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Evaluation of Ocular In-Situ Hydro-gel Formulated prednisolone for Controlling Experimentally Induced Uveitis In Rabbits

Amany H.M. Elshazly*[1], Ghada G. El-Hossary [1], Amany Abdel-Ghaffar [2], Atef M. Mahmoud[2], Leqaa Moemen[2] , Marwa Elfoly [3], Amal A Elgohary [4] and Ahmed Mostafa Ata[2]

[1] *Pharmacology Department, Research Institute of Ophthalmology, Giza, Egypt.*

[2] *Biochemistry Department, Research Institute of Ophthalmology, Giza, Egypt.*

[3] *Ophthalmology Department, Research Institute of Ophthalmology, Giza, Egypt.*

[4] *Ocular Physiology Department. Research Institute of Ophthalmology, Giza, Egypt.*

Abstract

ONE OF THE MAJOR limitations in ophthalmic delivery is the poor bioavailability of drugs from conventional eye drops. pre-corneal drug loss factors include rapid tear turnover, transient residence time, and the relative impermeability of corneal epithelium. occasionally, systemic absorption of drugs may result in systemic side effects. to overcome these issues, controlled drug delivery systems were developed, including different types of in situ gel-forming systems based on different mechanisms (temperature, ph, or ion activated) using different polymers that have been explored for sustained ocular delivery. this study aims to formulate ocular drugs as loaded ionic and ph-activated in situ hydrogel formulas for controlled drug delivery systems aiming to increase the pre-corneal residence time and reduce the frequency of application, limit side effects, and improve patient adherence to therapy. this study will conduct biochemical and ophthalmological tests for combined lecithin, alginic acid, and chitosan-loaded prednisolone eye drops in an animal model of induced uveitis. This study will be conducted to improve ocular bioavailability, and conjunctival sac residence time, reduce frequency of application, and maximize the clinical efficacy of the used topical drug for the treatment of ocular uveitis. A modified formulation of lecithin and combined hydrogel loaded with prednisolone will be formed and evaluated. Animal models of ocular uveitis will be established to test for ophthalmological efficacy of the new formulations. Ocular tissue samples will be obtained for biochemical studies to confirm the clinical efficacy and investigate biochemical changes.

Keywords: lecithin, chitosan, alginic acid, eye drops, uveitis.

Introduction

The achievement and maintenance of the ideal drug concentration at the site of action within the eye is one of the main challenges in ocular delivery. Extending the ocular residence time of pharmaceuticals for topical application to the eye has been the subject of an investigation into a variety of ophthalmic dosage forms, such as solutions, ointments, gels, and polymeric inserts. These dose forms have improved the corneal contact time to varied degrees. However, due to vision impairment (ointments, for example) or patient noncompliance (inserts, for example), they have not gained widespread acceptance [1]. The pre-corneal loss variables, which include fast tear turnover, nonproductive absorption, temporary resident duration in the cul-de-sac, and relative impermeability, are primarily responsible for the poor bioavailability of medications from conventional eye drops [2].

Occasionally, the medicine drained through the nasolachrymal duct may absorb systemically and cause some unwanted side effects. Owing to these physiological and anatomical limitations, the amount of dosage that is absorbed by the eyes is very small (less than 1%)[1]. This compels the physician to advise frequent high-concentration, pulse-type dosage, which can lead to several ocular product side effects. Occasionally, the medicine drained through the nasolacrimal duct may absorb systemically and cause some unwanted side effects. Owing to these

*Corresponding authors: Ahmed Mostafa Ata, E-mail: ahmed.mostafa1412@gmail.com Tel.: +2 010 9696 87 99
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physiological and anatomical limitations, the amount of dosage that is absorbed by the eyes is very small (less than 1%) [1]. This compels the physician to advise frequent high-concentration, pulse-type dosage, which can lead to several ocular product side effects.

Recent decades have seen a focus on controlled drug delivery methods, such as hydrogels, liposomes, nanoparticles, and membranes, to address these problems [3].

A particularly interesting class of drug delivery systems, hydrogels have been applied to numerous medical specialties, such as wound healing, immunology, cardiology, and oncology. A cross-linked polymer network with a significant amount of water makes up hydrogels [4].

Hydrogels with a high water content (usually 70–99%) resemble tissues in terms of physical makeup and can be highly biocompatible, making it easier to encapsulate hydrophilic medicines [4]. Furthermore, there is less chance of drug denaturation and aggregation when exposed to organic solvents because they are normally generated in aqueous solutions [5].

Hydrogels are solid-like due to their cross-linked polymer network, which also prevents other proteins from penetrating them. As a result, it is thought that by diffusing enzymes inward, hydrogels shield bioactive therapies from premature destruction [5]. Creating a loaded hydrogel formulation for the medication improves its bioavailability and pre-corneal residence duration. The current formulation attempts to slowly elute the medication and sustain a high local concentration of the medication in the surrounding tissues for a considerable amount of time [6].

Ocular delivery systems are especially interested in in situ gel-forming systems that are injected as drops into the eye and go through a sol-gel transition in the cul-de-sac. Polymers used in situ gelling systems display sol-to-gel phase transitions in the cul-de-sac as a result of environmental variations in particular physicochemical parameters such as pH, temperature, and ionic strength [7].

The polymers used in the current work as ophthalmic muco-adhesive gels include chitosan and sodium alginate. Sodium alginate is a naturally occurring linear, unbranched hydrophilic polysaccharide that forms a gel instantly when it interacts with the divalent cations in lachrymal fluid (pH 7.4) to generate calcium alginate [8].

A naturally occurring, biodegradable polymer, chitosan improves mucoadhesive permeability. At pH 5.6, chitosan is still liquid; at pH 7.0, it turns into a gel. Because of these characteristics, chitosan is a perfect polymer for in situ formulation [9].

As a copolymer, hydroxypropyl methylcellulose (HPMC) is added to improve viscosity and help achieve prolonged drug administration. With its high swelling capacity, HPMC is a semisynthetic, inert, viscoelastic polymer that is non-ionic, nontoxic, and an excellent carrier for medicinal applications [10].

Furthermore, due to their biocompatibility, other natural phospholipids, such as lecithin, are frequently employed in the pharmaceutical industry. Pharmaceutical products containing lecithin can preserve cell-membrane fluidity, which aids in the absorption of drugs from lecithin-based formulations [11]. Because of this, the lecithin-based drug delivery method improves the absorption of medications that are not well absorbed. Lecithin-chitosan nanoparticles, which form between the positively charged chitosan and the negatively charged lecithin, have shown promise as medication carriers. These muco-adhesive nanoparticles work together to stick to the corneal and conjunctival surfaces' mucin layer. The medicine produced from the nanoparticles remains accessible on the ocular surface for extended periods because the mucin layer holds onto them for a long time [12].

To create a sustained ocular drug delivery system, the current study will combine chitosan, sodium alginate, lecithin, and hydroxyl propyl methyl cellulose (HPMC). With long-term stability, biocompatibility, muco-adhesiveness, and prolonged drug release within the anterior surface of the eye, all of these are anticipated to prolong the ocular drug delivery on the external surface of the eye. Animal models raised in experiments to simulate ocular uveitis will be used to develop the condition and administer the tested medications.

Uveitis is an inflammatory ocular condition that causes a progressive loss of visual field and even loss of vision. Glucocorticoid steroids, administered as oral medication, posterior sub-tenon injection, or topical eye drops, are the usual means of treatment [13]. However, topical medications, particularly glucocorticoids, when applied often and over an extended period can have unwanted local adverse effects. These side effects are the main reason for treatment failure since they reduce patient compliance and persistence [14].

The objective of the present study is to develop an ionic and pH-activated in situ gelling drug delivery system composed of sodium alginate, chitosan, and lecithin to be compared with plain eye drops aiming to increase the bioavailability of the used drugs, decrease the frequency of application, and maximize the clinical efficacy and patient compliance. Moreover, decreasing doses is linked to reduced toxicity of long-term drug applications. Particle size and zeta potential of the formulas will be tested along with clinical efficacy in an experimentally induced model of uveitis. Moreover,

Tissue samples will be obtained for biochemical studies to confirm the clinical efficacy of the tested drug. The topical drug chosen for prophylaxis and alleviation of experimentally induced uveitis is prednisolone.

Material and Methods

Chemicals and drugs needed for hydro-gels and lecithin-loaded drugs:

1-Sodium alginate, an ophthalmic gel forming muco-adhesive polymer. 0.2%, 2-carboxymethyl cellulose (0.5%) as a copolymer, 3- Chitosan 0.5%, 4- Polyethylene glycol 400. 5- Tween 80. 6- Sodium tripolyphosphate (TPP), 7-Benzalkonium chloride (as preservative), 8- Soybean oil granular l- alpha – Lecithin, and 9- prednisolone acetate .

The chemicals and drugs will be obtained from Sigma-Aldrich (USA).

Experimental animals

The experimental study was carried out on New Zealand albino male and female rabbits weighing 1500 to 2000 grams and obtained from the laboratory animal research house, Research Institute of Ophthalmology (RIO). The rabbits were housed in stainless steel cages with free access to drinking water and food. Animals were maintained under standard conditions of ventilation, temperature (25±2°C), humidity (60-70%), and light/dark condition (12/12hrs). Animal care and protocols were by the guidelines and approval of the Ethical Committee of RIO. The experiment was performed by the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The eyes of animals were examined by using a slit lamp (Carl Zeiss) before induction of diseases and those with any defect were excluded.

Formulation of the hydrogels loaded drugs

Preparation of Chitosan-Alginate hydrogel-loaded prednisolone

A 30 ml chitosan solution with a concentration of 1.5 mg/ml was dissolved with 1% acetic acid using a magnetic stirrer at 500 rpm. The drug was dissolved in 7.5 ml Polyethylene glycol 400 and 0.4 ml Tween 80 with a magnetic stirrer at 1000 rpm to yield a final concentration of 1% Prednisolone in the hydrogel-loaded formula. The two solutions were mixed at 1000 rpm for 30 min.

A stock of 0.05 mg/ml alginic acid and 0.75 mg/ml sodium tripolyphosphate (TPP) was prepared and 12 ml of it was then added to the chitosan-drug mixture drop-wise during stirring at 1000 rpm. After adding all the alginic acid and TPP mixture, the final formula was mixed at 1000 rpm for 30 min (15).

After the mixing period, powdered carboxymethyl cellulose (0.5%) as a copolymer was added to the mixture gradually to yield a final concentration of 1% and gel consistency.

Preparation of Chitosan-Alginate- lecithin loaded prednisolone The Lecithin powder and drug were dissolved in a molar ratio (7:2) in a quantity of 50 ml of chloroform-methanol mixture (2:1) contained in a round bottom flask, with gently shaking until complete dissolution. The Final concentration of Prednisolone is 1%. The mixture was then charged in a rotary vacuum evaporator and evaporated to dryness at 50 C and 150 rpm. The dried lipid film was hydrated by drop-wise addition of 40 ml distilled water at 60 C and 150 rpm for 1 h. The resulting vesicle suspension was then homogenized for 5 min at 20K rpm at 3 cycles. 10 ml of chitosan in 1% acetic acid was prepared to give a final ratio of (1:3) chitosan: lecithin (W/W). After complete dissolution, alginic acid was added with a concentration of 0.05 mg/ml and mixed for 30 min at 1000 rpm using a magnetic stirrer. Then the pH was adjusted to 5 with sodium hydroxide.

The 10 ml of chitosan-alginic acid was mixed with the liposomal suspension already prepared at 1000 rpm for 5 min. Then the whole formula was homogenized for 5 min at 20K rpm at 5 cycles. In the end, 1% carboxymethyl cellulose contained, 5 ml Polyethylene glycol 400, and 0.4 ml Tween 80 were mixed with the final formula using a homogenizer at 1000 rpm for 15 min(16).

Zeta potential and particle size distribution measurement:

The prepared particles were analyzed for their mean particle size and size distribution in terms of the average diameters and polydispersity index by photon correlation spectroscopy using particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) at a fixed angle of 173° at 25° C. Samples were analyzed in triplicate. The same equipment was used for the determination of zeta potential.

Induction of anterior uveitis:

Anterior uveitis was induced by a single intravitreal injection of 10 µL complete Freund's adjuvant in the right eyes only. Rabbits were anesthetized by Ketamine (Ketalar; Warner L: Ambert) in a dose of 2 mg/kg intra-muscular and topically by benoxinate hydrochloride. The lid was retracted with a wire speculum and a calliper was applied to the limbus to identify the site of injection opposite the pars plana. The superior rectus was grasped by fixation forceps. The tip of a 10 µL Hamilton needle (Alltech) was inserted 4 mm from the limbus in the upper temporal part of the globe through the pars plana and directed towards the center of the globe to inject the adjuvant in the mid-vitrous. Animals were followed up for two weeks for manifestations of anterior uveitis. The study was

conducted on the right eyes of rabbits and the left eyes served as normal.

Animal groups of anterior uveitis:

Eight animal groups were used; each group consisted of 10 animals. An anterior uveitis model was induced only in the right eye of each rabbit. The first group was used as a negative control (no uveitis model was induced). Before starting the experiment, all animals were examined by a slit lamp to exclude any signs of ocular inflammation. The tested animal groups were as follows: G2 is the anterior uveitis model induced by 10 µl Freund s adjuvant intravitreally, G3 is the model uveitis group treated with prednisolone hydrogel formula, G4 is the model uveitis group treated with prednisolone hydrogel and lecithin loaded formula, G5a is the model uveitis group treated with commercial prednisolone eye drops (1%). The eye drops were applied once daily for 14 days.

Ophthalmological examination:

Examination, photography, and clinical scoring for inflammation will be done on days 2, 7, and 14 after experimental uveitis induction using a slit lamp (Carl-Ziess, Germany) with focal illumination. The examiner was blindfolded. The scoring system for experimental uveitis was done according to the criteria described by Bellot (1996) [17]. The total score for each eye was obtained by the addition of the partial scores assigned to every sign observed. Normal eyes had a score of zero.

Scoring system for experimental uveitis is shown in table 1. The total score (16 points maximum) is obtained by the addition of the partial scores assigned to every sign observed.

Electroretinography (ERG) examination for the anterior uveitis model animals

Before recording, rabbits were adapted to darkness overnight. Under a dim red light. Then animals were anesthetized by intra-peritoneal injection of20mg/kg ketamine hydrochloride (TRITTAU-Germany) and 5mg/kg lignocaine hydrochloride (Xylocaine, Astra-Zeneca, Sweden). Benoxinate hydrochloride 0.4% was instilled into the eye as a topical anesthetic (Benox, EIPICO, Egypt)). Animas were maintained on a heated pad at 37 °C. Pupils were dilated by topical application of 1% cyclopentolate hydrochloride (Plegica, Egypt)). Flash ERG recording was performed monocularly using a mini-Ganzfeld stimulator (Neuro-ERG, Neurosof, Ivanovo, Russia. The device conforms to the standards approved by the International Society for Clinical Electrophysiology of Vision (ISCEV). Recordings were obtained using conjunctival silver-coated hook electrodes. A stainless steel needle electrode was inserted under the scalp just outside the outer canthus and served as the reference electrode. A stainless steel needle electrode was inserted in the animal tail and served as ground. Under dark-adapted conditions, scotopic ERG was recorded in response to light flash stimuli. The light intensity was 0.0 log cd•m− 2 for 5 ms at a frequency of 0.5 ms. Ten consecutive recordings were averaged for each light presentation. Photopic responses were obtained after light adaptation for 10 min, Light adapted single flash ERG response was obtained by 0.5 of 0.0 log cd•m− 2 flash intensity. Signals were amplified and filtered (2-200Hz). The wave amplitudes were evaluated and analyzed.

Immunological and biochemical investigations of anterior uveitis experiment.

At the end of the experiment of induced uveitis (14th day), animals were sacrificed by an intramuscular injection of pentobarbital (150 mg/kg). Eyes were enucleated and ocular tissue samples (iris and ciliary body) were dissected out, weighed, and stored at -80^{o} C for later biochemical investigations. The following levels were evaluated:

1-Tumour necrosis factor -α (TNF-α). 2-interleukin-1 beta (IL-1 β). 3-superoxide dismutase (SOD). Levels were assessed in the soluble fraction of tissue homogenate using enzyme-linked immunosorbent assay ELISA Kits for rabbits by the manufacturer's instructions. Kits were obtained from Sigma-Aldrich (USA).

Determination of trans-corneal penetration of the tested formulas (In Vivo Study):

Nine groups of rabbits, 3 animals each (6 eyes), were used to determine trans-corneal penetration of drugs. A single dose of each drug formula (50 ul) was instilled into the corneal surface of each eye. During drug administration, the lower eyelid was pulled gently away from the eye for collection of instilled formulations in the lower cul-de-sac of the eyelid. To ensure mixing the dispersions with lacrimal fluid, the lower eyelid was lifted back and forth once over the cornea. At time intervals 1, 12, and 24h after instillation, an aliquot of 100–200 ul of the aqueous humor was aspirated from the anterior chamber of one eye in each group using a 26-gauge needle syringe. The aqueous humor samples were stored at −20 C and assayed for HPLC analysis to measure drug concentration at the determined time intervals (after 1 hour, 12, and 24 hours after topical instillation). Then, the formulations were compared regarding penetration rate and residence time. The above experiment was repeated three times.

Preparation of samples for HPLC analysis:

Samples were left to melt at room temperature. Samples from each group were pooled together and then 400 ul of each was added to 1 ml of ethyl acetate. Control samples were prepared using various drug concentrations added to blank aqueous humor samples. Samples and standards were vortexed for 5 min and then centrifugated at 2500 ×g for 10 min

(4°C) to allow phase separation, the clear supernatant was transferred to a 5 mL glass tube. This volume was evaporated (45◦ C) to dryness in a vacuum concentrator and then reconstituted with 200 µL of methanol to be injected into HPLC.

HPLC analysis conditions:

The wavelength is 248 nm for prednisolone. The column Kromasil is 3.5 and 5um um, 4.6x150 mm. The Mobile phase is 0.1% OPA: Acetonitrile, 45:55, 70:30 and the mode of elution is isocratic. The flow rate is 1ml/min and the temperature is 25. Wavelength: 248 nm for Prednisolone 255 nm for quercetin and 278 nm for diclofenac.

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using the Statistical Package for Social Sciences program (SPSS) (software version 25; SPSS Inc., IBM Corp., NY, USA, 2017). The quantitative variables are presented as means and standard deviations (SDs). Repeated measures analysis of variance (ANOVA) and Benforroni test were used to compare results. The level of significance was taken at a P value < 0.05.

Results

Particle size and zeta potential of the tested formulas

Zeta potential is a measure of the surface electrical charge of the particles and is used to characterize colloidal drug delivery systems. The magnitude of zeta potential indicates the stability of the colloidal systems. As zeta potential increases, towards positive or negative values, the repulsion phenomenon between particles will be greater leading to a low tendency for the particles to come together and, consequently, more stable colloidal dispersion. The zeta potential for prednisolone-containing neutral liposomes is – 5.7 mV on average. In this study, the measured zeta potential for prednisolone-loaded hydrogel (alginic acid and chitosan) shown in table 2, was 8.34±0.312mV indicating a positive charge shift and a mean particle size of 598.0±159.1 nm. Therefore it is clear that the addition of hydrogel shifted zeta potential from a slightly negative value to a slightly positive one.

Such a slight positive value is suitable for ocular drug delivery systems in that it doesn't cause either ocular irritation or excess tear flow. Consequently, those particles will not be diluted or drained through the inner canthus. Adding lecithin to the formula caused a more positive shift of the charges reaching 22.9±1.92 mV (which means more stability in tear film) and a particle size distribution decreased slightly to 512.3±105.2 nm which means more ability of intracellular distribution. That explains the notable ability of this formula to treat induced ocular inflammation with a notable increase in the duration of action (the smaller positively charged nanoparticles had more ability for absorption through the negatively charged corneal epithelium).

Determination of trans-corneal penetration of the tested formulas (In Vivo Study):in vivo ophthalmic absorption of prednisolone

The plots of concentration against the time of prednisolone in rabbits' aqueous humor after instillation of 50 µl of either prednisolone 1% loaded chitosan-Alginate hydrogel(P1), prednisolone 1% loaded chitosan-Alginate-lecithin(P2) or commercial prednisolone (P3) eye drops into the rabbits' eyes are exhibited in Fig. 1 and table 3. Prednisolone mean concentrations of loaded formulas (P1, P2) were noticeably higher comparable to commercial eye drops at the examined time points. Their mean levels after 24 hours were 33.407±3.601, and 48.547±6.63 ng/ml respectively. These values were still higher than the range of minimum effective concentration (MEC) of prednisolone acetate 28.4 ng/ml, (McGhee et al 1990. On the other hand, 24 hours level of commercial eye drops reached 4.908±0.768ng/ml.

The longer time of residence and higher content observed for both diclofenac and prednisolone indicated that loading hydrogel and lecithin could increase their absorption into the eyes. That may be attributed to the high muco-adhesive properties and absorption enhancer properties of chitosan, alginic acid, and lecithin used in the formula.

Results of the anterior uveitis experiment

Ophthalmological results:

Results of the ophthalmological examination are shown in Table 4 and figs (2-6).

When subjected to slit lamp examination all normal control eyes (G1) had a score of zero when examined on days 2,7,14 from the start of the experiment. In group 2 intravitreal injection of 10 ul Freund s adjuvant induced anterior uveitis with clinical score of 9.5000+2.58844. Treatment of the anterior uveitis model with (prednisolone-loaded hydrogel G3, prednisolone-loaded hydrogel, and lecithin G4, unloaded prednisolone eye dropsG5) showed marked significant improvement as compared to the untreated group. However, the loaded formulas (G3, G4) showed more noticeable responses reaching almost normal scores with insignificant differences from the normal control group.

Immunological and biochemical analysis results of ocular tissues (iris and ciliary body):

The levels of IL-1β, α-TNF and the activity of SOD in uveitis of all experimental groups treated with prednisolone formulas (table 5)

In the normal control (G1) group levels of IL-1B were 0.027 ± 0.0023 (ng/g tissue) on the 14th day of the experiment. In the model untreated group (G 2)

taking an intravitreal injection of 10 ul Freunds adjuvant) a significantly increased level of IL-1B to 0.047 ± 0.0034 (ng/g) is measured. Treatment of the anterior uveitis model with (prednisolone-loaded hydrogel G3, prednisolone-loaded hydrogel, and lecithin G4, unloaded prednisolone eye dropsG5) showed marked significant improvement as compared to the untreated group. However, the loaded formulas (G4) showed more noticeable responses reaching almost normal scores with insignificant differences from the normal control group.

In normal control (G1) Levels α TNF were 0.057 ± 0.008 (ng/g) on 14 th day of the experiment. In the untreated group (G 2) intravitreal injection of 10 ul Freund s adjuvant have significantly increased Levels of α TNF to 0.165 ± 0.024 (ng/g). Treatment of anterior uveitis models in G3, G4, and G5 showed marked significant improvement of α TNF levels as compared to the untreated group. However, the loaded formula (G4) showed more noticeable responses reaching almost normal scores with insignificant differences from the normal control group.

In normal control (G1) activity of the SOD enzyme was 0.0138 ± 0.018 (ng/g) on 14 th day of the experiment. In group 2 intravitreal injection of 10 ul Freund s adjuvant have significantly increased Levels of activity of SOD enzyme to 0.25 ± 0.021 (ng/g). Treatment of anterior uveitis models in G3, G4, and G5) showed marked significant improvement as compared to the untreated group reaching levels of. However, the hydrogel and lecithin-loaded formulas (G3, G4) showed more noticeable responses reaching almost normal scores with insignificant differences from the normal control group.

Results of electroretinogram (ERG) of the retina in uveitis experiment

Results are illustrated in Tables (6,7,8) and Figs. (7,8). The full-field ERGs recordings from the control (G1) untreated uveitis model (G2) and treated rabbits were obtained under dark (DA) and light-adapted conditions (LA). In the untreated uveitis model (G2), the DA response showed a significant decrease in both A wave amplitudes. The LA single flash b-wave response also showed a significant decrease in amplitude in comparison with the control group (G1).

In G3 (animals treated with hydrogel-loaded prednisolone) and G4 (animals treated with hydrogel and lecithin-loaded prednisolone), the photopic b-wave amplitude mean values were significantly higher than that of the uveitis model group (G2). At the same time, there were no statistical differences when compared to the control group. Again, for scotopic ERG, both a-wave and b-wave amplitudes were significantly improved and no statistical differences were found in comparison with control animals. In addition, for any ERG parameters measured, there was no significant difference between G3 and G4.

In animals treated with commercial prednisolone (G5), there was a significant increase only in scotopic b-wave amplitude as compared to G2, while other ERG parameters showed no significant difference to that in the untreated uveitis model.

Discussion

By preparing eye medications as loaded ionic and pH-activated in situ hydrogel formulae, the present study offers a novel solution to the problems with traditional ophthalmic drug delivery systems. With a focus on treating ocular uveitis, these formulations seek to decrease application frequency, increase patient adherence to medication, prolong pre-corneal residency duration, and improve drug absorption [18].

Regarding the studied formulae' trans-corneal penetration, zeta potential, and particle size, the results were promising. The hydrogel formulations had favorable features for particles, as seen by a positive change in zeta potential, which suggests enhanced stability and decreased likelihood of particle aggregation [19]. Furthermore, compared to commercial eye drops, the hydrogel-loaded formulations treated rabbits showed noticeably higher prednisolone concentrations in their aqueous humor. This suggests that improved therapeutic efficacy is a result of prolonged drug release and enhanced ocular absorption [20].

When compared to untreated uveitis models and rabbits treated with commercial prednisolone eye drops, the results of the ophthalmological examination show a significant improvement in the clinical scores of the rabbits treated with the hydrogel-loaded prednisolone formulations. Reduced vascular engorgement and inflammation were seen using a slit lamp examination; the formulae including lecithin and hydrogel had the greatest effects. These results imply that the new formulations successfully reduce the symptoms of uveitis and encourage the healing of ocular tissue. Recent research also has shown the better efficacy and longer duration of chitosan-deoxycholate-loaded prednisolone in treating ocular inflammations than the unloaded prednisolone [21]. However, the present tested formulae add the beneficial effect of adding alginic acid to chitosan. Alginic acid adds to the properties of chitosan (as a mucoadhesive and permeation enhancer) due to instantaneous gel formation by interaction with divalent cations present in lachrymal fluid [(8,9].

Hydroxy Propyl Methyl Cellulose (HPMC) is also used as a copolymer and a viscosity enhancer to

further aid in the accomplishment of sustained drug delivery [10].

Furthermore, lecithin, a naturally occurring phospholipid, is added to the second tested formula in an attempt to preserve cell-membrane fluidity, which will aid in the absorption of drugs from formulations containing lecithin [11]. It has been shown that the lecithin-chitosan nanoparticles, which form between the positively charged chitosan and the negatively charged lecithin, are potentially useful drug carriers. These muco-adhesive nanoparticles work together to stick to the corneal and conjunctival surfaces' mucin layer. The medicine produced from the nanoparticles remains accessible on the ocular surface for extended periods because the mucin layer holds onto them for a long time [12].

Additional evidence for the therapeutic effectiveness of the hydrogel-loaded prednisolone formulations comes from biochemical studies of ocular tissues. The treated groups exhibited a considerable decrease in the levels of pro-inflammatory cytokines, namely IL-1β and α-TNF, which eventually approached levels similar to those of the normal control group. Furthermore, in animals given the hydrogel-loaded formulations, the antioxidant enzyme superoxide dismutase (SOD) activity was brought back to almost normal levels. These findings confirm the innovative drug delivery system's therapeutic potential by indicating the inhibition of oxidative stress and inflammation, two important elements in the pathophysiology of uveitis [22].

The hydrogel-loaded prednisolone formulations' therapeutic efficacy is further supported by the ERG results. When compared to untreated uveitis models, animals treated with these formulations exhibited a considerable improvement in both scotopic and photopic ERG values. Notably, the hydrogel and hydrogel-lecithin-loaded formulations did not differ much, suggesting similar efficacy. Though not as much, commercial prednisolone eye drops also demonstrated some improvement.

The results of this study highlight the potential of in situ hydrogel formulations in enhancing drug delivery and therapeutic outcomes, and they have important implications for the treatment of ocular uveitis. Optimizing the formulations, looking into different pharmacological combinations, and examining long-term safety and efficacy in clinical settings are possible areas of future research. Furthermore, comparative analyses with alternative traditional and innovative drug delivery methods may offer important new perspectives on the relative benefits of in situ hydrogels for ocular drug delivery.

Conclusion

The hydrogel-loaded prednisolone eye drops showed encouraging outcomes in terms of their physicochemical characteristics, ocular penetration, therapeutic efficacy, and safety record when used to treat rabbits' experimentally induced uveitis. These results demonstrate the potential benefits of in situ hydrogel.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Scoring system for experimental uveitis, The total score (16 points maximum) is obtained by the addition of the partial scores assigned to every sign observed

Score	Vascular signs	Pupillary signs	Exudative signs
	A- Conjunctival vessels	A-**pupil size**	**A-presence of fibrin**
0	*Normal	*Normal	*Not observed
1	*Mild dilation	*Mild miosis	*Traces of fibrin on the pupil border
2	*Moderate dilation	*Miosis	*Cyclitic membrane
3	*Diffuse dilation		
	B- Perikeratic vessels	**B-reactivity**	**B- presence of hypopion**
0	*Not observed	*Normal	*Not observed
1	*Incomplete vascular ring *Complete ring	*Slow	*Observed
2		*Not reactive	
	C- Iris vessels		**C-Keratic precipitates**
0	*Normal		*Not observed
1	*Isolated dilated vessels		*Observed
2	*Multiple dilated vessels		
3	*All vessels dilated		

TABLE 2. Particle size and zeta potential of the tested formulas

Formula	Particle size	Unit
Prednisolone 1%-Chitosan-Alginate hydrogel	598.0±159.1	nm
Prednisolone 1%- Lecithin- Chitosan-Alginate hydrogel	512.3±105.2	nm
Formula	**Zeta potential**	**Unit**
Prednisolone 1%-Chitosan-Alginate hydrogel	8.34±0.312	mV
Prednisolone 1%- Lecithin- Chitosan-Alginate hydrogel	22.9±1.92	mV

TABLE 3. The mean concentrations (ng) of instilled drugs against time (hours) in rabbits' aqueous humor after instillation of both loaded formulas and unloaded commercial drugs

Drug	1hour	12 hours	24h
P 1	261.520±10.588	213.547±10.674	33.407±3.601
P2	392.029±22.075	365.564±35.46	48.547±6.63
P3	97.242±27.028	73.342±3.426	4.908±0.768

Prednisolone 1% loaded chitosan-Alginate hydrogel(P1) , prednisolone 1% loaded chitosan-Alginate-lecithin(P2) and commercial prednisolone (P3). (n = 6, value ± SD

TABLE 4. Clinical scores on day 14 (G1 is the normal group, G2 is the anterior uveitis model induced by 10 µl Freund s adjuvant intravitreally, G3 is the model uveitis group treated with prednisolone hydrogel formula, G4 is the model uveitis group treated with prednisolone hydrogel and lecithin formula, G5 is the model uveitis group treated with commercial prednisolone formula. The eye drops were applied once daily for 14 days.

Groups	Mean ±SD	P1 value	P2 value
G1	0.0000. ±0.0000		0.000
G2	9.5000±2.58844	0.000	
G3	1.5000±1.64317	1.000	0.000
G4	1.1667±1.16905	1.000	0.000
G5	4.6667±1.36626	0.000	0.000

Data are expressed as mean + SD , n=6 , percent improvement compared to model untreated group(G2) ,P1: Compared to group 1, P2: Compared to group 2

TABLE 5. The levels of IL-1β, α-TNF and the activity of SOD in uveitis of all experimental groups treated with prednisolone formulas (G1 is the normal group, G2 is the anterior uveitis model induced by 10 µl Freund s adjuvant intravitreally, G3 is the model uveitis group treated with prednisolone hydrogel formula, G4 is the model uveitis group treated with prednisolone hydrogel and lecithin formula, G5 is the model uveitis group treated with commercial unloaded prednisolone formula. The eye drops were applied once daily for 14 days).

	IL-1β (ng /g. tissue)	α-TNF (ng /g. tissue)	SOD (ng /g. tissue)
Group 1: Control	0.027 ± 0.0023^{a}	0.057 ± 0.008^{a}	0.138 ± 0.018^{a}
Group 2: Uveitis model	0.047 ± 0.0034^{b}	0.164 ± 0.024^{b}	0.25 ± 0.021^{b}
Group 3: Prednisolone+ hydrogel	0.035 ± 0.004^{c}	0.092 ± 0.008^{c}	0.156 ± 0.015^{a}
Group 4: Prednisolone+ hydrogel+ lecithin	0.027 ± 0.0046^{a}	0.061 ± 0.009^{a}	0.14 ± 0.013^{a}
Group 5: Prednisolone Market	0.037 ± 0.006^{c}	0.094 ± 0.0038^{c}	0.2 ± 0.023^{c}

Data are represented as means ±SD; n=6 for each group. Different letters within the same column represent statistically significant values ($p<.05$) while the same letters represent statistically non-significant values.

TABLE 6. Mean values (±SD) of retinal Photopic b waves amplitudes (µv) of (ERG) of control (G1), untreated uveitis model (G2) and treated groups (G3) (G4) (G5)

	Controls	Model	G3	G4	G5
Mean ±SD	58.0333 ±2.39472	24.9500 ±10.95933	55.3833 ±17.63978	52.0000± 12.29602	30.6833± 12.21465
P1		0.001*	1.000	1.000	0.006*
P2			0.002*	0.007*	1.000
P3				1.000	0.017*
P4					0.055

Data are expressed as mean ± SD, n=6 *Significant difference when (P<0.05). G1 is normal control, G2 is the model untreated uveitis, G3 is uveitis treated with hydrogel loaded prednisolone, G4 is uveitis treated with hydrogel lecithin loaded prednisolone and G5 is uveitis treated with commercial unloaded prednisolone).

P1= G2, G3, G4 compared to G1 P2= G3, G4, G5 compared to G2P3 = G4, G5 compared to G3 P4 = G5 compared to G4

TABLE 7. Mean values (±SD) of retinal scotopic a wave mixed response waves amplitudes (µv) of (ERG) of control (G1), untreated uveitis model (G2), and treated groups (G3), (G4)and (G5)

	Controls	Model	G3	G4	G5
Mean± SD	47.2000±7.40810	24.7333±9.53471	40.7333±11.24040	40.3500±13.16127	28.7333±4.90985
P1		0.005*.	1.000	1.000	0.029*.
P2			0.084	0.099	1.000
P3				1.000	0.419
P4					0.483

Data are expressed as mean ± SD, n=6 *Significant difference when (P<0.05).
(G1 is normal control, G2 is the model untreated uveitis, G3 is uveitis treated with hydrogel loaded prednisolone, G4 is uveitis treated with hydrogel lecithin loaded prednisolone and G5 is uveitis treated with commercial unloaded prednisolone)
P1= G2, G3, G4 compared to G1 P2= G3, G4, G5 compared to G2
P3 = G4, G5 compared to G3 P4 =G5 compared to G4

TABLE 8. Mean values (±SD) of retinal scotopic b wave mixed response waves amplitudes (µv) of (ERG) of control (G1), untreated uveitis model (G2), and treated groups (G3), (G4)and (G5)

	Controls	Model	G3	G4	G5
Mean±SD	99.5000±4.97755	34.7667±10.58200	88.5000±13.58116	88.0500±18.15894	63.0667±12.26029
P1		0.000*.	1.000	1.000	0.000*.
P2			0.000*	0.000*	0.007*.
P3				1.000	0.019*.
P4					0.022*.

Data are expressed as mean ± SD, n=6 *Significant difference when (P<0.05).
(G1 is normal control, G2 is the model untreated uveitis, G3 is uveitis treated with hydrogel loaded prednisolone, G4 is uveitis treated with hydrogel lecithin loaded prednisolone and G5 is uveitis treated with commercial unloaded prednisolone)
P1= G2, G3, G4 compared to G1 P2= G3, G4, G5 compared to G2
P3 = G4, G5 compared to G3 P4 =G5 compared to G4

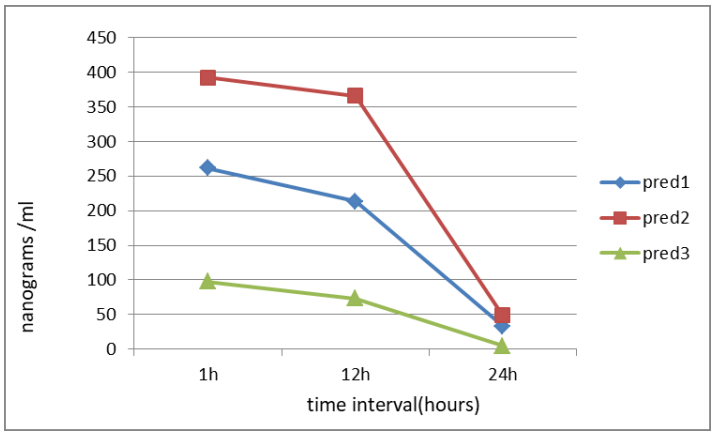

Fig. 1. Plot of concentration against the time of prednisolone in rabbits' aqueous humor after instillation of Prednisolone 1% loaded chitosan-Alginate hydrogel (P1), prednisolone 1% loaded chitosan-Alginate-lecithin(P2) and commercial prednisolone (P3). (n = 6, value ± SD)

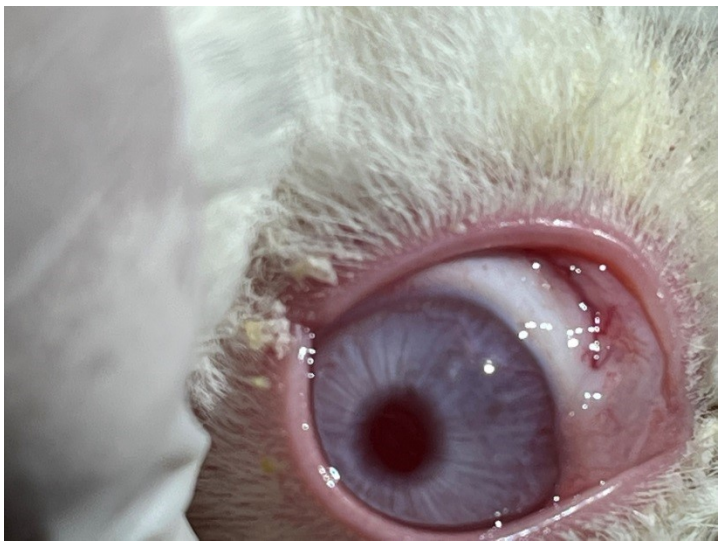

Fig. 2. Normal control group (G1) showing the anterior segment of the rabbit's eye with the normal iris and conjunctival vessels and clear anterior chamber. The normal dilated and reactive pupil is also noted.

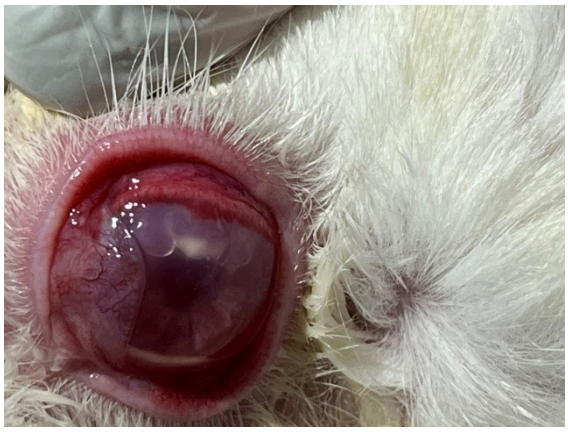

Fig. 3. Untreated anterior uveitis model (group 2) showing highly engorged iris vessels with anterior chamber fibrin formation in front of the lens, diffuse conjunctival redness with complete perihepatic ring vessels, miotic non-reactive pupil also observed.

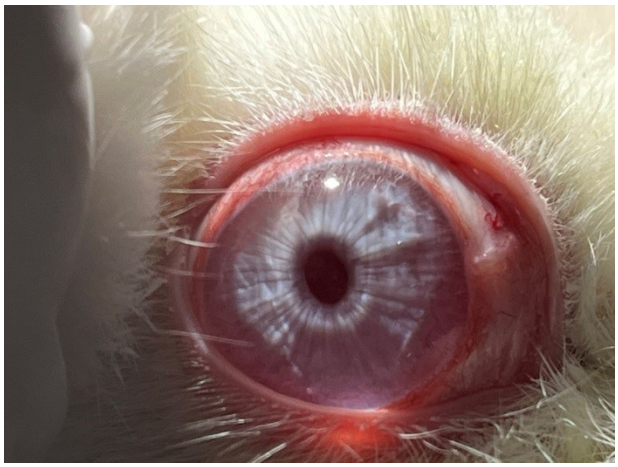

Fig. 4. Anterior uveitis model (group 3) treated with prednisolone loaded hydrogel 1% eye drops once daily showing mildly engorged iris vessels with clear anterior chamber and normal reactive pupil in front of the lens.

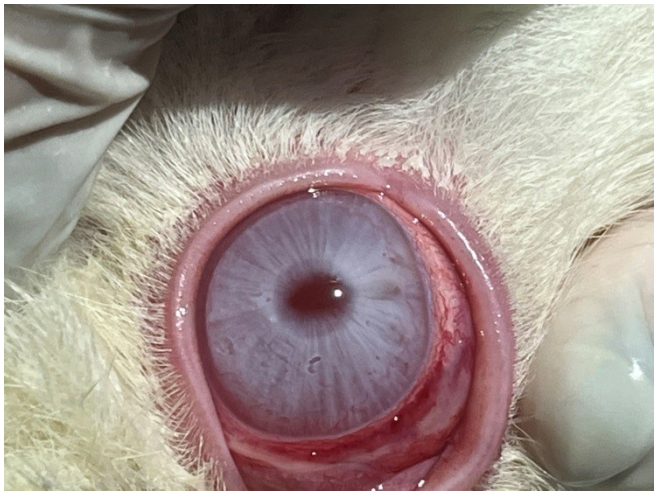

Fig. 5. Anterior uveitis model (group 4) treated with prednisolone-loaded hydrogel and lecithin 1% eye drops once daily showing markedly improved vascular engorgement of iris and conjunctival vessels with the clear anterior chamber and normal reactive pupil in front of the lens.

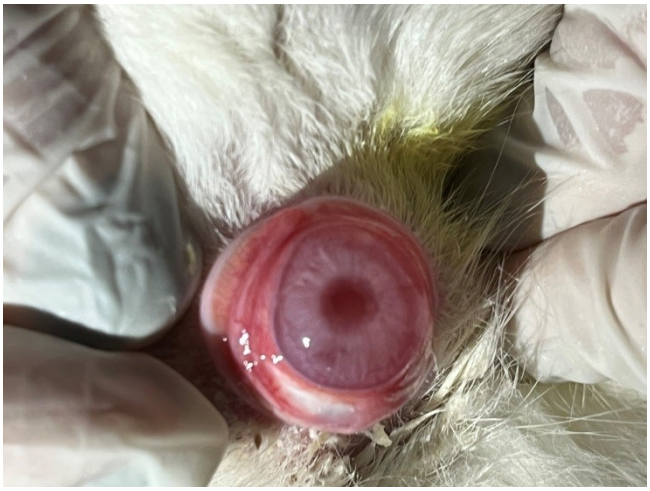

Fig. 6. Anterior uveitis model (group 5) treated with unloaded prednisolone (market) 1% eye drops once daily showing moderately engorged iris and conjunctival vessels with unobserved anterior chamber due to fibrin formation in front of the lens. The pupil is normal and reactive.

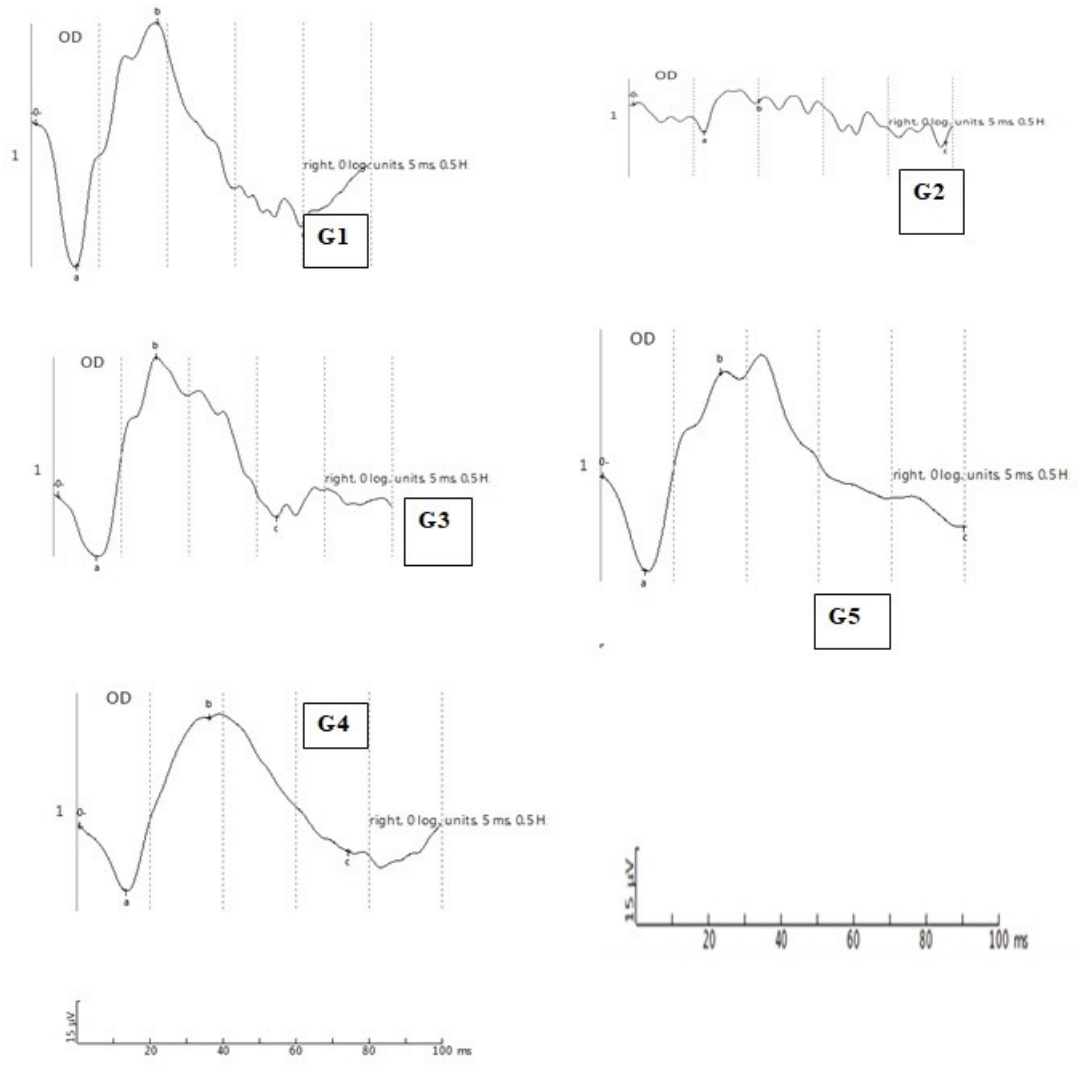

Fig. 7. Dark-adapted electroretinographic (ERG) recordings of selected rabbit eyes in control group (G1), untreated uveitis model (G2), animals treated with hydrogel loaded (G3)prednisolone), animals treated with hydrogel lecithin(G4), animals treated by commercial prednisolone (G5), the ERG mixed response demonstrated reduced a- and b-wave amplitudes in G2 group. Dramatic improvement in G3 and G4. G5 showed also moderate improvement.

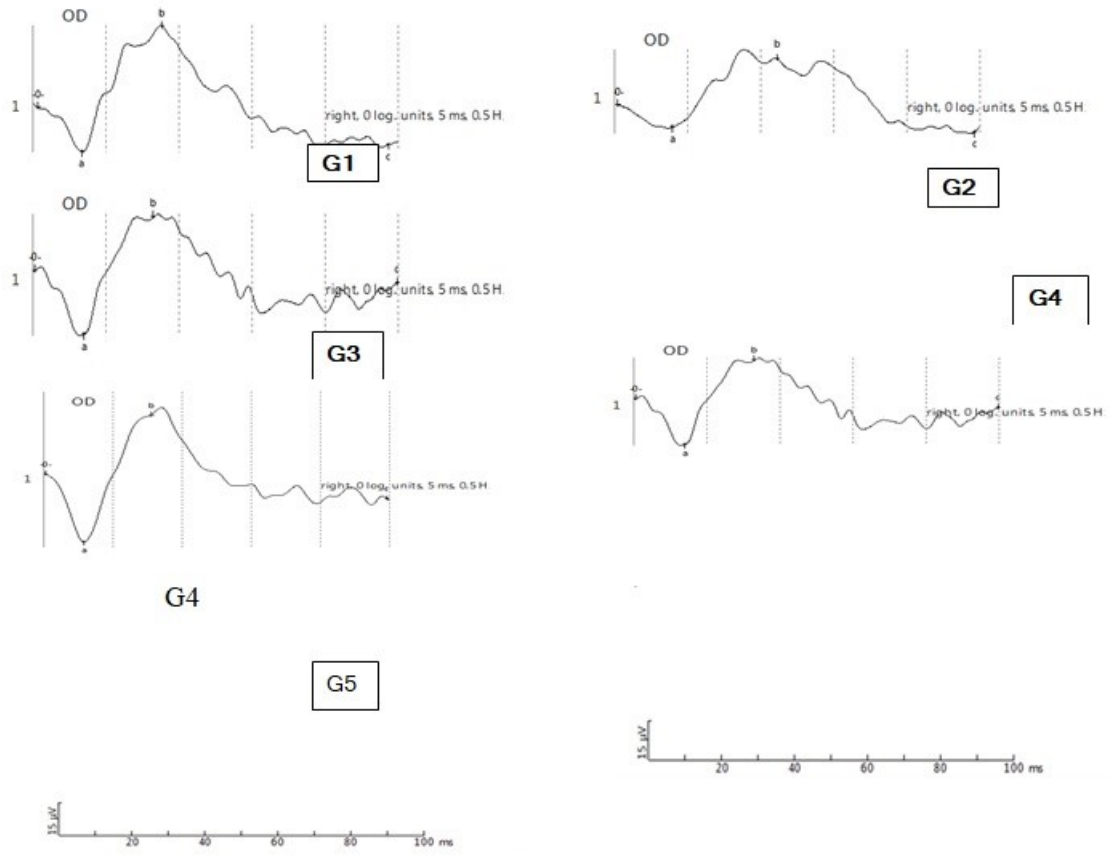

Fig.8. Dark-adapted electroretinographic (ERG) recordings of selected rabbit eyes in the control group (G1), untreated uveitis model (G2), animals treated with hydrogel loaded (G3) prednisolone, animals treated with hydrogel lecithin (G4), animals treated by commercial prednisolone (G5). The ERG light-adapted response demonstrated reduced b-wave amplitudes in the G2 group. Dramatic improvement in G3 and G4. G5 showed also moderate improvement.

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تقييم هلام مائي موضعي للعين يحتوي على بريدنيزولون لمكافحة التهاب العنبية المستحث تجريبياً في الأرانب

اماني حسن الشاذلي[1] ، غادة غانم الحصري[1]، اماني عبد الغفار[2]، عاطف محمد محمود[2]، لقاء مؤمن[2]، مروة الفولي[3]، امل الجوهري[4] و احمد مصطفى عطا[2]

[١] قسم علم الأدوية، [٢] قسم الكيمياء الحيوية، [٣] قسم طب العيون، [٤] قسم فسيولوجيا العين - معهد بحوث أمراض العيون – الجيزة – مصر.

المخلص

من القيود الرئيسية في توصيل الأدوية للعين هو ضعف التوافر الحيوي للأدوية من قطرات العين التقليدية. تشمل عوامل فقدان الدواء قبل القرنية سرعة دوران الدموع، وقت الإقامة العابر، وعدم نفاذية القرنية النسبي. في بعض الأحيان، قد يؤدي الامتصاص العام للجسم للأدوية إلى آثار جانبية.

للتغلب على هذه المشكلات، تم تطوير أنظمة توصيل الأدوية المضبوطة، بما في ذلك أنواع مختلفة من أنظمة تصنيع الهلام موضعيا مبنيا على آليات مختلفة (الحرارة، الرقم الهيدروجيني، أو التنشيط الأيوني) باستخدام بوليمرات مختلفة تم استكشافها للتوصيل الموضعي. تهدف هذه الدراسة إلى صياغة الأدوية العينية كتركيبات هلامية مائية موضعية و يمكن تنشيطها أيونياً وبال Ph و محملة بالدواء لأنظمة توصيل الأدوية المحملة بهدف زيادة وقت الإقامة بالقرنية وتقليل تكرار التطبيق، والحد من الآثار الجانبية، وتحسين التزام المريض بالعلاج. ستجري هذه الدراسة اختبارات بيوكيميائية و اختبارات اكلينيكية لقطرات عين البريدنيزولون المحملة بمزيج من الليسيثين وحمض الألجينيك والكيتوزان في نموذج حيواني للتهاب العنبية المستحث.

الكلمات الدالة: ليسيثين، الكيتوزان، وحمض الألجينيك، قطرة للعين، التهاب العنبية