Section A-Research paper

Formulation, characterization, *ex-vivo* and *in-vivo* evaluation of in-situ gel containing Gingko Biloba Extract for the treatment of Glaucoma

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Abstract:

In this research work herbal drugs Gingko Biloba extract were used to prepare in-situ gel for glaucoma. A total of six formulations were prepared and evaluated for different evaluation parameters. The percent drug release for all formulations was carried out in Franz diffusion cell using simulated tear fluid the formulation, G5 showed a maximum release of 98.88 % in a total of 8 hours. The results of *ex-vivo* studies indicated that 82.68% of the drug permeated from the formulations, demonstrating a sustained effect. The ocular irritancy test conducted on the ocular in situ gel yielded an overall score of zero, indicating the absence of any signs of ocular irritation, such as redness, increased tear production, or swelling (edema). Also, in-situ gel of Gingko Biloba Extract (G5) formulations demonstrated a good percentage reduction in IOP, with a value of 24.94±1.64 mmHg. The in-situ gel formulation achieved a C_{max} of 8.9 mg/mL after 4 hours, which was approx. 10 times higher than the C_{max} of the Timolol eye drop (0.908 mg/mL) obtained after 2 hours. This indicates that the in-situ gel formulations achieved a significantly higher peak concentration in the aqueous humor compared to the Timolol eye drop.

Keywords: In-situ Gel, Glaucoma, Gingko Biloba, In-Vivo studies.

Introduction:

In recent years, there has been a notable focus on the advancement of novel methodologies for drug administration. The utilization of controlled drug delivery has emerged as a promising approach to augment the efficacy and safety of traditional drug administration techniques by facilitating accurate spatial and temporal localization within the human body [1]. Pharmaceutical researchers encounter an intriguing and demanding endeavor in the realm of ocular drug delivery. The eye, as the essential anatomical structure responsible for the sense of vision, is prone to a range of diseases, including glaucoma, dry eye syndrome, conjunctivitis, and cataract. The distinctive anatomical and physiological features, along with the defense mechanisms, of this organism present challenges in achieving effective drug delivery to the intended target location. The development of an efficient ophthalmic drug delivery system necessitates a comprehensive comprehension of the anatomical and physiological aspects of the eye [2].

Glaucoma is a significant cause of blindness worldwide, and it can be categorized into different types, including angle-closure glaucoma (ACG) and open-angle glaucoma (OAG). Glaucoma is known to be more prevalent among certain populations, such as women and Asians. However, the exact reasons for these demographic differences are not fully understood. The main characteristic

of glaucoma is an increase in intraocular pressure (IOP) within the eye. This elevated pressure can lead to progressive damage to the optic disk and subsequent vision loss. It is important to note that glaucoma often develops without noticeable symptoms in its early stages, which is why regular eye examinations are crucial for early detection and treatment [3-4]. The underlying cause of glaucoma is believed to involve an imbalance between the production and drainage of aqueous humor, the fluid inside the eye. Agents used to treat glaucoma aim to reduce IOP, typically targeting either the reduction of aqueous humor production or the improvement of its drainage through the trabecular meshwork or the uveoscleral pathway. Lowering IOP is considered the primary modifiable risk factor for glaucoma, and various treatment options are available. These include medications in the form of eye drops, laser therapy, and surgical interventions. The choice of treatment depends on the type and severity of glaucoma, as well as individual patient factors [5]. Among the number of various novel drug delivery systems available for the treatment of Glaucoma, these days herbal drugs are proven to be a promising approach.

In recent years, there has been a growing interest in exploring the potential of herbal medicines possessing antioxidant and anti-inflammatory properties as a viable alternative for the treatment of glaucoma. Glaucoma is a multifaceted ocular disorder distinguished by optic nerve impairment, frequently correlated with heightened intraocular pressure (IOP). Conventional therapeutic approaches predominantly prioritize the reduction of intraocular pressure (IOP) as a means to mitigate progressive harm. However, there is an emerging inclination to investigate supplementary therapeutic methodologies, such as the utilization of natural products [6].

Preclinical and certain clinical investigations have demonstrated promising advantages of herbal remedies possessing antioxidant and anti-inflammatory characteristics in the context of glaucoma [7].

In recent times, there has been a growing interest in the utilization of in situ gel-forming systems for enhancing the retention duration and facilitating controlled release of drug compounds within the ocular environment. This is primarily due to the ability of these systems to enhance the bioadhesive properties of ophthalmic solutions. The utilization of in situ gel-forming systems in ocular drug delivery exhibits considerable potential, as it offers advantages such as sustained drug release, extended residence time, and improved drug absorption [8].

In recent years, there has been considerable interest in the advancement of in situ gel systems, primarily attributed to the various benefits they provide as polymeric systems. The advantages encompassed in this context comprise the ease of administration, decreased frequency of administration, enhanced patient compliance, and improved comfort. In situ gels are capable of

undergoing gelation at the site of application in response to different stimuli, including alterations in pH, temperature, or solvent composition. These triggers play a role in facilitating the transition of the liquid formulation into a gel state, thereby enabling localized and sustained drug delivery. Through the utilization of these mechanisms, in situ gel systems possess significant potential for enhancing drug delivery strategies and enhancing patient outcomes [9].

For the efficient management of glaucoma and to minimize the occurrence of bilateral blindness resulting from progressive glaucoma. The field of medicine has witnessed a biological revolution, opening up new possibilities for therapeutic interventions. In the pursuit of controlling elevated intraocular pressure (IOP) associated with glaucoma, novel treatment approaches utilizing both synthetic and herbal drugs are being explored. Synthetic drugs such as Latanoprost, Timolol, Brimonidine, Travoprost, Bimaprost, and Pilocarpine are commonly used in the treatment of glaucoma. These drugs work by reducing aqueous production in the ciliary body or increasing aqueous humor outflow through various pathways. However, long-term use of these synthetic agents can lead to side effects such as bradycardia, tachyphylaxis, blurred vision, and pigmentation issues. Additionally, these drugs can be quite expensive for patients. Herbal drugs are favored over synthetic drugs due to their cost-effectiveness, non-toxic nature, and safety profile. Currently, there is no herbal formulation available in the market for the treatment of glaucoma. Therefore, this study aimed to develop an effective herbal formulation specifically designed for the treatment of glaucoma. Given the delicate nature of the eye, a safer herbal formulation is often preferred over a synthetic alternative.

The present study aimed to develop an in-situ gel of the herbal drug ginkgo biloba. This combination with synergistic effect was specifically designed to study its potential as an intraocular pressure (IOP) lowering agent and its efficacy in treating glaucoma. The development of an herbal in situ gel with synergistic composition holds promise as it may offer a natural and potentially safer alternative for glaucoma treatment.

Materials and Methods

Materials

In the present research study, various chemicals, solvents, and reagents were utilized for conducting experiments and analyses. The Gingko Biloba extract was purchased from Vihan Herbal and Food Ingredients, India. The polymers HPMC K4M, Carbopol 934, Sodium chloride, Sodium hydroxide, and Benzalkonium chloride were purchased from HiMedia Laboratories, Mumbai, India. All the other chemicals, solvents, and reagents used were of analytical grade.

Methods

Preparation of pH-triggered in-situ gel for Gingko Biloba Extract:

For preparing an in-situ gel, aqueous solutions of Carbopol 934P were formulated at various concentrations. These solutions were then assessed for their overall appearance and physical characteristics, including clarity and color, under both physiological conditions (pH 7.4 ± 0.2, 37◦C) and non-physiological conditions (pH 4.8 ± 0.2, 25◦C). A precise amount of ginkgo biloba extract, Sodium chloride (used as an isotonic modifier), and benzalkonium chloride (added as a preservative) were combined in distilled water (75 mL) to create an aqueous solution (Table 1). The HPMC solution was subjected to continuous stirring while the prescribed quantity of Carbopol 934P was added, and left to hydrate overnight. Subsequently, the polymer solution was mixed with the aqueous drug solution, ensuring constant stirring until a homogeneous dispersion was achieved and a clear solution was formed. The pH was adjusted to a value of 7.4 by employing a 0.5 M solution of sodium hydroxide [10].

Table 1: Formulation design of Gingko Biloba *in-situ* gels.

Ingredients	Formulation code					
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
Ginkgo biloba extract (g)	0.6	0.6	0.6	0.6	0.6	0.6
HPMC K4M (g)	0.4	0.4	0.4	0.4	0.4	0.4
Carbopol-934 (g)	0.05	0.1	0.15	0.2	0.25	0.3
Sodium chloride (g)	0.9	0.9	0.9	0.9	0.9	0.9
0.1N NaOH (ml)	q.s	q.s	q.s	q.s	q.s	q.s
Benzalkonium chloride (%)	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water q.s (ml)	100	100	100	100	100	100

Characterization

The following characterization was carried out

pH

The pH values of the ocular in situ gel formulations were measured by using a calibrated digital pH meter. The calibrated pH meter's electrode was submerged into the sample of the in-situ gel formulation. The reading was let to reach a state of equilibrium, and thereafter, the pH value was recorded [12].

Viscosity

The viscosity of the gel formulations was measured using a Brookfield Viscometer (DV-II + pro model with spindle no. 61). To evaluate the pre-gelation viscosities of the formulations at pH 6, 10 grams of the gel formulation was transferred into a clean and dry beaker. Spindle was lowered into the gel in the beaker, ensuring that it was fully immersed and there were no air bubbles trapped around the spindle. The spindle was rotated at 10, 20, 30, 40, and 50 rpm. The viscosity readings displayed on the Brookfield Viscometer were recorded at each rpm setting [13].

Gelling capacity

The gelling capacity of the in-situ gel formulation was evaluated to determine its ability to undergo sol-gel transition, transforming from a liquid state to a gel when administered to the cul-de-sac of the eye. Simulated Tear Fluid (STF) was prepared, which mimics the composition of tears. A drop of the in-situ gel formulation was placed into 2 ml of the prepared STF solution in a vial. The vial containing the mixture was then placed in a temperature-controlled thermostat set at 37±2°C, maintaining the physiological temperature of the eye [14]. The duration required for gelation, which serves as an indicator of gel formation, was recorded. Additionally, the time required for the gel to disintegrate was also recorded.

In vitro release studies

In the in vitro release study, a Franz diffusion cell was used, in-situ gel formulations were placed in the donor compartment and freshly prepared simulated tear fluid was placed in the receptor compartment. A dialysis membrane, previously soaked in simulated tear fluid overnight, was positioned between the donor and receptor compartments. 1 ml volume of the formulation was accurately pipetted into this equipment. The rotation speed of the Franz diffusion cell was set at 50 rpm to facilitate efficient diffusion. The temperature of the medium in the diffusion cell was maintained at 37 ± 0.5°C to replicate physiological conditions [15].

Aliquots of 1 ml were withdrawn from the receptor compartment at specified time intervals (0.5, 1, 2, 4, 6, and 8 hours). The samples were filtered through 0.45 μm syringe filters and were subjected to HPLC analysis to determine the extract concentrations.

***Ex-vivo* drug permeation study**

The *ex-vivo* permeation investigation was conducted utilizing a modified Franz diffusion cell, with the cornea of male New Zealand albino rabbits. These rabbits were transported to the

laboratory under cold conditions, and immersed in normal saline solution at a temperature of 4°C. One end of the setup was used as a sample port, whilst the other end served to secure the separated cornea and the generated in situ gel formulations. To keep the permeation research at a temperature of 37±0.2°C, a magnetic stirrer was used. The cornea that had been detached was subjected to treatment using a volume of 20 mL of in situ gels, as well as a reference formulation. Aliquots of the medium were extracted from the sampling port at 1, 2, 4, 6, and 8 hours. These aliquots were then substituted with fresh media of equal volume to ensure a constant volume throughout the experiment. The samples underwent High-Performance Liquid Chromatography (HPLC) analysis to determine the concentration of the liberated extract [16].

Sterility test

Aseptic procedures were used to evaluate the sterility of the selected formulation, G5. The formulation was introduced into a medium containing soybean casein digest, and the resulting combination was subjected to incubation at a temperature of 35°C for a minimum duration of 14 days, to facilitate potential bacterial proliferation. During the incubation phase, the sterility of the formulation was assessed by visually examining the transparency of the medium. The lack of observable proliferation in the culture medium throughout the 14-day monitoring duration confirms the sterility of the formulation, suggesting its absence of bacterial contamination and appropriateness for ocular administration [17].

***In-Vivo* Studies**

This study included male albino rabbits from New Zealand, who were in good condition and had an average weight ranging from 2.5 to 3.0 kg. The objective of the study was to examine the ocular pharmacokinetics of extracts using an in-situ gel formulation. The Institutional Animal Ethics Committee (CPCSEA) of the Pinnacle Biomedical Research Institute (PBRI) has provided authorization for the implementation of animal experimentation with reference number PBRI/IAEC/PN-22013. Throughout the duration of the experiment, the rabbits were subjected to isolation inside restraining cages, where they were provided with unrestricted access to food and beverages. The restrictions on allowable eye movement and lag durations were not imposed. Prior to the commencement of the investigation, ocular abnormalities were not detected by the use of external and slit-lamp screening methods.

Ocular irritation test

The Draize test was performed to assess the ocular irritancy of formulated substances. A group of seven male albino rabbits from New Zealand were subjected to the application of optimized in

situ gel formulations to evaluate ocular discomfort. A small amount (50µl) of the in-situ gel was applied to the lower conjunctival sac of one eye of each test animal. The other eye serves as a control and was left untreated. The animals were observed at regular intervals, such as 1 hour, 2 hours, 4 hours, and 8 hours after the application. During these observations, any signs of ocular irritation, such as redness, swelling, discharge, or changes in the cornea or conjunctiva, are noted. The degree of ocular irritation was assessed using the Draize scoring system. The observations were recorded and scored based on the severity and reversibility of the observed effects. The recorded scores were analyzed to determine the ocular irritation potential of the in-situ gels. On a scale from 0 (no irritation) to +3 (maximum irritation and redness), the conjunctiva's congestion, edema, discharge, and redness were ranked [18].

Intraocular pressure (IOP) measurement

The study protocol was reported to the Institutional Animal Ethics Committee (CPCSEA) of the Pinnacle Biomedical Research Institute (PBRI) for the implementation of animal experimentation with reference number PBRI/IAEC/PN-22013. The design of the study was a single-dose cross-over with a one-week washout period. The rabbits were housed in accordance with normal animal housing conditions, in an air-conditioned room with a temperature of 22±0.5^{o}C, alternate light and dark cycles, and a standard meal and water supply [19].

Rabbit eyes were induced with glaucoma using a steroid induction approach. The eyes of twelve rabbits were treated with 0.5% dexamethasone eye drops three times daily for two weeks. Using a Schiotz tonometer, intraocular pressure was monitored twice each week at midday. After each installation, the eye was blinked three times. Using a Schiotz tonometer, the IOP of the treated eye and the control eye was measured at 0.5, 1, 2, 4, 6, and 24 hours after instillation. The percentage decrease in IOP at each time interval was calculated using the following formula:

$$\%\ \text{decrease in IOP} = \frac{\text{IOP control eye} - \text{IOP treated eye}}{\text{IOP control eye}}$$

Experiment data were input into the program Kinetica VR 2000 (Innaphase Company, Philadelphia, PA). Maximum percentage decrease in IOP (% Dec. IOP max), Time for maximum percentage decrease in IOP (Tmax), mean residence time (MRT), and area under percentage decrease in IOP against time curve from 0 to 24 hr were determined for each plot (AUC0–24 hr). SPSS software (SPSS Inc., Chicago, IL) was used to determine the significance of the difference in these parameters between the selected in situ gel formulations [20].

***In-vivo* pharmacokinetics studies**

In vivo pharmacokinetic studies were conducted to investigate the absorption, distribution, metabolism, and elimination of a drug. The parameters evaluated were maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the concentration-time curve (AUC), elimination half-life (t1/2), clearance (CL), and volume of distribution (Vd) [21]. For investigation, 16 New Zealand rabbits were chosen at random and divided into two groups: the experimental group and the control group. The administration of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) intramuscularly rendered the rabbits unconscious. The right lower conjunctival sac of each rabbit in the experimental group was treated with 50 µL of a selected G5 in situ gel formulation, while those in the control group received an Timolol eye drop. Two drops of tetracaine HCl (1%, w/v) were used to anesthetize the rabbits locally. After 0.5, 1, 2, 4, 6, and 8 hours after delivery, samples of aqueous humor were taken from the anterior chamber using a 1.0 ml syringe with a 29 G needle HPLC was used to examine the materials obtained [21]. Using SPSS software, the outcomes were analyzed (SPSS Inc., Chicago, IL).

Stability Studies for Optimized In-Situ Gels

For ophthalmic preparations, the study was conducted for short term duration of 3 months. The optimized formulations were stored in glass vial and kept in the stability chamber at room temperature of 25 ± 2°C and 60 ± 5 % Relative humidity (RH) and at accelerated temperature of 40 ± 2°C and 65 ± 5 % Relative humidity (RH). The parameters namely appearance, clarity, pH and drug content were evaluated for 1, 2 and 3 months to verify the stability of optimized formulations [22].

Results and Discussion

pH

The pH of the formulations (G1-G6) prepared in the study falls within the range of 6.15 to 6.56. the pH of the formulations remained within the desired range and was not influenced by the polymers indicating that the formulation process was well-controlled, and the selected polymers did not significantly alter the pH of the resulting gels. Results are shown in table 2.

Table 2: pH of the prepared ocular *in situ* gel formulations

S. No.	Formulation code	pH

1.	G1	6.15±0.04
2.	G2	6.54±0.09
3.	G3	6.51±0.06
4.	G4	6.50±0.10
5.	G5	6.56±0.08
6.	G6	6.53±0.06

Viscosity

The viscosity testing of all batches was performed, and the results are summarized in Table 3 to Table 4 (Graph G5 & G6). All 6 formulations exhibited high viscosity under conditions of low shear rates. The viscosity and rheological behavior of the formulations were evaluated both before and after the addition of simulated tear fluid (STF) using a Brookfield programmable DV-II + pro model with spindle no. 61 at different shear rates.

Table 3: Pre-gelation viscosity studies of ocular *in situ* gel of Gingko Biloba Extract

Rpm	Viscosity in cps (before gelling) ±SD*					
	G1	G2	G3	G4	G5	G6
10	212±2.11	192±1.98	306±2.16	376±2.43	432±2.89	684±3.10
20	111±0.93	118±1.06	290±1.11	298±1.55	336±2.18	498±2.56
30	104±1.67	108±1.24	210±2.18	246±2.12	314±2.27	306±2.78
40	86±1.20	99±1.19	108±1.17	109±1.34	250±2.69	264±1.19
50	72±0.98	86±1.14	92±1.49	96±1.05	201±1.35	224±1.78

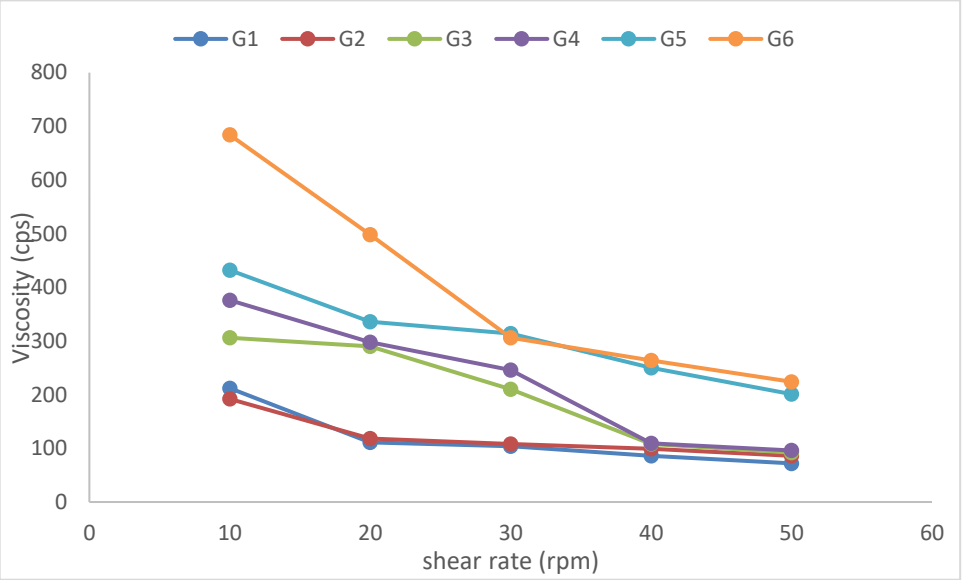
*Average of 3 determinations, SD = standard deviation

Table 4: Post-gelation viscosity studies of ocular *in situ* gel of Gingko Biloba Extract

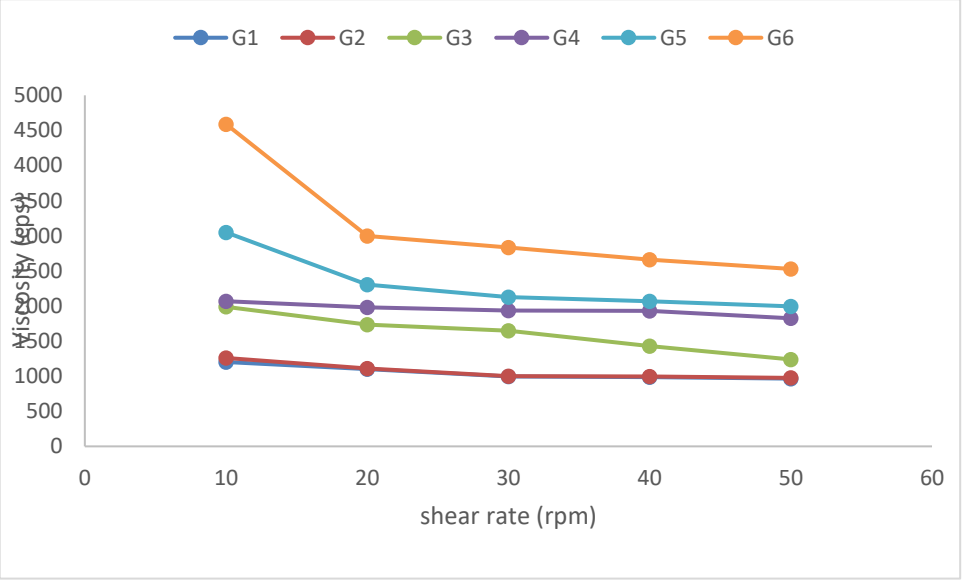
Rpm	Viscosity in cps (Post-gelation) ±SD*					
	G1	G2	G3	G4	G5	G6
10	1201±2.94	1259±3.09	1987±4.18	2067±4.56	3048 ±5.15	4590±5.85

20	1100±2.35	1111±2.46	1733±3.45	1980±3.52	2303±4.65	2998±4.45
30	993±2.13	998±2.38	1646±3.46	1933±3.54	2128±3.65	2833±4.41
40	988±1.79	994±1.76	1427±2.87	1930±2.84	2066±3.66	2659±3.86
50	965±1.46	975±1.63	1238±1.96	1824±2.45	1994±2.55	2527±2.28

*Average of 3 determinations, SD = standard deviation



Graph 1: Pre-gelation viscosity of ocular *in situ* gels of Gingko Biloba Extract



Graph 2: Post-gelation viscosity of ocular *in situ* gels of Gingko Biloba extract

Gelling capacity

Formulation G1, comprising 0.05g of Carbopol 934 showed no gelling ability at the pH of simulated tear fluid (STF) (pH 7.4) due to its low viscosity. However, as the concentrations of Carbopol 934 were increased, the formulations retained their liquid state at room temperature and the formulated pH. Upon exposure to the pH of STF, the gelling capacity of the formulations improved due to the increased viscosity. Among all the formulations, G5, G6 exhibited exceptional gelling capacity, denoted by '+++'. It rapidly formed a robust gel that remained stable for an extended period (more than 6-8 hours) (Table 5).

Table 5: *In vitro* gelling capacity of ocular *in situ* gel of Gingko Biloba Extract

S. No	Formulation Code	Gelling capacity
1	G1	-
2	G2	+
3	G3	++
4	G4	++
5	G5	+++
6	G6	+++

- indicates no gelation, + indicates gelation occurred after a few minutes and dissolved rapidly, ++ indicates immediate gelation and remained up to a few hours, and +++ indicates immediate gelation and remains for an extended period.

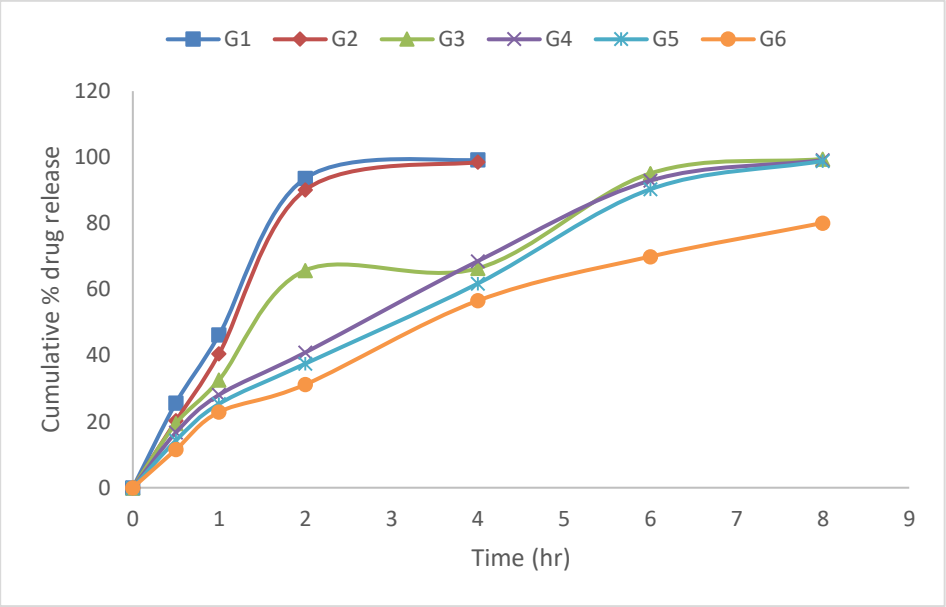
In vitro release studies

The *in-vitro* permeation study was conducted using the Franz diffusion cell to assess the drug release of all the batches (G1 to G6). The results showed that the percentage of drug release varied for each formulation over time. Formulations G1 to G2 released 90% of the drug within 2hrs. However, formulation G6 released 80% of the drug in 8 hrs. The graphical representation of the percentage cumulative drug release (% CDR) versus time is shown in Graph 3 and Table 6, providing a visual depiction of the drug release patterns of each formulation over the study period. Among all the in situ gel formulations, formulations G5 demonstrated the best performance.

Table 6: *In-vitro* release studies of ocular *in situ* gel of Gingko Biloba Extract

Time	Cumulative % release

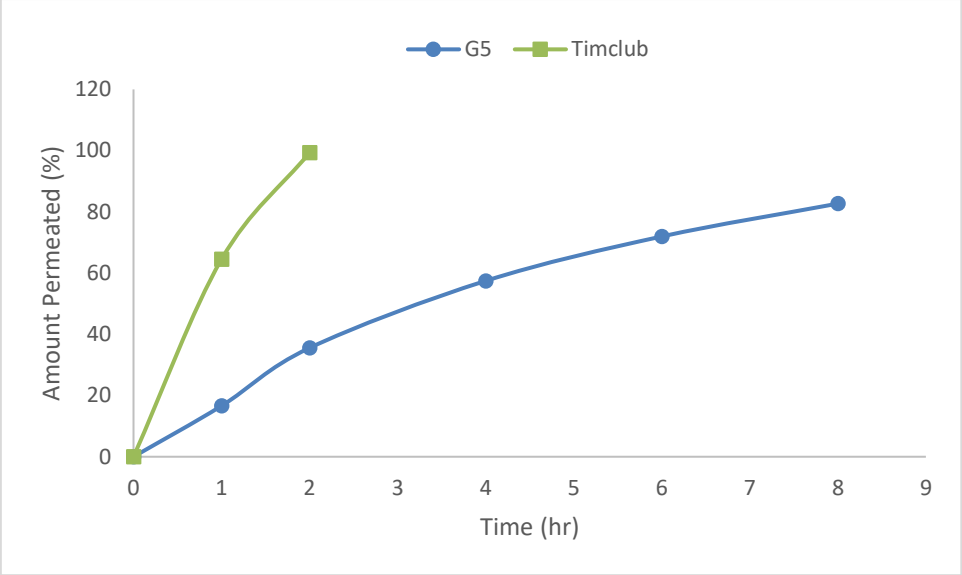
(hrs)	G1	G2	G3	G4	G5	G6
0	0	0	0	0	0	0
0.5	25.68	20.28	19.45	16.72	14.31	11.56
1	46.26	40.55	32.62	28.11	25.38	22.91
2	93.65	90.13	65.78	40.92	37.54	31.24
4	99.25	98.54	66.39	68.57	61.79	56.6
6	-	-	95.11	92.94	90.32	69.95
8	-	-	99.48	99.12	98.88	80.12



Graph 3: In vitro release studies of ocular *in situ* gel of Gingko Biloba Extract

***Ex-vivo* drug permeation study**

The ex-vivo drug diffusion study was conducted to evaluate the drug permeation from the selected formulation (G5) over a period of 8 hours. The results indicated that 82.68% of the drug permeated from the formulation, demonstrating a sustained effect. The data, represented in Graph 4 clearly shows that a significantly higher amount of drug permeated from the selected formulations.



Graph 4*: Ex vivo* corneal permeation study of Timolol eye drop, *in situ* gel of Gingko Biloba Extract (G5)

Sterility test

The sterility test was conducted on formulation G5. The results of the sterility test revealed that there was no evidence of microbial growth in the soyabean casein digest medium throughout the 14-day incubation period. The absence of visible turbidity or any signs of contamination in the formulation indicated that it remained clear and sterile. Based on these findings, the formulation G5 were confirmed to be free from any microbial contamination, meeting the requirements for a sterile ocular product. The results are shown in Table 7.

Table 7: Sterility test on ocular *in situ* gel of Gingko Biloba Extract (G5)

Formulation code	Incubation days							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**14**
G5	-	-	-	-	-	-	-	-

"-" sign indicates no growth.

***In-Vivo* Studies on Ocular *In Situ* Gel of Gingko Biloba Extract (G5)**

Ocular irritation test

The ocular irritancy test conducted on the ocular in situ gel formulations containing Gingko

Biloba Extract (G5) yielded an overall score of zero, indicating the absence of any signs of ocular irritation, such as redness, increased tear production, or swelling (edema). These results suggest that the excipients used in the formulation are safe for topical application in the eye (Figure 1).

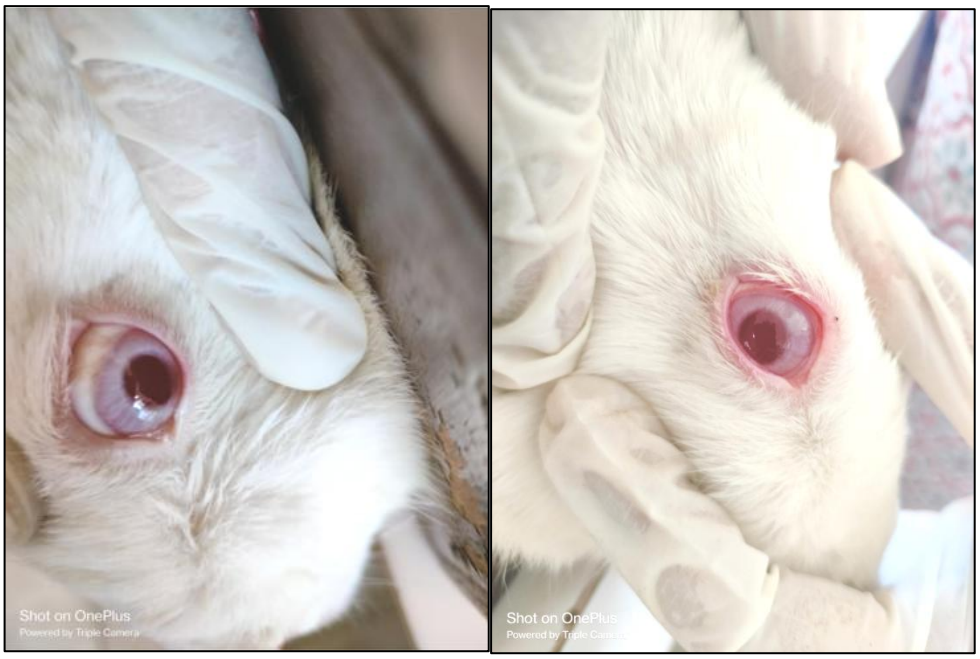


Figure 1: Testing of ocular irritation of in situ gel formulations containing Gingko Biloba Extract.

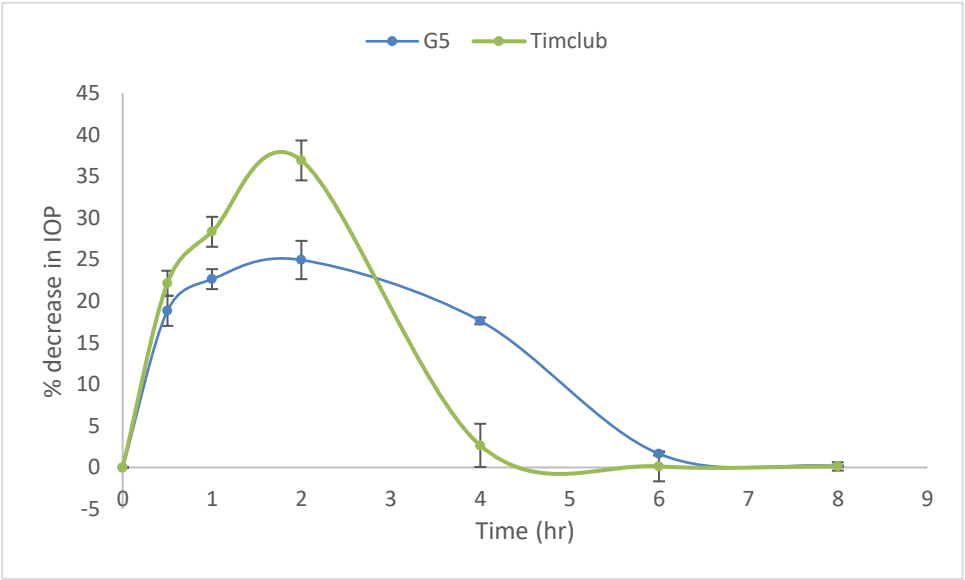
Intraocular pressure (IOP) measurement

Figure 2 and graph 5 showing the average percentage reduction in intraocular pressure (IOP) as a function of time for the Timolol eye drop, in situ gel of Gingko Biloba Extract (G5) formulations. Glaucoma induction increased the IOP of rabbit eyes from the baseline value of 15.9± 2.34 mmHg to 26.6±2.42 mmHg. Maximum IOP reduction of 18.9± 2.60 mmHg following ocular administration of Timolol eye drop was seen at 2 hours; by 5 hours, IOP had recovered to pre-administration levels. After 2 hours of ocular administration, the in-situ gel of Gingko Biloba Extract (G5) formulations demonstrated the good percentage reduction in IOP, with a value of 24.94±1.64 mmHg. There was a significant reduction in IOP after using the selected formulations, and this effect was maintained until the end of the trial. The Timolol eye drop reduced IOP max by a mean of 36.91±2.68 while it was 28.94±1.64 for G5 formulations. Following a 2-hour Tmax, all the formulations showed a steady reduction in response. In comparison to Timolol eye drop, the tested formulation had a considerably greater area under the percentage decrease in IOP response curve (AUC0-24h = 5.104).

The MRT was around 8.20 times higher with the in-situ gel formulations than with the Timolol

eye drop, suggesting a substantial difference between them. This suggests that the extended pharmacological effect was around eight times greater in the developed formulation.

Figure 2: IOP measurement after application of in situ gel formulations containing Gingko Biloba Extract

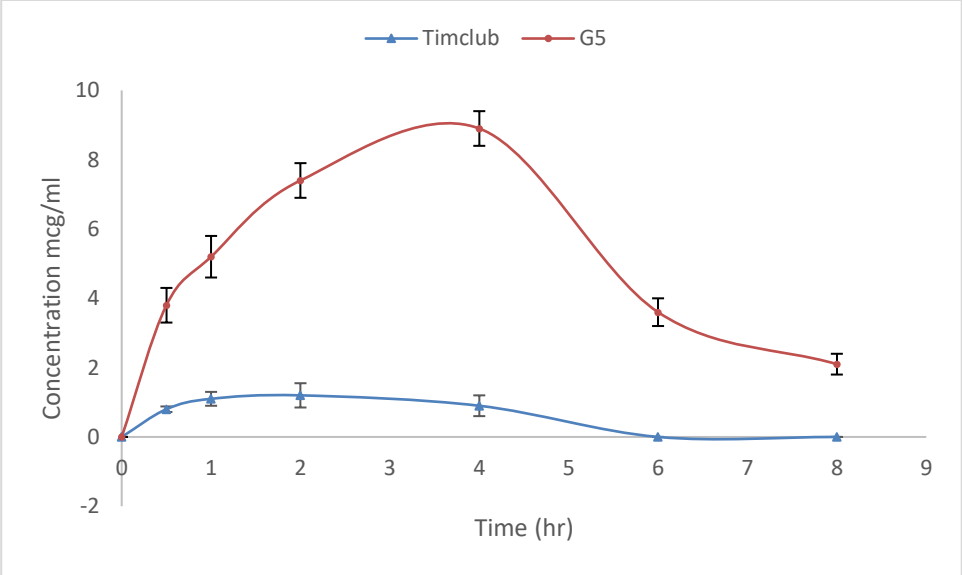


Graph 5: Percentage decrease in IOP as a function of time.

In-vivo pharmacokinetics studies

After the topical administration of the developed in situ gel formulations and Timolol eye drop, the levels of drugs in the aqueous humor of rabbits were measured over different time intervals, as shown in graph 6. The pharmacokinetic parameters, including C max (maximum concentration),

T_{max} (time to reach C_{max}), AUC (area under the concentration-time curve), k (elimination rate constant), MRT (mean residence time), and $T_{1/2}$ (half-life), were calculated for both the in-situ formulation and the drug suspension, and the results are presented in Table 8. The in-situ gel formulations achieved a C_{max} of 8.9 mg/mL after 4 hours, which was 10 times higher than the C_{max} of the Timolol eye drop (0.908 mg/mL) obtained after 2 hours.



Graph 6: Concentration vs time curve of in situ gel formulations and Timolol eye drop in aqueous humor of albino rabbits.

Table 8: Pharmacokinetic parameters of in situ gel formulations and Timolol eye drop

Pharmacokinetic parameters	**Gingko Biloba Extract in situ gel formulation G5**	**Timolol eye drop (Timclub)**
C_{max} (mcg/ml)	8.9 ± 1.32	0.908 ± 0.048
T_{max}	4hr	2hr
AUC 0–8 (mcg.h/mL)	18.2 ± 1.86	3.04 ± 0.26
K (h-1)	0.628 ± 0.025	0.668 ± 0.215
$T_{1/2}$ (h)	1.12 ± 0.08	0.901 ± 0.164
MRT (h)	3.68 ± 0.11	2.4 ± 0.05

Stability Studies

During the test duration, the in-situ gel of Gingko Biloba Extract (G5) formulations exhibited good physical form and homogeneity. The stability analysis was performed by subjecting the

formulation to various temperatures for a specific period. The results of the stability analysis indicated that the selected in situ gel of Gingko Biloba Extract (G5) formulations remained highly stable under these conditions. The stability data is presented in Table 9.

Table 9: Stability data in-situ gel of Gingko Biloba Extract (G5) formulations

Storage period	**4ºC**		
	Viscosity (cps)	**pH**	**% drug content**
1 month	205±0.58	6.25±0.08	98.63±1.81
2 months	202±1.26	6.19±0.06	98.24±1.62
3 months	202±1.58	6.19±0.08	98.01±1.85
Storage period	**25±2°C & 60 ± 5%RH**		
1 month	204±1.62	6.56±0.06	99.08±1.56
2 months	201±1.35	6.48±0.04	98.48±1.28
3 months	201±0.98	6.48±0.05	98.05±1.16
Storage period	**40±2°C & 65 ± 5%RH**		
1 month	204±1.62	6.56±0.05	99.15±1.05
2 months	202±1.34	6.38±0.08	98.06±2.16
3 months	202±1.81	6.35±0.06	97.01±2.48

Conclusion

The research work concluded that the formulated preparation of an in-situ gel with herbal drug extract proves to be a suitable approach in the treatment of glaucoma. The formulation G5 demonstrated the best release of the drug 98.88% after 8 hours. The results of Ex-Vivo studies indicated that 82.68% of the drug permeated from the formulation, demonstrating a sustained effect. The results of in-vivo pharmacokinetic studies resulted in a C_{max} of 8.9 mg/mL after 4 hours, which was 10 times higher than the C_{max} of the Timolol eye drop (0.908 mg/mL) obtained after 2 hours. The overall results have made this herbal in-situ gel a promising candidate in treating glaucoma.

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