Trimetazidine Topical Delivery Prevents Carrageenan Induced Retinal Damage and Glucose Induced Lens Opacification by Decreasing Drop of Protein Level

Rakesh Swain

Siksha O Anusandhan University School of Pharmaceutical Sciences

Souvik Nandi

Siksha O Anusandhan University School of Pharmaceutical Sciences

Rudra Narayan Sahoo

Siksha O Anusandhan University School of Pharmaceutical Sciences

Sujata Mohapatra

Siksha O Anusandhan University School of Pharmaceutical Sciences

Subrata Mallick (✉ profsmallick@gmail.com)

Siksha O Anusandhan University School of Pharmaceutical Sciences <https://orcid.org/0000-0002-6606-1223>

Research Article

Keywords: Trimetazidine ocular delivery, carrageenan induced retinal damage, Glucose induced stress, Cataract prevention

Posted Date: September 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-890324/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Carrageenan induced oxidative stress develops retinal damage and increases lipid peroxidation. Glycation of the lens proteins increases modified proteins and may affect the lens structural integrity and opacification, after increasing glucose concentration in the lens. Trimetazidine (TZ) has been reported to have cytoprotective anti-ischaemic activity after systemic administration in an animal model. Trimetazidine ophthalmic film formulation has been prepared using HPMC as the hydrogel-forming polymer. The cytoprotective role of trimetazidine has been studied on carrageenan-induced retinal damage of rat eye by histological and Malondialdehyde (MDA) assay applying sterilized film formulation. Trimetazidine solution was used in combination with a high concentration of glucose solution (55 mM) for examining the protective effect from cataract formation. Formulation showed controlled and extended ophthalmic permeation of the drug for about 5 h. Damage of retinal cells in the ganglionic layer has been decreased significantly in the trimetazidine-treated group. Drug-treated control group also showed significant disappearance of pyknotic cells. The addition of trimetazidine to the glucose medium practically retarded the lens opacification by reducing the over-production of reactive oxygen species (ROS) in increased sugar concentration. Presence of trimetazidine probably decreased the drop of protein level in lens under stressed condition and greater preventive effect has been noticed with TZ concentration of 240 µg/ml rather than 120 µg/ml. Trimetazidine ophthalmic film formulation could prevent retinal damage and cataract formation.

Introduction

Oxidative stress plays a vital role in developing retinal diseases such as cataract, glaucoma, diabetes retinopathy, macular degeneration, uveitis etc. In oxidative stress, an excess amount of reactive oxygen species (ROS) generated lead morphological and functional impairments in retinal ganglionic cells [1, 2]. The carrageenan-induced ocular inflammation has been well accepted oxidative stress model which increases lipid peroxidation with an end product of MDA and also reduces the antioxidant level during the acute phase of inflammation in cells [3]. In the acute inflammation process, the leukocyte emigration (diapedesis) occurs and the inflammatory mediators including neutrophils adhere to the endothelium releasing protease and reactive oxygen species responsible for cell damage [4].

Loss of lens transparency associated with multi-factorial oxidative stress such as hypertension, smoking, diabetes and others leads to cataract formation. The aetiology involved in hypertensive cataract is the decrease in Na^+K^+ Adenosine Triphosphate activity in the lens epithelium [5]. Oxidative damage of the lenticular component occurs due to the aromatic compounds present in the inhaled smoke [6]. The oxidative stress prevents glutathione and other protective antioxidants from reaching the nucleus of the lens which leads to cataract formation [7]. In some cases mutations in enzymes of cholesterol metabolism bring about increased accumulation and redistribution of cholesterol inside these cells causing cataract formation [8]. Increased reactive oxygen species level (ROS) and decreased antioxidant efficiency are the major causes of the pathogenesis of cataract formation in chronic diabetic condition. In diabetic condition excess amount of sugar is reduced by aldose reductase forming sorbitol (an alcoholic

form of sugar) followed by fructose formation. Glucose moves freely across the lens and also the retina cell membrane due to the insensitivity of insulin [9]. But the produced sorbitol does not diffuse through the membranes easily causing oxidative stress, osmotic damage and increased sodium ion accumulation leading to cataract formation [10, 11].

Deamidation, glycation truncation, and oxidation have all been identified as covalent modifications associated with lens protein damage by proteomic analysis [12, 13]. Deamidation is one of the most common crystallin degradation mechanisms, giving a negative charge to the protein by converting glutamine residues to glutamate. Asparagine is likewise deamidable, and both residues are altered in cataractous aggregates [14, 15]. Several oxidation sites on the crystallins have been discovered, with tryptophan, cysteine, and methionine residues being targeted. In aged cataractous lenses, certain cysteine residues on b/g-crystallins were shown to be oxidized [16]. Experiments indicating that the presence of an oxidised crystalline peptide enhanced aggregation of both b and g-crystallins imply that truncation events may induce more significant for protein destabilization [17]. Due to oxidative stress, ROS can attack the amino acid residues, resulting in oxidation of side chains and/or breakage of polypeptide bonds. This affects the non-covalent interaction that holds the protein together correctly, causing protein destabilization and perhaps causing the protein to unfold. Uncontrolled glucose level in the lens can directly cause protein glycation and affects the chaperone function by destabilizing α -crystallin generating adducts, and alters the biological characteristics of the protein.

TZ a class of metabolic modulator is commonly used as an anti-anginal agent [18]. It exhibits a short elimination half-life of about 3 to 4 h and needs frequent oral administration of 3 times daily [19-21]. Cytoprotective anti-ischaemic activity of TZ has been reported after intraperitoneal [22] and oral [23] administration. Trimetazidine inhibits Na^+ , Ca^+ accumulation, cell acidosis at the molecular level and decreases the production of free radicals, and expected to retard the progression of cataract formation if delivered topically [23, 24]. Trimetazidine has been reported to act as a protective agent in retarding selenite induced lens opacification in neonatal rats (age 8 to 10 days) before eyelid opening after ip administration [25, 26]. Selenite induced cataract model studies are laborious and time-consuming also (72 to 96 hours) [27]. After eyelid opening selenite cannot induce lens opacification even in high dose administration [28]. In vitro glucose inducing cataract model could provide a fast and effective model on the isolated lens of goat [29].

Present research was undertaken to develop a thin hydrogel-forming trimetazidine film formulation for ocular administration in the cul-de-sac. The cytoprotective role of trimetazidine has been studied on lipopolysaccharide-induced retinal damage in a rat eye model. The effects were analyzed by Thiobarbituric acid reactive substances (TBARS) assay and histological study [30, 31]. Current study was also focused on investigating the protective effect of TZ if any in a glucose-induced in-vitro cataract model on isolated goat eye lens after local application.

Experimental

Materials

Trimetazidine dihydrochloride was collected as a free sample from Medley Research Centre (Mumbai, Maharashtra), Hydroxypropyl methylcellulose (HPMC K 15M) was purchased locally from Burgoyne and Co (Mumbai, India). Triethanolamine was purchased from HIMEDIA, Mumbai, India. All other chemical reagents used were of analytical grade.

Animal

Institutional animal ethics committee (IAEC) approval has been taken for this animal experiment [Approval No. IAEC/SPS/SOA/04/2020] for Control and Supervision of Experiments on Animals (CPCSEA) Guidelines, India. Westar male rats between 170-200 gm were taken and kept under constant environmental condition of 22 ± 1 °C (RH 50-60 %) with natural light. Animals were fed normal laboratory diet and water ad libitum. All the animals were healthy and free from clinically ocular abnormalities.

Fabrication of Trimetazidine Ocular Film

HPMC K 15M was swelled in the presence of an adequate amount of water (1 gm/40 ml) in refrigeration condition for 12 h. The swelled gel was stirred magnetically for 24 h. Trimetazidine and triethanolamine were dissolved in a small amount of water and poured into the HPMC dispersion with continuous stirring for 3-4 hour. Trimetazidine ocular film formulation was prepared by casting and solvent evaporation method on Petri plate (Tarsons, diameter: 90 mm). Drying was done at 40 °C in an incubator until the consecutive weighings were not varying. The thickness of the film formulation was measured by a digital micrometer (Mitutoyo, Japan). Folding endurance was conducted by repeated folding a piece of film at the same place until it breaks. The surface pH of the film was measured by dipping the pH meter's glass electrode inside the swelled segment of the film formulation [32].

Infrared Spectroscopy Analysis

Infrared spectroscopy analysis of trimetazidine and the prepared TZ film formulation was carried out in FTIR-Spectrophotometer (Model-JASCO FTIR-4100, Japan) by using the KBr pellet method.

Differential Scanning Calorimetry (DSC)

Differential scanning analysis of pure trimetazidine and the film was performed by differential calorimeter (DSC-1, Mettler Toledo; Software- Star E, SNR- 18289) within a range of 30-300 °C in a constant heat flow of 10 °C per minute in continuous nitrogen purging of 50 ml per min.

Dissolution study

Assessment of the in vitro drug release was done by USP type-II (Paddle type) dissolution apparatus (Electrolab TDT067 Dissolution tester). Accurately weighed a suitable cut piece of film was glued properly onto a glass slide by using adhesive (cyanoacrylate) and merged completely inside the dissolution vessel containing 200 ml phosphate buffer (pH 7.4) as dissolution medium. Dissolution test was carried out at

temperature 34.0 ± 0.5 °C with 50 rpm paddle rotation speed. Samples were withdrawn as per predetermined time intervals and analyzed spectrophotometrically at λ_{max} 269 nm after filtering through a 0.45 µm syringe driven filter [32].

Ex-vivo corneal permeation study

Goat eyeballs were acquired from a local slaughterhouse within 1 h after sacrificing. These were properly rinsed with distilled water followed by phosphate buffer (pH 7.4). The cornea along with about 5 mm wide scleral ring was excised from ocular globe of goat and again rinsed with phosphate buffer solution. A pre-weighed circular cut piece of film was placed on the middle of the epithelial corneal surface and faced towards the donor compartment side of the diffusion chamber with an effective surface area of 1.56 cm². Endothelial side compartment was filled with phosphate buffer (pH 7.4, 200 ml) and ex vivo corneal permeation was carried out for six hour at 34 ± 2 °C under steady stirring speed of 50 rpm. At the regular time interval, 10 ml of samples were withdrawn after filtering through a 0.45 µm syringe driven filter. Absorbance was recorded at 269 nm using a Ultra-Violet visible spectrophotometer (JASCO V-630). The experiment was triplicated and mean $\pm$ SD were recorded [32].

Oxidative stress

Wistar male rats were divided into three groups containing 3 animals each. Group-I was served as a test group (a small piece trimetazidine film (2 mg/Kg dose) previously sterilized by UV exposure, followed by carrageenan injection after 1 h), Group-II was served as + ve control group (not drug-treated), and Group-III was served as a control group. Lipopolysaccharide (carrageenan solution 20 µl, 2 % w/v) was injected into the sub-conjunctiva (upper palpebral region) in the rat eye for induction of oxidative stress [33]. Before commencing sub-conjunctival injection, the rats were anaesthetized with an intraperitoneal injection (0.1 ml/100 g of rat weight of ketamine-xylazine mixture at 91 and 9.1 mg/kg respectively) [34]. The sign of reddening of the conjunctiva followed by tearing was visualized. The rats were euthanized within 1 h of carrageenan injection under a higher dose of thiopental sodium anaesthesia. Then the eyeballs were removed to carry out histology and Malondialdehyde (MDA) (a product of lipid peroxidation) assay.

Histopathological examination

Histological evaluation was performed by taking the rat eyes which were enucleated and firmly place in paraffin. The sections were obtained in the inferior portion and the vertical meridian of the eyeball. Each paraffin block was sectioned with 5 µm and staining was done using hematoxylin and eosin. The histopathology of the specimens was examined using a photo microscope Olympus make model Magnus MLX-TR with a digital camera.

Determination of lipid peroxidation

Lipid-peroxidation was carried out to measuring the MDA according to the Thiobarbituric acid (TBA) test described by Yeh et al. 1990 with the following modification [30]. After treatment, the whole eyeballs were homogenized with a one-tenth lens weight of 6 % perchloric acid (HClO_4) at 0 °C by using a glass homogenizer. The sample was then centrifuged for 15 minutes at 4 °C and 200 μl of supernatant was separated in a test tube. In the test tube 42 μl of sodium acetate (5 mol/l), 200 μl of 8 % sodium lauryl sulphate and 200 μl of thiobarbituric acid (0.8 %) were added. The tube was then placed into a heating block at 95 °C for 50-60 min and cooled with tap water. Butanol and pyridine mixture (15:1) of 2.5 ml and distilled water 0.5 ml was added over the sample mixture and centrifuged at 4000 revolutions per min. At λ_{max} of 532 nm the absorbance of the organic layer was measured and MDA level was estimated in nmol per g on wet tissue.

Lens culture

Fresh goat eyeballs were acquired from a local slaughter house and stored in refrigeration condition (about 4 °C). The extracapsular method was adapted for the removal of the lens then collected lens were incubated in simulated aqueous humour (NaHCO_3 0.5 mM, KCl 5 mM, $\text{NaH}(\text{PO}_4)_2$ 0.5 mM, CaCl_2 0.4 mM, NaCl 140 mM, MgCl_2 2 mM and Glucose 5.5 mM, pH 7.8) at room temperature for 4 h. Streptomycin 250 mg % and penicillin 32 mg % were combined with the culture media to avoid microbial growth [35]. This healthy normal isolated lens was used as control (Group-I). A high concentration glucose of 55 mM was used to induce cataract of the isolated lens (Group-II) [35]. In the drug-treated group trimetazidine solution was used in combination with glucose solution for examining the protective effect of cataract formation. Here, Group-III Group-IV were served as treatment group with trimetazidine (120 $\mu\text{g/ml}$, and 240 $\mu\text{g/ml}$) incubation of lens both in combination with glucose (55 mM) respectively.

Lens protein Content

The protein estimation of the samples was carried out as per Lowry et al. 1951 technique, with bovine serum albumin serving as the standard [36].

Results And Discussion

Film properties

Prepared films were found of uniform thickness ($86 \pm 5 \mu\text{m}$) with an almost transparent appearance. Surface pH was found (7.0 to 7.2) to be very much compatible and nonirritant to the eye. Prepared film formulations have shown good folding-endurance (>200) and not brittle and soft enough to produce sufficient flexibility upon placing in the ocular cul-de-sac because of the presence of triethanolamine as a plasticizer.

FTIR

The FTIR spectrum of trimetazidine showed the characteristic bands of –CH stretching, -NH₂ bending, -OH stretching, -CH bending, and -C-O stretching at 2923, 1600, 3489, 1415, and 1099 cm⁻¹ respectively (Fig. 1a) [37, 38]. The spectrum obtained of the film formulation indicates the presence of the characteristic peaks of the drug at almost the same wavenumber. In the film formulation (F) the characteristic -NH₂ bending peak at 1600 shifted to 1587 cm⁻¹ with a decreased intensity. The broadening of the absorption band in the range of 3400-3200 cm⁻¹ was observed due to the presence of polymer. Shifting and broadening in the film formulation is a major evidence of H-bond formation due to the interaction between drug and polymer [37].

Thermal analysis

Thermal analysis of pure trimetazidine showed a sharp melting endothermic peak at 236.13 °C with the onset of melting of 228.48 °C confirming its crystalline characteristics (Fig. 1B). Shifting of the endothermic melting peak with significantly reduced enthalpy is a sign of amorphous formation of the drug crystals to a marked extent in the formulated film [39]. It might have happened owing to the arresting of the drug amorphous state into the HPMC matrix [40]. A wide endothermic shouldering in between 80 to 100 °C was observed due to the water evaporation from the film-matrix formulation containing HPMC as the hydrogel-forming polymer matrix.

Drug release and corneal permeation

The result of drug release experiment of TZ from HPMC film in the dissolution medium (34 °C, pH 7.4) is shown in (Fig. 1C). Drug release from the film has been continued for more than 180 min in a controlled manner. Sustained ex vivo corneal permeation was also noticed to be continued more than 5 h as depicted in (Fig. 1D). Polymeric hydrogels are playing an important role in the recent development of mucosal drug delivery system [41]. Hydroxypropyl methylcellulose (HPMC) is widely used in ocular drug delivery because of its mucoadhesivity, biocompatibility and biodegradability. HPMC can increase the retention time at the site of application on the mucosal layer of the cul-de-sac resulting in decreased nasolacrimal drainage and improved ocular bioavailability. This polymer has also a role in ocular protection and tolerability by avoiding conjunctival dehydration [3]. It is absorbed tear fluid from the adjacent environment and entangles with the mucin of the conjunctiva which is resulting in a gel layer followed by slowly releasing the entrapped drug.

Histopathologic examination

The histopathologic specimens of all three groups are depicted in (Fig. 2). Distinguished edema (inner plexiform layer), degeneration of retinal ganglionic cell layer, and appearance of pyknotic cells in the inner nuclear layer of induced rat eye retina were noticed. Retinal cell damage was clearly understood when compared with the control group of histological specimens without drug treatment. The severity of damage of retinal cells in the ganglionic layer has been decreased significantly in the drug-treated group. The number of pyknotic cells were found also very few in the inner nuclear layer in the drug pretreated

group. The cytoprotection activity of this drug might have acted by increasing metabolic process in the cells followed by inhibition of Ca^{+2} , Na^{+} accumulation. It helped in decreasing cell acidosis as well as the production of free radicals.

Determination of lipid peroxidation

The enzymatic assay was performed to determine the MDA level induced by oxidative damage [42]. Malondialdehyde was measured as a marker of the free radical initiated oxidative stress damage. The results of estimated MDA level in rat eye are reported in (Fig. 3). The mean MDA level of the positive control (carrageenan-induced and without drug treatment), negative control (non induced and without drug treatment), and test (carrageenan-induced and with drug treatment) clearly indicate the cytoprotective effect on ocular tissue. The MDA level of drug-treated group was significantly reduced as a comparison to the without drug-treated group. Trimetazidine was inhibited the boosting of MDA label of the retinal epithelial tissue undergone by oxidative stress. It decreased the oxidative stress that happened during the carrageenan injection, indicated as a partial reduction in ROS [43].

Physical appearance of lens

Oxidative stress condition has been induced in the lenses after incubating isolated lenses in glucose medium of 55 mM. In oxidative stressed condition, denaturation and aggregation of lens proteins have been progressed resulting in the precipitation and opacification of the lens. Accumulation of sorbitol in the induced lens produced opacity.

During oxidative stress, the lens proteins denature which forms disulfide cross-linking causing disulfide and mixed disulfide bond formation, resulting in protein aggregation and precipitation leading to lens opacification after increasing glucose concentration in the lens [44]. The use of trimetazidine acted as an antioxidant and retarded the formation of cataract. The preventive role of trimetazidine hydrochloride has been demonstrated photographically in (Fig. 4a-d). Trimetazidine inhibits beta-oxidation of free fatty acids and stimulates glucose oxidation, (conversion of glucose into energy further reduces glucose amount locally) in the lens and possibly inhibits the cataract formation.

Histology of lens

The histopathological profile of the lens is depicted in (Fig. 5a-b). In the control group, the presence of normally configured anterior epithelium, lens fibers and capsule are clearly visible. But, in the case of induced group, anterior epithelium is almost absent [45]. According to the distribution of fibres in induced group, the fragmentation of fibers have occurred [46] while in drug treated group, retardation of the epithelial cell loss and fibers fragmentation was clearly visible. In diabetic condition, accumulation of sorbitol occurs which leads to increases the water volume in lens followed by raises the osmotic pressure and rupturing of the lens fiber.

Lens protein Content

Effect of Trimetazidine on protein level in isolated goat lens has been depicted in Fig. 6. Under oxidative stress condition lens protein denatures and forms disulfide cross-links resulting in protein aggregation and lens opacification. In TZ-treated group the protective effect has been observed by preventing protein denaturation and aggregate formation. Presence of trimetazidine might have decreased the drop of protein level in lens under stressed condition. The protein level of cataract induced lens (Group-II) has decreased significantly as compared to control lens (Group-I) at p value 0.0075. Cataract induced lens protein has statistically significant with treated lens protein Group-III ($p=0.0190$) and group-IV ($p=0.0114$). In presence of trimetazidine lens protein level has not been decreased significantly in both the Group-III ($p=0.0509$) and Group-IV ($p=0.2530$) at concentration 120 $\mu\text{g/ml}$ and 240 $\mu\text{g/ml}$ respectively. Better preventive effect has been observed with TZ conc. 240 $\mu\text{g/ml}$ compared to 120 $\mu\text{g/ml}$.

Conclusions

The polymeric film of trimetazidine was produced of adequate strength and flexibility to meet the patient compliance. DSC thermogram revealed that the crystalline drug was almost amorphised in the film formulation. Controlled *ex vivo* ocular permeation of the drug was observed from the film formulation for about 5 h. Trimetazidine exhibited a marked effect in combating the oxidative stress and pyknotic cell formation. The degenerative changes due to oxidative stress of carrageenan has also been prevented. Trimetazidine ocular delivery prevented carrageenan induced retinal damage and glucose induced lens opacification *in vitro* to a promising level. Presence of trimetazidine prevented the protein denaturation and aggregate formation and also decreased the drop of protein level in the lens under stressed condition.

Declarations

ETHICAL STATEMENT:

Ethics approval and consent to participate: This study was approved by animal ethics committee (IAEC) [Approval No. IAEC/SPS/SOA/04/2020] for Control and Supervision of Experiments on Animals (CPCSEA) Guidelines, India.

Consent for publication: All authors read and approved the final version of the manuscript.

Availability of data and materials: A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Competing interests: The authors declare no conflict of interest associated with the manuscript.

Funding: Not applicable

Authors' contributions: Rakesh swain and Subrata Mallick have given substantial contribution to the conception and design of the manuscript. Souvik Nandi and Rudra Narayan Sahoo assisted during the in vivo, ex vivo studies. Rakesh swain has prepared the final manuscript which was further reviewed by Sujata Mohapatra. Subrata Mallick was in charge of the whole operation, rectification and approval of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements: The authors are grateful to the Department of Science & Technology, Ministry of Science & Technology, New Delhi, India, for providing INSPIRE fellowship to Rakesh Swain (IF 180581). The authors are also very much grateful to the President, Siksha O Anusandhan (Deemed to be University) for providing other facilities. The authors are also grateful to Medley Pharmaceutical Ltd. for the gift sample of Trimetazidine Dihydrochloride.

References

1. Masuda T, Shimazawa M, Hara H. Retinal diseases associated with oxidative stress and the effects of a free radical scavenger (Edaravone). *Oxid Med Cell Longev*. 2017.
2. Hironaka K, Inokuchi Y, Fujisawa T, Shimazaki H, Akane M, Tozuka Y, Tsuruma K, Shimazawa M, Hara H, Takeuchi H. Edaravone-loaded liposomes for retinal protection against oxidative stress-induced retinal damage. *Eur J Pharm Biopharm*. 2011;79(1):119-25.
3. Mohapatra R, Mallick S, Nanda A, Sahoo RN, Pramanik A, Bose A, Das D, Pattnaik L. Analysis of steady state and non-steady state corneal permeation of diclofenac. *RSC Adv*. 2016;6(38):31976-87.
4. Imhof BA, Dunan D. Basic mechanism of leukocyte migration. *Horm Metab Res*. 1997;29(12):614-21.
5. Miller D, Tijerina ML, Mayman C. In vitro production of steroid cataract in bovine lens: Part I: Measurement of optical changes. *Acta Ophthalmol*. 1979;57(6):1101-6.
6. West S, Munoz B, Vitale S, SCHEIN O, Maguire M, Bressler N. Watermen Study-II-Smoking and Nuclear Opacities. *Invest Ophthalmol Vis Sci*. 1992;33(4):1097-1097.
7. Gupta VB, Rajagopala M, Ravishankar B. Etiopathogenesis of cataract: an appraisal. *Indian J Ophthalmol*. 2014;62(2):103.
8. Cenedella RJ. Cholesterol and cataracts. *Surv Ophthalmol*. 1996;40(4):320-37.
9. Sharma DS, Wadhwa S, Gulati M, Kadukkattil Ramanunni A, Awasthi A, Singh SK, Khursheed R, Corrie L, Chitranshi N, Gupta VK, Vishwas S. Recent advances in intraocular and novel drug delivery systems for the treatment of diabetic retinopathy. *Expert Opin Drug Deliv*. 2020:1-24.
10. Murata M, Ohta N, Sakurai S, Alam S, Tsai JY, Kador PF, Sato S. The role of aldose reductase in sugar cataract formation: aldose reductase plays a key role in lens epithelial cell death (apoptosis). *Chem Biol Interact*. 2001;130:617-25.
11. Aukunuru JV, Sunkara G, Ayalasomayajula SP, DeRuiter J, Clark RC, Kompella UB. A biodegradable injectable implant sustains systemic and ocular delivery of an aldose reductase inhibitor and ameliorates biochemical changes in a galactose-fed rat model for diabetic complications. *Pharm Res*. 2002;19(3):278-85.

12. Hains PG, Truscott RJ. Post-translational modifications in the nuclear region of young, aged, and cataract human lenses. *J Proteome Res.* 2007;6:3935–43.
13. Zhang Z, Smith DL, Smith JB. Human beta-crystallins modified by backbone cleavage, deamidation and oxidation are prone to associate. *Exp Eye Res.* 2003;77:259–72.
14. Wilmarth PA, Tanner S, Dasari S, Nagalla SR, Riviere MA, Bafna V, Pevzner PA, David LL. Age-related changes in human crystallins determined from comparative analysis of post-translational modifications in young and aged lens: does deamidation contribute to crystallin insolubility? *J Proteome Res.* 2006;5:2554–66.
15. Hains PG, Truscott RJ. Age-dependent deamidation of lifelong proteins in the human lens. *Invest Ophthalmol Vis Sci.* 2010;51:3107–14.
16. Hains PG, Truscott RJ. Proteomic analysis of the oxidation of cysteine residues in human age-related nuclear cataract lenses. *Biochim Biophys Acta.* 2008;1784:1959–64.
17. Udupa EG, Sharma KK. Effect of oxidized betaB3-crystallin peptide on lens betaL-crystallin: interaction with betaB2-crystallin. *Invest Ophthalmol Vis Sci.* 2005;46:2514–21
18. Reymond F, Steyaert G, Carrupt PA, Morin D, Tillement JP, Girault HH, Testa B. The pH-partition profile of the anti-ischemic drug trimetazidine may explain its reduction of intracellular acidosis. *Pharm Res.* 1999;16(5):616-24.
19. Wang L, Feng R, Gao J, Xi Y, Huang G. Generic sustained release tablets of trimetazidine hydrochloride: preparation and in vitro–in vivo correlation studies. *Asian J Pharm Sci.* 2016;11(3):417-26.
20. Aminabhavi TM, Nadagouda MN, Joshi SD, More UA. Guar gum as platform for the oral controlled release of therapeutics. *Expert Opin Drug Deliv.* 2014;11(5):753-66.
21. De Robertis S, Bonferoni MC, Elviri L, Sandri G, Caramella C, Bettini R. Advances in oral controlled drug delivery: the role of drug–polymer and interpolymer non-covalent interactions. *Expert Opin Drug Deliv.* 2015;12(3):441-53.
22. Mohand-Said S, Jacquet A, Lucien A, Espinasse-Berrod MA, De Silva MF, Sahel J. Protective effect of trimetazidine in a model of ischemia-reperfusion in the rat retina. *Ophthalmic Res.* 2002;34(5):300-5.
23. Kaszuba-Bartkowiak K, Nowak MS, Jurowski P, Gos R. The role of trimetazidine in the protection of the retina. *Arch. Med. Sci.* 2007;3(3):S65.
24. Dézsi CA. Trimetazidine in practice: review of the clinical and experimental evidence. *Am J Ther.* 2016;23(3):e871.
25. Fang W, Ye Q, Yao Y, Xiu Y, Gu F, Zhu Y. Protective effects of trimetazidine in retarding selenite-induced lens opacification. *Curr Eye Res.* 2019;44(12):1325-36.
26. Huang L, Jia W, Chang C, Effects of antioxidants or free free radical scavengers on cataract formation induced by selenium. *Chinese Journal of Biochemistry and Molecular Biology.* 1989.
27. Huang LL, Zhang CY, Hess JL, Bunce GE. Biochemical changes and cataract formation in lenses from rats receiving multiple, low doses of sodium selenite. *Exp Eye Res.* 1992;55(5):671-8.

28. Shearer TR, Ma H, Fukiage C, Azuma M. Selenite nuclear cataract: review of the model. *Mol Vis*. 1997;3(8).
29. Isai M, Elanchezhian R, Sakthivel M, Chinnakkaruppan A, Rajamohan M, Jesudasan CN, Thomas PA, Geraldine P. Anticataractogenic effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, in an experimental animal model. *Curr Eye Res*. 2009;34(4):264-73.
30. Yeh LA, Ashton MA. The increase in lipid peroxidation in diabetic rat lens can be reversed by oral sorbinil. *Metabolism*. 1990;39(6):619-22.
31. Castorina C, Campisi A, Di Giacomo C, Sorrenti V, Russo A, Vanella A. Lipid peroxidation and antioxidant enzymatic systems in rat retina as a function of age. *Neurochem Res*. 1992;17(6):599-604.
32. Pramanik A, Sahoo RN, Nanda A, Mohapatra R, Singh R, Mallick S. Ocular permeation and sustained anti-inflammatory activity of dexamethasone from kaolin nanodispersion hydrogel system. *Curr Eye Res*. 2018;43(6):828-38.
33. Nanda A, Sahoo RN, Pramanik A, Mohapatra R, Pradhan SK, Thirumurugan A, Das D, Mallick S. Drug-in-mucoadhesive type film for ocular anti-inflammatory potential of amlodipine: Effect of sulphobutyl-ether-beta-cyclodextrin on permeation and molecular docking characterization. *Colloids Surf B*. 2018;172:555-64.
34. <https://animal.research.uiowa.edu/iacuc-guidelines-anesthesia>
35. Kurmi R, Ganeshpurkar A, Bansal D, Agnihotri A, Dubey N. Ethanol extract of *Moringa oliefera* prevents in vitro glucose induced cataract on isolated goat eye lens. *Indian J Ophthalmol*. 2014;62(2):154.
36. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*. 1951;193:265-75.
37. Chiş A, Kacsó I, Borodi G, Bratu I. Complexul de incluziune al trimetazidinei cu β -cyclodextrina. *Clujul Med*. 2011;84(2).
38. Mandal S, Kumar SS, Krishnamoorthy B, Basu SK. Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. *Braz J Pharm*. 2010;46(4):785-93.
39. El-Nahas HM, Hosny KM. Chitosan-based floating microspheres of trimetazidin dihydrochloride; preparation and in vitro characterization. *Indian J Pharm*. 2011;73(4):397.
40. Panda B, Parihar AS, Mallick S. Effect of plasticizer on drug crystallinity of hydroxypropyl methylcellulose matrix film. *Int J Biol Macromol*. 2014;67:295-302.
41. Kirchhof S, Goepferich AM, Brandl FP. Hydrogels in ophthalmic applications. *Eur J Pharm Biopharm*. 2015;95:227-38.
42. Marcocci C, Kahaly GJ, Krassas GE, Bartalena L, Prummel M, Stahl M, Altea MA, Nardi M, Pitz S, Boboridis K, Sivelli P. Selenium and the course of mild Graves' orbitopathy. *N Engl J Med*. 2011;364(20):1920-31.

43. Cargnoni A, Ceconi C, Bernocchi P, Parrinello G, Benigno M, Boraso A, Curello S, Ferrari R. Changes in oxidative stress and cellular redox potential during myocardial storage for transplantation: experimental studies. *J Heart Lung Transplant*. 1999;18(5):478-87.
44. Zhao W, Devamanoharan PS, Varma SD. Fructose induced deactivation of glucose-6-phosphate dehydrogenase activity and its prevention by pyruvate: implications in cataract prevention. *Free Radic Res*. 1998;29(4):315-20.
45. Demirbolat I, Ekinci C, Nuhoğlu F, Kartal M, Yıldız P, Geçer MÖ. Effects of Orally Consumed *Rosa damascena* Mill. Hydrosol on Hematology, Clinical Chemistry, Lens Enzymatic Activity, and Lens Pathology in Streptozotocin-Induced Diabetic Rats. *Molecules*. 2019;24(22):4069.
46. https://ntp.niehs.nih.gov/nnl/special_senses/eye/lncat/index.htm

Figures

Figure 1

(a): FTIR spectrograms; (b): DSC thermogram of trimetazidine dihydrochloride and film formulation; (c): Dissolution and (d): Permeation profiles of trimetazidine dihydrochloride and film formulation across cornea at 34 °C

Figure 2

Carrageenan injection in the upper palpebral region for oxidative stress induction in the rat eye (a): placebo group after 1 hour treated with polymeric film without drug; (b) positive control group after one hour after treated with polymeric film with drug; (c) normal rat eye

Figure 3

Trimetazidine effect on eye ball Malondialdehyde level of carrageenan induced (oxidative stress) rats. Group 1, control rats; Group 2, untreated carrageenan induced rats; group 3, carrageenan induced rats treated with trimetazidine. Each value was repeated three times. The value for stress induced rat is higher from normal control value at significance level of $*P<.001$. The value for the trimetazidine treated rats is almost similar without any remarkable difference from normal control rat ($†P<0.050$). The value for trimetazidine treated rats with carrageenan induced rats is different at a level significance of $‡P<0.050$.

Figure 4

(a) Group I: Complete cataractogenesis incubation in glucose 55 mM (Absolute loss of transparency); (b) Group II: Incubation of normal lens in glucose 5.5 mM (Transparency maintained); (c) Group III: Treatment group incubation in glucose 55 mM + 240 $\mu\text{g/ml}$, lens opacity developed slightly; (d) Group IV: Treatment group incubation in glucose 55 mM + 120 $\mu\text{g/ml}$, more lens opacity developed as compared to Group III

Figure 5

Histological photographs of goat lens sections stained with hematoxylin and eosin; (a) Group-I: Normal lens histology, presence of anterior epithelium (black arrow) with lens fibre normal orderly configuration and fibre nuclei (yellow arrow); (b) Group-II cataract induced lens histology, fragmentation of lens fibre (yellow arrow), absence of anterior epithelium (black arrow); (c) Group-III: Treated lens histology, appearance of anterior epithelium (black arrow) and retardation fragmentation of fibre (yellow arrow)

Figure 6

Effect of Trimetazidine on protein level on isolated goat lens; all values are mean $\pm$ SD, $n=3$; Statistical comparison was performed using student t-test. Four groups are consider here; (a) Group-I served as control group, (b) Group-II served as cataract induced group, (c) Group III: Treatment group incubation in glucose 55 mM + 120 μ g/ml (d) Group IV: Treatment group incubation in glucose 55 mM + 240 μ g/ml, Group-I is statistically significant than Group-II at significance level $p = 0.0075$, where Group-I is not significantly difference with treatment Group-III (0.0509) and Group-IV (0.2530). Group-II is significantly higher from the treatment group-III (0.0190) and group-IV (0.0114) at the bracketed significant level.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.tif](#)