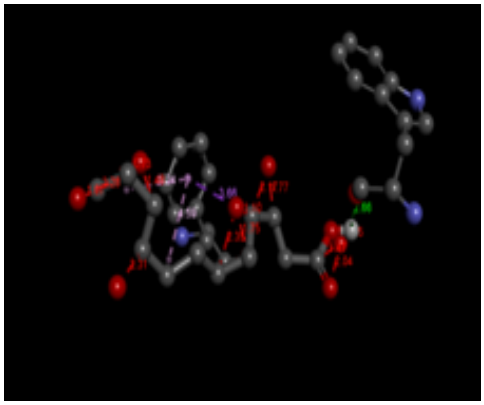

FIG. 8: 3D STRUCTURE OF CATECHIN WITH 1I90 WITH BINDING AFFINITY – 7.1

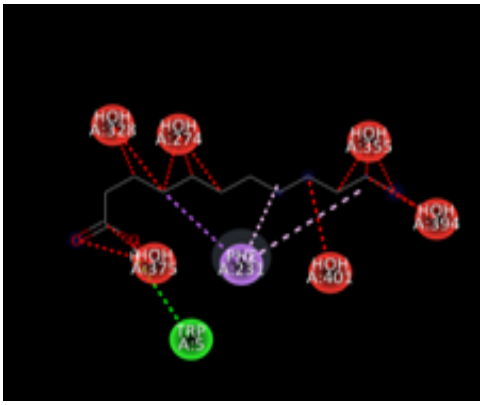

FIG. 9: 3D STRUCTURE OF CATECHIN WITH 1I90 WITH BINDING AFFINITY – 7.1

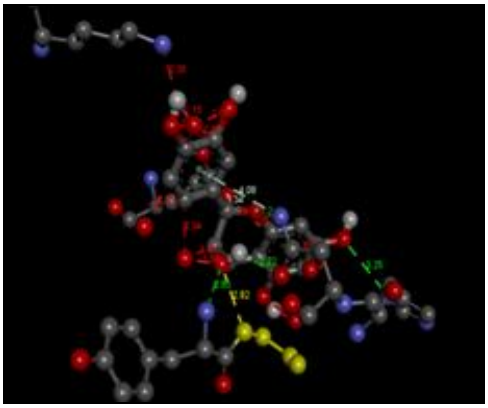

FIG. 10: 3D STRUCTURE OF EPICATECHIN WITH 1I90 WITH BINDING AFFINITY – 7.2

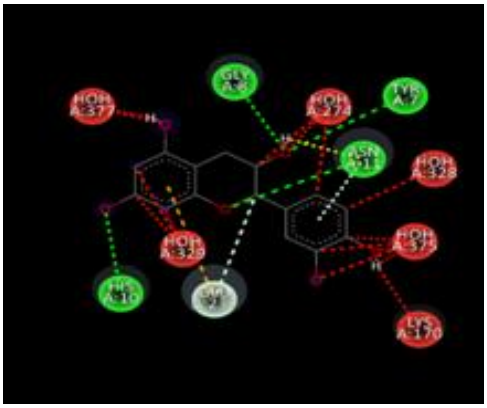

FIG. 11: 2D STRUCTURE OF EPICATECHIN WITH 1I90 WITH BINDING AFFINITY – 7.2

PXRD: XRD spectrum of *Camellia sinensis* and *Areca catechu* shows blunt characteristic peaks which shows that it is amorphous in nature as shown in **Fig. 12** and **13.**

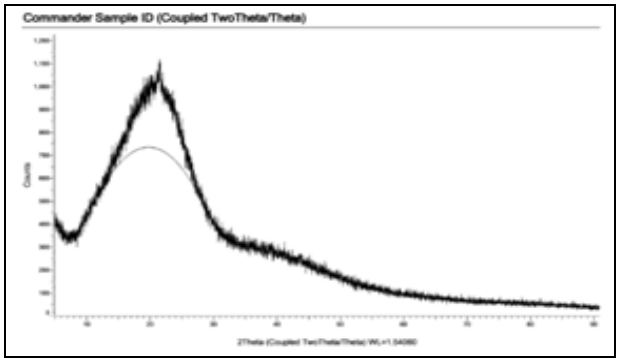

FIG. 12: *CAMELLIA SINENSIS* PXRD GRAPH

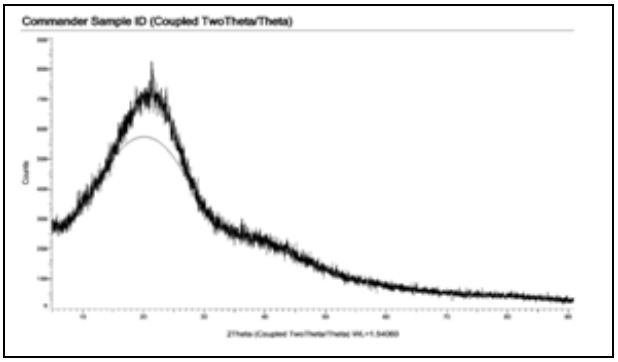

FIG. 13: *ARECA CATECHU* PXRD GRAPH

Phytochemical Screening of *Camellia sinensis* and *Areca catechu:*

TABLE 2: PHYTOCHEMICAL SCREENING OF *CAMELLIA SINENSIS* AND *ARECA CATECHU*

Sr. no.	Secondary metabolites	Test	*Camellia sinensis*	*Areca catechu*
1	Alkaloids	Wagner's test, Hager's test	+	+
2	Saponins	By shaking	-	-
3	Glycoside	Baljet test, Legal's test	+	+
4	Carbohydrates	Fehling's test, Molisch's test	+	+
5	Phenolic compounds	Lead acetate test	+	+
6	Tannins	Lead acetate test	+	+
7	Flavonoids	Sulphuric acid test	+	+
8	Steroids and sterols	Libermann- Burchard test	+	+

Determination of Absorbance Maxima of *Camellia sinensis* Extract: For the characterization of pure extract by UV spectroscopy, it is important to know the wavelength of maximum absorption. The spectrum of pure extract in ethanol and the maximum absorption was found at 279nm as shown in **Fig. 14.**

TABLE 3: CALIBRATION CURVES CONSTANT FOR *CAMELLIA SINENSIS* IN ETHANOL

Regression equation data	***y= mx+ c***
Slope (m)	0.150
Intercept (c)	0.0057
Correlation coefficient (R^2)	0.9996

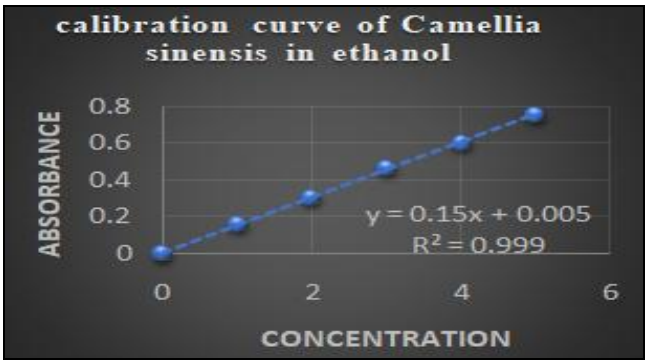

FIG. 14: CALIBRATION CURVE OF *CAMELLIA SINENSIS* IN ETHANOL

Determination of Absorbance Maxima of *Areca catechu* Extract: For the characterization of pure extract by UV spectroscopy, it is important to know the wavelength of maximum absorption. The spectrum of pure extract in ethanol and the maximum absorption was found at 274 nm as shown in **Fig. 15.**

TABLE 4: CALIBRATION CURVES CONSTANT FOR *ARECA CATECHU* IN ETHANOL

Regression equation data	***y= mx+ c***
Slope (m)	0.1271
Intercept (c)	0.00147
Correlation coefficient (R^2)	0.9979

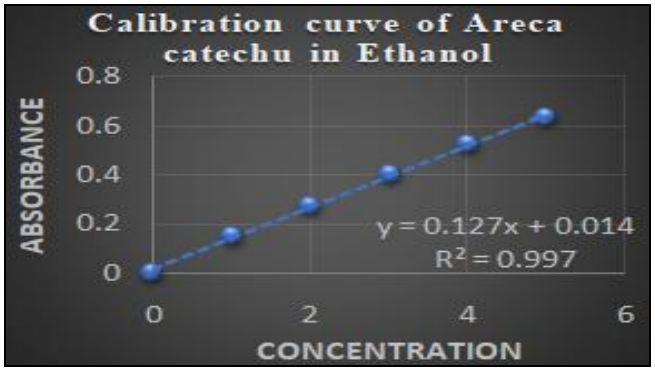

FIG. 15: CALIBRATION CURVE OF *ARECA CATECHU* IN ETHANOL

Fourier Transforms Infrared (FTIR) Spectroscopy of *Camellia sinensis* Extract:

TABLE 5: FUNCTIONAL GROUPS PRESENT IN *CAMELLIA SINENSIS* EXTRACT [6]

Appearance of peak	Functional groups	Characteristic frequency	Observed Frequency	Compound class
Strong, broad	O – H Stretching	3550 - 3200	3386.63	Alcohol
medium	C – H stretching	3000 - 2840	2923.53, 2856.73	Alkane
Weak	C – H Bending	2000 - 1650	1696.30	Aromatic compound
Strong	C = C stretching	1620 - 1610	1618.75	α,β-unsaturated ketone
strong	C – O stretching	1275 - 1200	1234.23	Alkyl aryl ether
Strong	C – O stretching	1205 – 1124	1198.37, 1144.65	Tertiary alcohol
strong	C – O stretching	1150 - 1085	1093.08	Aliphatic ether

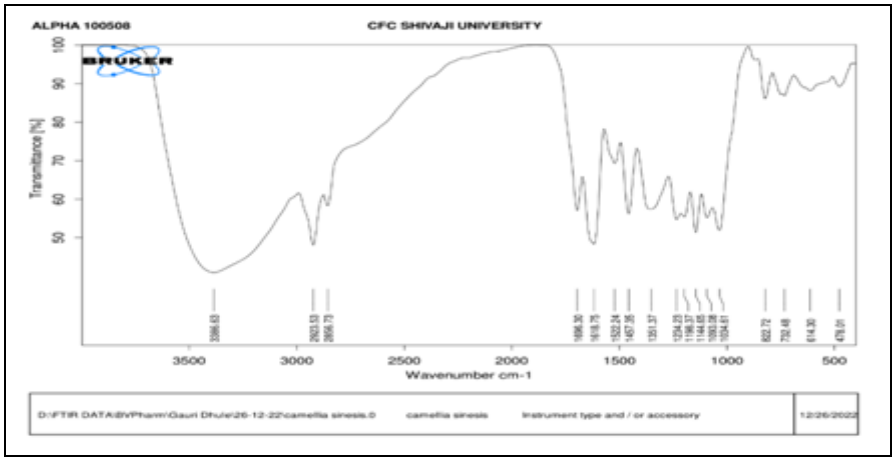

FIG. 16: FTIR OF *CAMELLIA SINENSIS*

Fourier Transforms Infrared (FTIR) Spectroscopy of *Areca catechu* Extract:

TABLE 6: FUNCTIONAL GROUPS PRESENT IN *ARECA CATECHU* EXTRACT

Appearance of peak	Functional groups	Characteristic frequency	Observed Frequency	Compound class
Strong, broad	O – H stretching	3550 - 3200	3410.83	Alcohol
medium	C – H stretching	3000 - 2840	2922.48	Alkane
strong	C = C stretching	1620 - 1610	1614.32	α,β-unsaturated ketone
Strong	N – O stretching	1550 - 1500	1520.64	Nitro compound
strong	C – F stretching	1400- 1000	1104.42	Fluoro compound

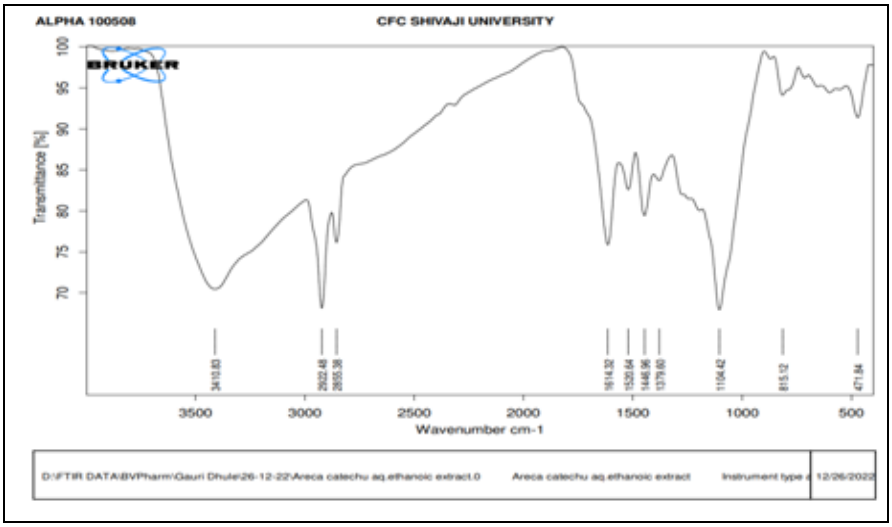

FIG. 17: FTIR OF *ARECA CATECHU*

Utilization of DOE:

ANOVA for Quadratic model

Response 1: pH

TABLE 7: ANOVA FOR QUADRATIC MODEL FOR PH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5902	5	0.1180	23.54	0.0046	significant
A-conc of poloxamer	0.1219	1	0.1219	24.32	0.0079	
B-conc of HPMC	0.2602	1	0.2602	51.88	0.0020	
AB	0.0484	1	0.0484	9.65	0.0360	
A^2	0.1596	1	0.1596	31.84	0.0049	
B^2	0.0288	1	0.0288	5.74	0.0746	
Residual	0.0201	4	0.0050			
Lack of Fit	0.0201	3	0.0067			
Pure Error	0.0000	1	0.0000			
Cor Total	0.6102	9				

Fit Statistics:

TABLE 8: FIT STATISTICS

Std. Dev.	0.0708	R^2	0.9671
Mean	6.96	Adjusted R^2	0.9260
C.V. %	1.02	Predicted R^2	0.7663
		Adeq Precision	12.6871

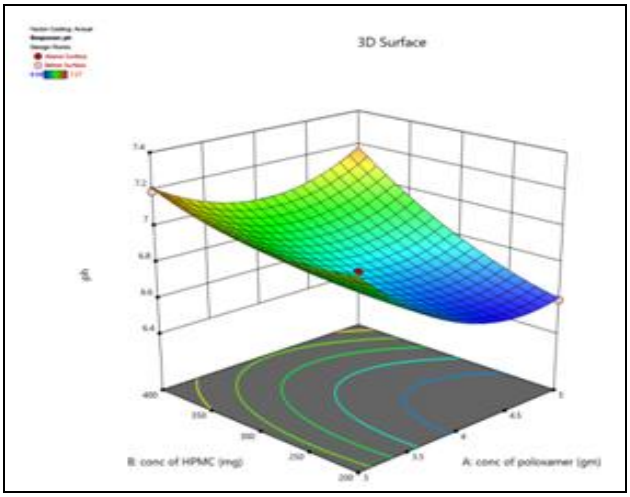

FIG. 18: 3D SURFACE PLOT OF RESPONSE 1: pH

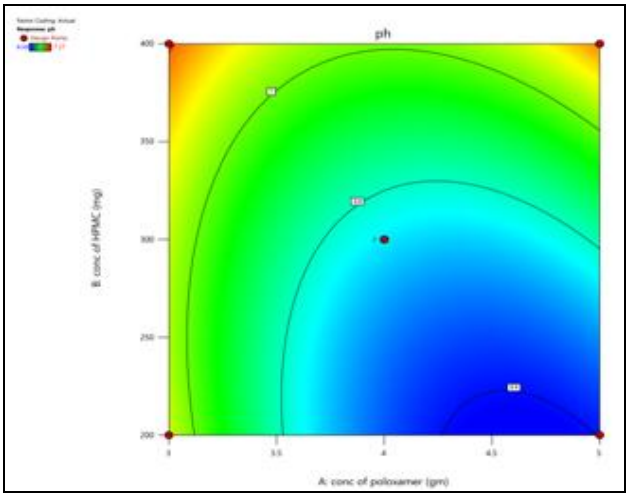

FIG. 19: COUNTER PLOT OF RESPONSE 1: pH

ANOVA for Quadratic Model:
Response 2: Viscosity:

TABLE 9: ANOVA FOR QUADRATIC MODEL FOR VISCOSITY

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0055	5	0.0011	79.41	0.0004	significant
A-conc of poloxamer	0.0025	1	0.0025	178.51	0.0002	
B-conc of HPMC	0.0014	1	0.0014	102.39	0.0005	
AB	0.0012	1	0.0012	88.36	0.0007	
A^2	0.0004	1	0.0004	25.25	0.0074	
B^2	0.0002	1	0.0002	12.88	0.0230	
Residual	0.0001	4	0.0000			
Lack of Fit	0.0001	3	0.0000			
Pure Error	0.0000	1	0.0000			
Cor Total	0.0056	9				

Fit Statistics:

TABLE 10: FIT STATISTICS FOR RESPONSE 2: VISCOSITY

Std. Dev.	0.0037	R^2	0.9900
Mean	0.1480	Adjusted R^2	0.9776
C.V. %	2.52	Predicted R^2	0.9291
		Adeq Precision	26.2759

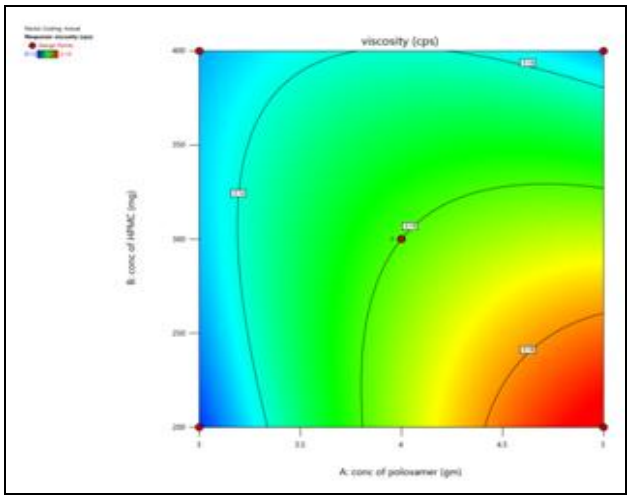

FIG. 20: COUNTER PLOT OF RESPONSE 2: VISCOSITY

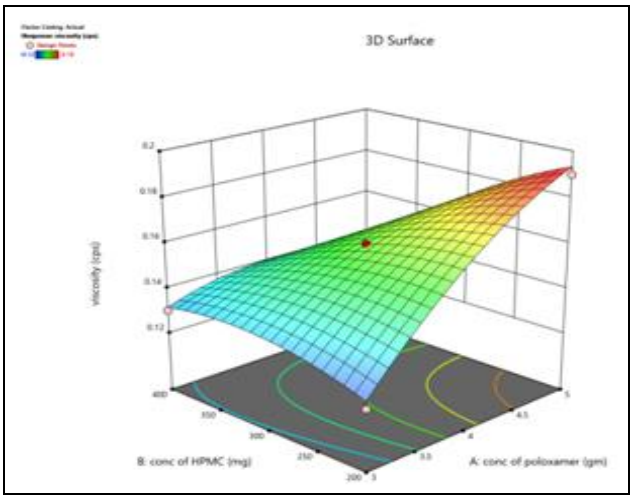

FIG. 21: 3D SURFACE PLOT OF RESPONSE 2: VISCOSITY

ANOVA for Quadratic Model:
Response 3: Gelling Temperature:

TABLE 11: ANOVA FOR QUADRATIC MODEL FOR GELLING TEMPERATURE

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	134.45	5	26.89	19.36	0.0066	significant
A-conc of poloxamer	41.92	1	41.92	30.19	0.0053	
B-conc of HPMC	0.0214	1	0.0214	0.0154	0.9071	
AB	2.25	1	2.25	1.62	0.2720	
A^2	60.07	1	60.07	43.26	0.0028	
B^2	68.64	1	68.64	49.43	0.0022	
Residual	5.55	4	1.39			
Lack of Fit	3.55	3	1.18	0.5924	0.7153	not
Pure Error	2.00	1	2.00			significant
Cor Total	140.00	9				

Fit Statistics:

TABLE 12: FIT STATISTICS FOR RESPONSE 2: GELLING TEMPERATURE

Std. Dev.	1.18	R^2	0.9603
Mean	35.00	Adjusted R^2	0.9107
C.V. %	3.37	Predicted R^2	0.7623
		Adeq Precision	11.6029

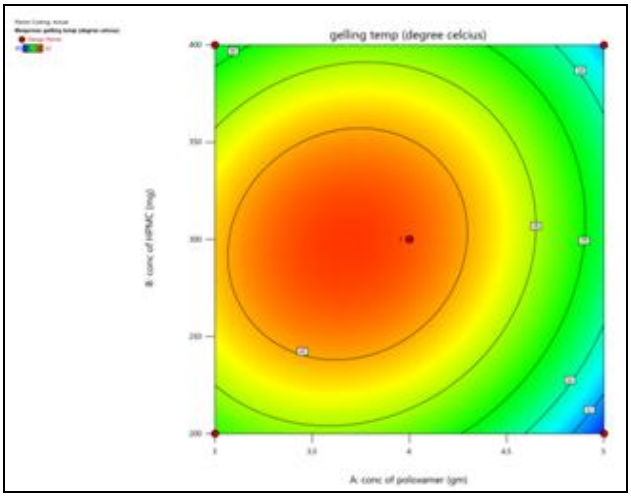

FIG. 22: COUNTER PLOT OF RESPONSE 3: GELLING TEMPERATURE

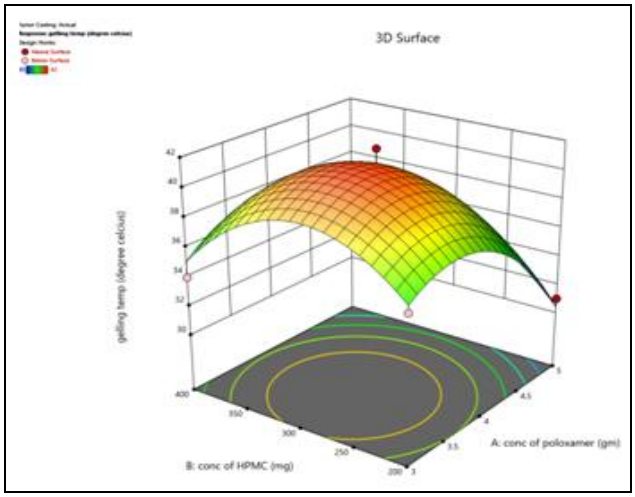

FIG. 23: 3D SURFACE PLOT OF RESPONSE 3: GELLING TEMPERATURE

Optimized Batch:

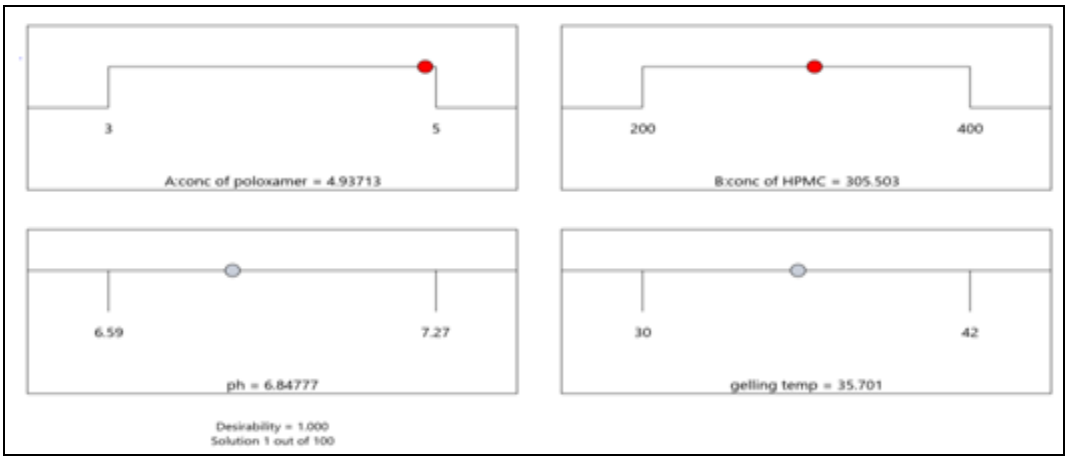

FIG. 24: OPTIMIZED CONCENTRATION OF POLOXAMER AND HPMC (OPTIMIZED BATCH)

Screening of *In-situ* Gel:

Appearance: The prepared formulation was found to be light brown in color, and clarity was found to be satisfactory against black and white background.

pH: The pH of the formulation wasfoundtobe7.4 which is the same as that of the pH of the tear.

***In-vitro* Gelation Study:** The optimized batch formed gel at 31.5(C without dilution with ATF and formed gel within a few seconds at 34°C after dilution with ATF. The prepared formulation formed gel within a few seconds and remained for an extended period of time was observed.

Isotonicity Study: The subjected formulation for the isotonicity study was exhibited no change in the shape of blood cells and damage was observed.

***In-vitro* Drug Release:** The drug release study was carried in Franz diffusion cell using a cellophane membrane and artificial tear fluid as a diffusion medium. The temperature was maintained on a magnetic stirrer with 20 rpm. The drug release was found to be prolong and sustained up to 24 hrs.

The percentage of drug release was found to be 91.68 %. Hence, formulation shows that prolong and sustained drug release can be possible.

TABLE 11: *IN-VITRO* DRUG RELEASE IN 24 HRS

Time (hrs)	%Release
1	5.10±1.12
2	10.96±0.92
3	17.53±0.65
4	24.04±1.23
5	30.28±1.08
6	35.20±0.21
7	39.56±0.42
8	46.76±0.95
9	59.23±1.25
10	64.75±0.38
18	81.23±1.06
24	91.68±0.86

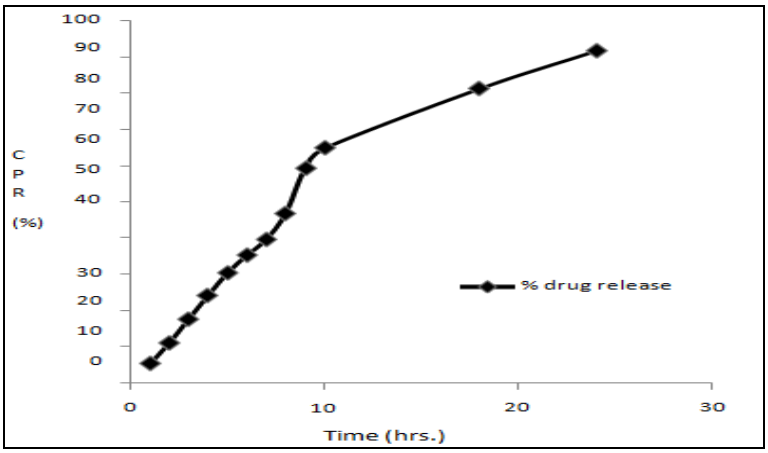

FIG. 25: CPR % V/S TIME

Eye Irritation Study: After the instillation of 50 µl prepared formulation in cul-de-sac of rabbit's eyes was observed up to 12 hrs. The result of prepared formulation indicated that formulated *in-situ* gel was found no any eye irritation observed up to 12 hrs but initially, slightly eye blinking was observed.

***In-vivo* Study:** The baseline was observed between 17 to 18.50 mmHg. After measurement of baseline glaucoma was induced by Dexamethasone subconjuctival injection.

The IOP was measured using a tonometer. After stable elevation of IOP, elevated IOP was lowered by giving treatment of Timolol maleate eye drop and formulated in situ gel. After 300 minutes, Timolol maleate reduced 23.07 % IOP and formulated in situ gel was reduced 18.51 % IOP.

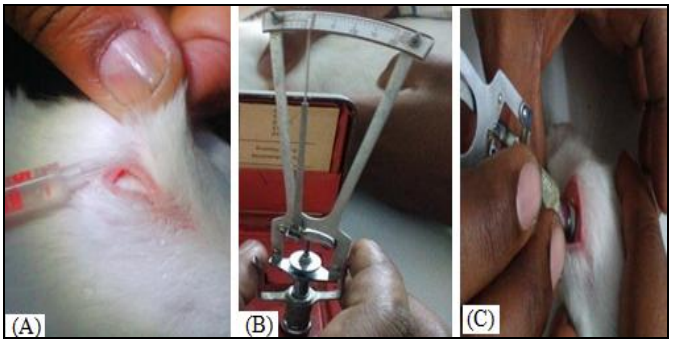

FIG. 26: *IN-VIVO* STUDY ON RABBIT EYE (A): GLAUCOMA INDUCTION, (B): TONOMETER, (C): IOP MEASUREMENT OF RABBIT EYE BY TONOMETER [7]

TABLE 12: IOP OF RABBIT EYE WITH DIFFERENT TIME INTERVALS

Group	IOP (mmHg) with time in minute							
	5	30	60	90	120	180	240	300
Control	16.50	17.00	16.50	17.00	16.00	16.00	17.50	16.50
Standard	25.00	26.00	24.50	24.00	23.00	22.00	21.50	20.00
Test	26.00	25.50	25.00	24.00	23.00	23.50	23.00	22.00

SEM Analysis: SEM analysis of optimized gel revealed a dehydrated gel structure having many pores with irregular shape and pore size.

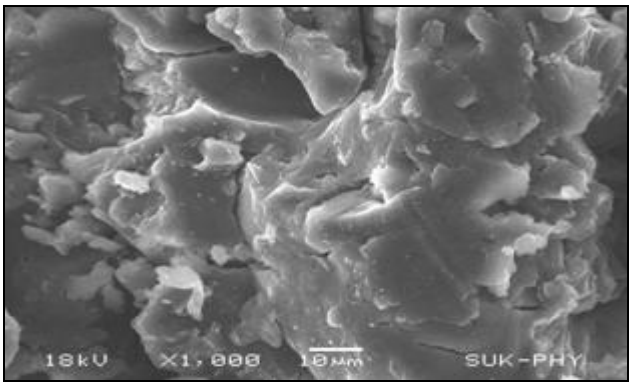

FIG. 27: SEM ANALYSIS OF OPTIMIZED GEL

TEM Analysis: TEM analysis of plain gel showed aggregated structure which may be due to aggregation of PF-127 polymeric chain and drug particles that indicates there is no drug loading occurred.

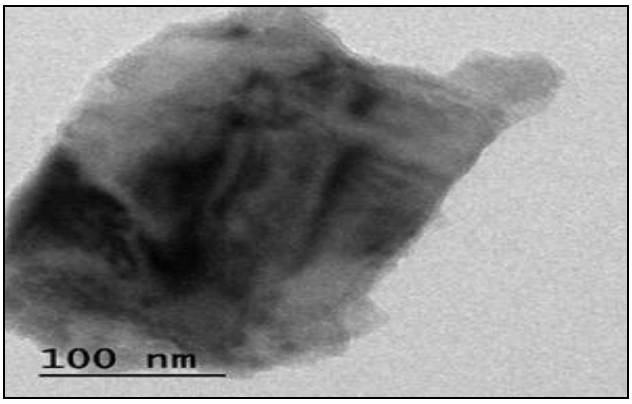

FIG. 28: TEM ANALYSIS OF OPTIMIZED GEL

TABLE 13: STABILITY STUDY OF 3 MONTHS

Parameters	Observations		
	After 30 days	After 60 days	After 90 days
Appearance	Lightbrown	Lightbrown	Lightbrown
pH	5.90	5.80	5.76
Gelling capacity	+++	+++	+++

Stability Study: The stability study was carried according to ICH guidelines at temperatures 40^{o}C and 75%RH for one month. After 30, 60, and 90 days appearance and gelling capacity were found as stable. After 30, 60, and 90 days pH was found to being the range of 5.7to 5.9 as shown in **Table 13.**

CONCLUSION: A thermoresponsive in situ gel system based on pluronic F127 and HPMC K4 m and extract of *Camellia sinensis* and *Areca catechu*. was successfully developed for ophthalmic drug delivery. The prepared formulation was transformed in to gel within a few seconds when instilled on eye surface and increased precorneal residence time of drug. The rheology study was found that formulated in situ gel was pseudoplastic behaviour. *In vitro* results indicated that in situ gel could prolong and sustain drug release up to 24 hrs. The developed formulation showed stable, isotonic nature and was successfully investigated on Dexamethasone induced glaucomatous rabbits for its intraocular hypotensive effect with no eye irritation. A prepared formulation was found 18.51% reduction of IOP. The resulting developed system will have good patient's acceptance.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Overby DR and Clark AF: Animal models of glucocorticoid-induced glaucoma. Experimental Eye Research 2015; 141: 15-22.

2. Luo Q, Zhang JR, Li HB, Wu DT, Geng F, Corke H & Gan RY: Green extraction of antioxidant polyphenols from green tea (*Camellia sinensis*). Antioxidan 2020; 9(9): 785.
3. Sharma PK & Chauhan MK: Optimization and characterization of brimonidine tartrate nanoparticles-loaded *in-situ* gel for the treatment of glaucoma. Current Eye Research 2021; 46(11): 1703-1716.
4. Pokkalath AS, Sawant A & Sawarkar SP: Herbal medicine for ocular diseases: An age old therapy and its future perspective. Journal of Drug Delivery Science and Technology 2022; 68: 102979.
5. Sharma DS, Gulati M, Singh SK, Singh PK & Wadhwa S: Role of novel drug delivery systems in overcoming the challenges associated with intraocular delivery of drugs: an overview. Multifunctional Nanocarriers 2022; 401-418.
6. Attri DS, Rathour A, Ray RK & Kumar V: Formulation and evaluation of hydrogel for topical drug delivery of *Zingiber officinale* Rosc. and *Withania somnifera* (L.) Dunal to increase the bioavailability of oils for the treatment of arthritis. Annals of Phytomedicine 2023; 12(1): 1-10.
7. Lal V & Verma A: Development and Evaluation of Ophthalmic Drop and In-situ Gel from Roots of *Boerhaavia diffusa*. Bri J of Pharm Res 2016; 11(1): 1-20.
8. Lee KK & Choi JD: The effects of Areca catechu L extract on anti-aging. International Journal of Cosmetic Science 1999; 21(4): 285-295.
9. Kim CY, Whittington DA, Chang JS, Liao J, May JA & Christianson DW: Structural aspects of isozyme selectivity in the binding of inhibitors to carbonic anhydrases II and IV. Journal of Medicinal Chemistry 2002; 45(4): 888-893.
10. Inamdar PRIYAL, Jelamvazir DS, Patel D & Meshram D: Phytochemical screening and *in-vitro* antifungal activity of Camellia sinensis. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(5): 148-150.
11. Robb CS, Geldart SE, Seelenbinder JA & Brown PR: Analysis of green tea constituents by HPLC-FTIR 2002.
12. Galassi F, Masini E, Giambene B, Fabrizi F, Uliva C, Bolla M & Ongini E: A topical nitric oxide-releasing dexamethasone derivative: effects on intraocular pressure and ocular haemodynamics in a rabbit glaucoma model. British J of Ophthalmology 2006; 90(11): 1414-1419.
13. Kersey JP & Broadway DC: Corticosteroid-induced glaucoma: a review of the literature. Eye 2006; 20(4): 407-416.
14. Conlon R, Saheb H & Ahmed IIK: Glaucoma treatment trends: a review. Canadian Journal of Ophthalmology 2017; 52(1): 114-124.
15. Digiuni M, Fogagnolo P & Rossetti L: A review of the use of latanoprost for glaucoma since its launch. Expert Opinion on Pharmacotherapy 2012; 13(5): 723-745.
16. Ahmad A, Ahmad SZ, Khalique N, Ashraf M & Alvi Y: Prevalence and associated risk factors of glaucoma in Aligarh, India populationbased study. DJO 2020; 31(1).
17. Weinreb RN, Aung T & Medeiros FA: The pathophysiology and treatment of glaucoma: a review. Jama 2014; 311(18): 1901-1911.
18. Kumar A, Malviya R & Sharma PK: Recent trends in ocular drug delivery: a short review. Eur J Appl Sci 2011; 3(3): 86-92.
19. Gasiunas K & Galgauskas S: Green tea a new perspective of glaucoma prevention. Int J of Ophthal 2022; 15(5): 747.
20. Ansari A, Mahmood T, Bagga P, Ahsan F, Shamim A, Ahmad S & Parveen S: Areca catechu: A phytopharmacological legwork. Food Fron 2021; 2(2): 163-83.
21. Rhee DJ, Katz LJ, Spaeth GL & Myers JS: Complementary and alternative medicine for glaucoma. Survey of Ophthalmology 2001; 46(1): 43-55.
22. Pathak S & Chopra H: Current Trends for Ophthalmic Drug Delivery: A Review.
23. Nitin Z: Ophthalmic in situ drug delivery system: a review. International Journal of Pharmaceutical Research and Development 2013; 5(5): 48-55.
24. Khule MR & Vyavahare SB: A Review: In-Situ gel drug delivery system. Int. J. Res. Education and Scientific Methods 2021; 9(3): 899-909.
25. Kumar KS, Bhowmik D, Paswan S & Srivastava S: Recent challenges and advances in ophthalmic drug delivery system. The Pharma Innovation 2012; 1(4): 1.
26. Jumelle C, Gholizadeh S, Annabi N & Dana R: Advances and limitations of drug delivery systems formulated as eye drops. Journal of Controlled Release 2020; 321: 1-22.
27. Patel JM and Shah KK: Formulation and evaluation of temperature dependent ophthalmic gel forming solution of Brimonidine Tartrate. In Journal of Pharmaceutical and Chemical Sciences 2012; 4: 1190-1199.
28. Malay KS and Raichur AM: Dual-drug delivery system based on in situ gel forming Nanosuspension of Forskolin to enhance antiglaucoma efficacy. American Association of Pharmaceutical Scientists 2010; 11: 322-335.
29. Pathare YS and Wagh VD: Herbal medicines and nutritional supplements used in the treatment of glaucoma. Res J of Pharma Biol and Chem Sci 2012; 3: 331-339.
30. Tare MS and Durgade AM: An in situ gel approach for ophthalmic drug delivery of andrographolide. Int Research J for Inventions in Pharma Sciences 2013; 1: 10-14.
31. Gupta SK: Oculo hypotensive effects of Foeniculum vulgare in experimental model of glaucoma. Indian Journal Physiol Pharmacol 2008; 52: 77-83.
32. Jie lou and wenjing Hu: Optimization and evaluation of a thermoresponsive ophthalmic in situ gel containing curcumin-loaded albumin nanoparticles. International Journal of Nanomedicin 2014; 9: 2517-2525.
33. Gabriel B and Shaker M: Current primary open angle glaucoma treatments and future direction. Clinical Ophthalmology 2012; 6: 1699-1707.
34. Mohamed H and Hamdy R: Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Industrial Crops and Products 2012; 3: 111-119.
35. Manzoor A and Rather A: Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian Journal of Chemistry 2012; 5: 125-132.
36. Sah KA and Verma VK: Phytochemicals and pharmacological potential of Nyctanthes arobortristis a comprehensive review. Int J of Research in Pharmaceutical and Biomedical Sciences 2012; 3: 420-426.

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