Advances in Bioresearch
Adv. Biores., Vol 13 (4) July 2022: 155-164

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.4.155164

Advances in Bioresearch

ORIGINAL ARTICLE

In vitro Anti-Cataractogenic Activity of *Eucalyptus globulus* and *In Silico* Docking studies of Isolated Rutin on Aldose Reductase Activity

Myrene R. Dsouza*., Devaprabha V.A. S., and Deepthi N. S.
Department of Biochemistry, Mount Carmel College Autonomous, Bengaluru - 560052, Karnataka, India
* For correspondence: myrene83@gmail.com

ABSTRACT
Even though surgery is the most successful cure for cataract, the development of alternative treatments is needed due to postsurgical complications involved. This study evaluated the in vitro anti-cataractogenic potential of 10% methanolic extract of E. globulus leaves against glucose-induced cataractogenesis in goat lenses. Freshly isolated goat lens was divided into five experimental groups: 55 mM glucose (Group I), 10 µg/ml EGE+55 mM glucose (Group II), 25µg/ml EGE+55 mM glucose (Group III), 50 µg/ml EGE+55 mM glucose (Group IV) and 12 ng/ml Enalapril+55 mM glucose. After 72 h incubation, biochemical parameters such as total protein, H_2O_2, malondialdehyde, reduced glutathione, and glutathione reductase was measured in the lens homogenate. It was observed that the extract with a concentration of 25 µg/ml protected the lens against glucose-induced oxidative damage. The lens had clarity comparable to those incubated with the standard drug Enalapril even after 48 h of treatment. The second aim was to explore the aldose reductase inhibitory activity of a Rutin, a bioactive isolated from E. globulus leaves. An aldose reductase inhibitor, Epalrestat, was used as the standard. AutoDock 4.2 was used to carry out in silico docking studies according to the Lamarckian genetic algorithm principle. For this study, parameters such asinhibition constant, binding energy, and intermolecular energy were ascertained. Further improvement of the potent aldose reductase inhibitor for diabetes treatment can be achieved from the molecular docking analyses.
Keywords: *cataract, eucalyptus, goat lenses, oxidative stress, Rutin, molecular docking*

Received 12.05.2022 Revised 21.06.2021 Accepted 23.07.2022

How to cite this article:
M R. Dsouza, Devaprabha V.A. S., and Deepthi N. S. *In vitro* Anti-Cataractogenic Activity of *Eucalyptus globulus* and *In Silico* Docking studies of Isolated Rutin on Aldose Reductase Activity. Adv. Biores. Vol 13 [4] July 2022. 155-164

INTRODUCTION

Cataract meaning "waterfall" refers to visual impairment due to lens opacification. Till date, surgery remains the only cost-effective intervention for cataract followed by replacement of the cloudy lens with artificial intra ocular lens[1, 2].Despite its reputation for success, it can cause complications like retinal detachment,posterior capsular opacification and cystoid macular edema that may result in irreversible blindness[3, 4].With cataract accounting for 51% of global blindness and approximately 20 million individuals suffering from post-surgical complications[3, 5], there is an urgent need for alternative measures to prevent and treat cataract.Several natural products and herbal medicines having antioxidant properties have been found to prevent induced cataractogenesis[5]. With expanding scientific evidence showing promising results in treating cataract with natural products, an ethnopharmacological approach maybe favorable. The discovery of these natural compounds in treatment is of utmost importance as they are easily available, cheaper, possess less to no drastic side effect and can be incorporated into diet or topical applications on large scale in developing countries [6]. The polyol pathway in the eye is highly expressed in people with diabetes mellitus. Only a smallamount of glucose undergoes metabolism via the polyol route under normal glycemic circumstances, since the bulk undergoes phosphorylation by hexokinase and the resultant product, glucose-6-phosphate, is used as a substrate in glycolysis or pentose phosphate pathway. However, glucose flow through the polyol pathway is considerably enhanced in diabetics because of persistent hyperglycemia. The polyol route can account for up to 33% of total glucose consumption in certain tissues, such as the eye [7]. A large quantity of glucose is redirected to the

polyol pathway in hyperglycemia, where aldose reductase (AR) converts glucose to sorbitol utilizing NADPH. As an osmolyte, sorbitol causes osmotic swelling, alterations in permeability of the membrane, leakage of glutathione, myoinositol, and the production of reactive oxygen species (ROS), all of which contribute to diabetic consequences such cataract, retinopathy, and neuropathy. Because NADPH is required to produce GSH from GSSG, the AR pathway's depletion of NADPH may compromise intracellular antioxidant defense. Sorbitol dehydrogenase converts sorbitol to fructose, resulting in the formation of NADH, which might contribute to an increase in ROS via NADH oxidase. NADPH is required for AR activity. GSH is necessary for NADPH regeneration. As a result, the activity of AR is indirectly influenced by GSH. Thepurpose of this analyses was to evaluate the activityof aldose reductase (AR) and glutathione reductase, as well as their function in oxidative stress and glucose-induced cataract. Inhibition of AR might be used to treat issues related to diabetes-induced cataract. Currently, carboxylic acid inhibitors (Ex., epalrestat), spirohydantoin derivatives (Ex., sorbinil), and succinimide compounds (Ex., ranirestat) are the most common kinds of AR inhibitors (ARI) [8, 9].Epalrestat is presently the only ARI accessible in India. Some medicines have been pulled off the market owing to safety concerns, while others are currently being tested in clinical studies [10]. As a result, novel ARIs must be developed and evaluated in terms of effectiveness, selectivity, and safety. *Eucalyptus globulus* contains large amounts of phytochemicals like flavonoids, tannins, propanoids, and alkaloids[11]. The extracts derived from the plant's leaf, stem, and core have shown to be successful in reducing H_2O_2-generated oxidative stress by boosting cellular viability, reduced glutathione content, and antioxidant enzyme activities while lowering ROS output and lipid peroxidation [12]. Purification of bioactives from the *E. globulus* leaves followed by computer-aided structure-based rational drug design and *in silico* docking elucidates how these molecules interact with the target macromolecule i.e., aldose reductase(AR). Auto Dock Tools predicts how small compounds attach to a given 3D-structured target enzyme. In addition to producing binding energies, the location of the ligand in the binding site of the enzyme may be seen in these docking experiments (Cosconati et al. 2010). It can be beneficial in the development of possible medication candidates as well as the development of new drugs.

MATERIAL AND METHODS

Plant material: *E. globulus* leaves were procured from30-year-old trees at the University of Agricultural Sciences, GKVK, Bengaluru. To eliminate dust and dirt, the leaves were washed with tap water, rinsed well with distilled water, and air dried in the shade. The dried samplewas then ground into a powder with an electric grinder and sieved. After that, the pulverized leaves were stored in airtight containers until use.

Preparation of extract and phytochemical screening: 10% methanolic extract was prepared by homogenization, and centrifuged at 6000rpm for 15 min at 4°C. To validate the existence of specific phytochemical components in the methanolic extract of *E. globulus* (EGE), chemical tests were performed according to standard protocols[13].

Preparation of lens culture: The anticataract potential of EGE was studied *in vitro* in goat eye lens induced by glucose. Goat eyeballs were obtained from anabattoir in Frazer town, Bengaluru within 2 h of slaughtering and transported at 0-4˚C to laboratory. The lenses were extracted by extra capsular removal and incubated in artificial aqueous humor containing 5.5 mM glucose [14]. 32 mg% Penicillin and 250 mg% Streptomycin were added to the culture media to prevent bacterial contamination.

Experimental design: 60 goat lenses were divided into the following five experimental groups:

Group I: Glucose 55 mM (toxic control)
Group II: EGE (10 µg/ml) + Glucose 55 mM
Group III: EGE (25 µg/ml) + Glucose 55 mM
Group IV: EGE (50 µg/ml) + Glucose 55 mM
Group V: Standard drug Enalapril (12 ng/ml) + Glucose 55 mM

Photographic assessment of lens opacity: To study the opacity of the lens after 72 h of incubation, lenses from the control and experimental groups were laid on a wire mesh with the posterior surface of the lens resting on the mesh[15]. The opacity of the lens was classified as below:

- Absence of opacity: 0
- Slight degree of opacity: +
- Presence of diffuse opacity: ++
- Presence of extensive thick opacity: +++

Preparation of lens homogenate: Lenses were homogenized in 10 volumes of 0.1M potassium phosphate buffer, pH 7.0, followed by at 10,000 rpm at 4°C for 30 min. The supernatant thus obtained was used to assay various biochemical parameters.

Study of anticataract potential of EGE

The protein content was determined from standard curve prepared with bovine serum albumin and expressed as mg/g lens tissue [16].The extent of lipid peroxidation was determined according to Heath and Packer[17]. Reduced glutathione (GSH) was measured according to Beutler et al., [18]. Hydrogen peroxide levels were ascertained according to the protocol of Velikova et al., [19]. The activities of guaiacol peroxidase (GPx) and glutathione reductase (GR) was estimated using time scan feature of the spectrophotometer according to Chance &Maehly [20] and Carlberg and Mannervik[21] respectively.

Extraction and isolation of active compound:20 g of the *E. globulus*leaf powder was extracted with 250 ml of 80% ethanol in a Soxhlet apparatus until exhaustion. The extract was filtered and concentrated by evaporation. 10 ml of the concentrated sample was mixed with 25 ml of distilled water and extracted with petroleum ether followed by chloroform. Each extraction used 50 ml of the respective solvent and was repeated thrice. The aqueous layer was collected and allowed to stand for 72 h at room temperature. A yellow precipitate was obtained that was filtered out and washed with chloroform: ethyl acetate: ethanol (50:25:25). The precipitate was then dissolved in hot methanol and filtered. The filtrate was evaporated to dryness. A yellow powder was obtained.

Identification of isolated Rutin: The partially purified Rutinsample from *E. globulus* was compared with standard using HPLC. The instrument was equipped with binary pump, dynamax C18 column and UV-Vis detector. 30 µl of the sample was used. Methanol:water (1:1) served as the mobile phase with a flow rate of 1 ml/min and run time of 30 min.Fractions were detected at 360 nm[22]. Methanol was used to dissolve the isolated rutin and the absorption peak was determined using UV-Vis spectrophotometerand compared with standard Rutin.

***In silico* structure prediction ofaldose reductase**: The protein sequence of aldose reductase was retrieved from UniProt (www.uniprot.org). It had the Accession Number: O60218. The conserved domain and catalytically important sites were determined using NCBI CDD [23]. Protein molecule structure was retrieved in ".pdb" format. H_2O molecules were deleted and only the final stage H atoms were added to the target protein molecule. Polar hydrogen atoms and Gasteiger charge were added to the whole enzyme. SWISS-MODEL (https://swissmodel.expasy.org) was used to obtain the structural 3D model if the enzyme. After this, a homology-based search was done using Protein Data Bank (PDB) repositories. The best suited template with the highest similarity was used to determine biological property and for generating the 3D model. Evaluation of the accuracy of the structure and the Ramachandran plot was confirmed using PROCHECK and ProSA-web [27].

Preparation of ligand molecule: NCBI PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the 3D structure of Rutin (PubChem CID: 5280805). The ligands were retrieved in 3D structure in ".sdf" format. This was then converted into ".pdb" format through online SMILES Translator (https://cactus.nci.nih.gov/translate/). The converted files were downloaded in ".pdb" format. These ".pdb" files were used to run different tools and software's.

Determination of *in silico* pharmacokinetics, bioactivity ADME properties: The computational prediction for rutin was performed through the online software: Molinspiration (https://www.molinspiration).com/), Pre-ADMET and prediction of ADME/Tox (https://preadmet.bmdrc.kr/), and SwissADME (https://swissadme.ch). Calculation of important drug-like properties such as polar surface area, logP, number of hydrogen bond, as bioactivity score for the drug targets such as kinase inhibitors, GPCR ligands, nuclear receptors and ion channel modulators was performed. The assessment of absorption, distribution, metabolism, and excretion (ADME) is paramount to the understanding of the behaviour of the drug molecule. Parameters such as the drug's ability to reach the target site in sufficient concentration and its period of bioactivity were assessed by Pre-ADMET.Swiss ADME was used to obtain predictive models for physicochemical properties, pharmacokinetics, and drug-likeness. The physicochemical parameters analyzed give a global description of the structure.

Preparation through Biovia Discovery Studio Visualizer: This software suite was used for deleting alternate conformations, analyzing missing atoms in incomplete residues, removing water molecules, protonating titratable residues, and modeling missing loop regions. After suitable changes, the crystal structure of the protein was saved in .pdb format. This protein molecule was used for docking.

Preparation of Grid Parameter File: The active site residues used for docking was taken from literature - ASP69, ASP352, GLU277 [25]. Grid maps were created with a spacing to allow ligand binding i.e., 0.375 Å. Adjustment of grid dimension to 50 × 50 × 50 points was done. The maps were then analyzed by Auto Grid and the interaction energy was assigned at each grid point. The interaction energy between every ligand atom and receptor was computed for the entire binding site. AutoDock Vina was used for docking.

Virtual Screening through PyRx: The PyRx software was used to screen the ligand interaction that possessed minimum binding energy with the protein target. The protein molecule was loaded in the

".pdb" format and then converted to the to ".pdbqt" format. The protein molecule was imported in .sdf format, energy of ligands was minimized and then the file was converted and saved to ".pdbqt" format. Docking was performed between protein target and ligand molecule and the interaction possessing minimum binding energy were screened for drug likeliness property analysis.

Docking through AutoDock Vina: The enzyme target in .pdb format was loaded on AutoDock Vina (https://cactus.nci.nih.gov/translate/). The protein was made ready by the addition of hydrogen polar atoms, deletion of water molecules, addition of Kollman charges and then saved in ".pdbqt" format. The grid box was selected for the area to be docked, AutoDock Vina was executed, and the results analyzed.

Statistical analysis: Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of 4 lenses in each group. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Cataract has been identified as leading cause of joint blindness in India. It is responsible for about 47.8% of blindness, accounting for 17.7 million cases of blindness (Singh et al., 2019). Even though cataract surgery helps to reduce vision loss, it is costly and out of reach for many people in developing countries.

Phytochemical screening: The methanolic extract of the pulverized leaves of *E. globulus* revealed the occurrence of alkaloids, terpenoids, tannins and flavonoids (Table 1).

Table 1: Phytochemical screening of *Eucalyptus globulus*

Phytochemicals	*Eucalyptus globulus*
Alkaloids	+
Flavonoids	+
Terpenoids	+
Tannins and phenolics	+
Saponin	-

Photographic evaluation of lens: The lenses of all five experimental groups were photographed after 72 h of incubation. Group I or toxic control lenses incubated in 55mM glucose alone were completely opaque. Group II lenses which were incubated in 10 µg/ml EGE along with 55mM glucose were comparatively less opaque than Group I. The lenses in Group III and Group IV which were incubated in 25 µg/ml and 50µg/ml of EGE respectively along with 55mM glucose showed better clarity when compared to Group II. Group V or positive control lenses incubated in 55mM glucose and standard drug Enalapril exhibited maximum clarity (Fig 1).

Table 1: Effect of methanolic extract of *E. globulus* leaves on glucose-induced cataractogenesis.

Sl. No	GROUPS	TREATMENT	DEGREE OF OPACITY
1	Group I	55mM glucose	+++
2	Group II	55mM glucose + 10 µg/ml EE	++
3	Group III	55mM glucose + 25 µg/mlEE	0
4	Group IV	55mM glucose + 50 µg/mlEE	+
5	Group V	55mM glucose + Enalapril (12ng/ml)	0

The gradation of lens opacities was graded as follows: 0 - Absence, + - Slight degree, ++ - Presence of diffuse opacity, +++ - Presence of extensive thick opacity

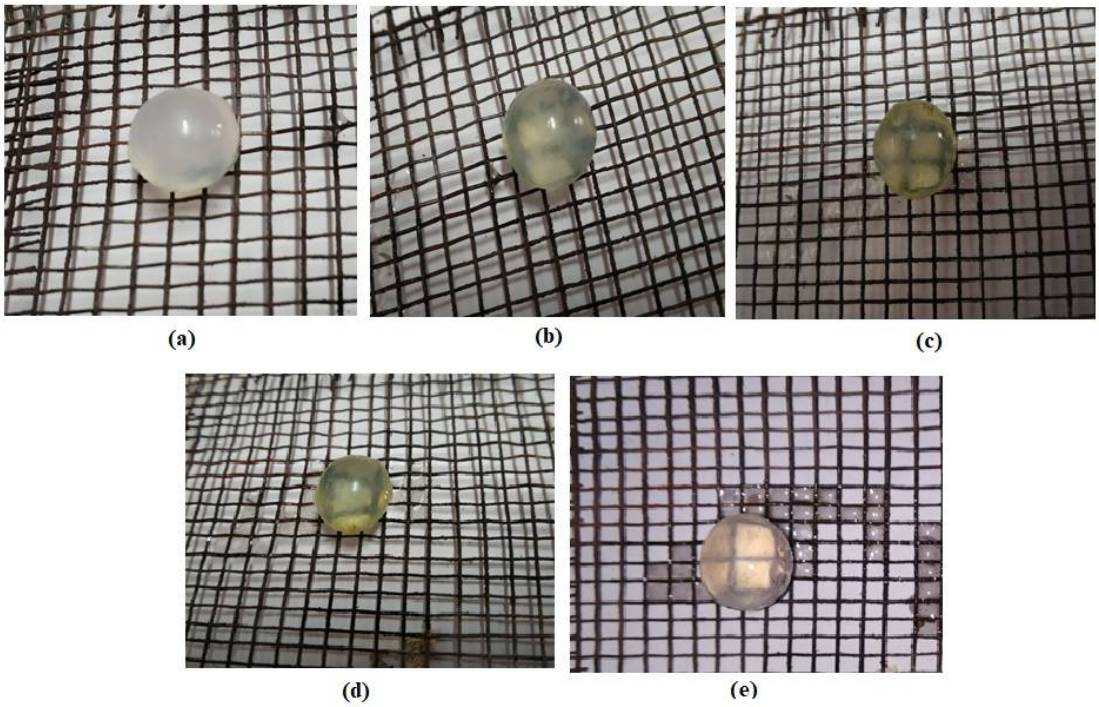

Fig 1: Photographic evaluation of lens opacity after 72 h incubation with 55mM glucose (a), 55mM glucose + 10 µg/ml EGE (b), 55mM Glucose + 25 µg/ml EGE (c), 50 µg/ml EGE + 55mM glucose (d), and 55mM glucose + 12 ng/ml Enalapril(e).

Effect of eucalyptus extract on oxidative stress markers: High concentrations of glucose is utilized by the lens in many pathways resulting in oxidative and osmotic stress [27]. The hyperosmotic effect brought about by the increased intracellular accumulation of sorbitol results in hydropic lens fibers which then disintegrate and form cataract [28].Sorbitol accumulates in the lens when the concentrations of glucose are high and aldose reductase is active [29]. In the hyperglycemia-induced lenses the ionic equilibrium is distorted and there is accumulation of Na^+ and loss of K^+ leading to swelling and opacification of the lens. These changes cause decrease in the number of water-soluble proteins and an increase in the number of insoluble protein due to protein aggregation (Table 2). This results in lens opacification. In the presence of phytochemicals such as flavonoids, tannins, terpenoids etc., the enzyme aldose reductase is inhibited and therefore there is no sorbitol accumulation [29].

Table 2: Effect of methanolic extract of *Eucalyptus globulus* on oxidative stress markers using isolated goat-lens model.

Parameter analyzed	GroupI (Control)	Group II	Group III	Group IV	Group V
Total protein (mg/g)	158.67±0.1675	167.45±5.05	167.74 ± 3.66	161.55 ± 6.15	153.37 ± 4.22
Malondialdehyde (nmole/g)	0.5733 ±0.101	0.589±0.158	0.309 ±0.048	0.921 ±0.051	0.546 ±0.000
H_2O_2(µmole/g)	0.060 ± 0.0019	0.0142 ± 0.0	0.0160 ± 0.0	0.0617 ± 0.0	0.0213 ± 0.0
Reduced GSH (mg/g)	1.49 ±0.141	1.53± 0.007	2.225±0.077	1.839± 0.015	1.384±0.090
Glutathione Reductase (IU/g)	0.00135±0.00	0.000082±0.00	0.002551±0.00	0.00103±0.00	0.000097±0.00
Guaiacol Peroxidase (IU)	0.00121±0.00	0.00018019±0.00	0.000284±0.00	0.000535±0.000	0.000166±0.00

Lipid peroxidation is an autocatalytic process which results in cell death. In this process, polyunsaturated lipids are oxidized to form lipid peroxides which can further undergo decomposition to form toxic carbonyl groups.Lens MDA formation maybe due to peroxidation of the lens plasma membrane lipids or might denotethe outcome of its relocation from the easilyperoxidizable retina.GSH is an important antioxidant present in the lens which plays a chief role in metabolism, catalysis, transport, scavenges free radicals and toxins.GSH in the lens maintains protein thiol groups in their reduced state, thuspreventing

the occurrence of high molecular weight protein aggregates [5]. It was seen that Group III had significantly higher levels of GSH when compared to the control lens (Table 2).The presence of increased levels of H_2O_2in lens is an indication of oxidative stress related with the formation of cataract.There is a considerable increase in levels of H_2O_2in the control while Group II and III containing glucose and 10 µg/ml and 25 µg/ml EGErespectively have lower levels of H_2O_2 (Table 2, Fig 3).
GR reduces lens permeable glutathione disulfide (GSSG) to lens impermeable glutathione (GSH), thereby trapping GSH within the lens. The lens incubated with EGE had higher levels of GSH when compared to GSSG for which one of the reasons being the activity of GR. In the lens, the GSH/GSSG ratio is usually high maintained by the glutathione redox cycle[30]. The increased activity of GR might be a contributing factor in lesser lens opacity in Group III (Table 2, Fig 4). When compared to the GPx activity in all other groups, Group IV was significantly high(Table 2, Fig 4). This indicates that the lenses in Group IV had high levels of harmful oxidants that triggered the expression of GPx. The reduction in Gpx activities in all other groups is indicative of other favorable and potent mechanisms that reduce the levels of such harmful oxidants in lenses treated with the extract.

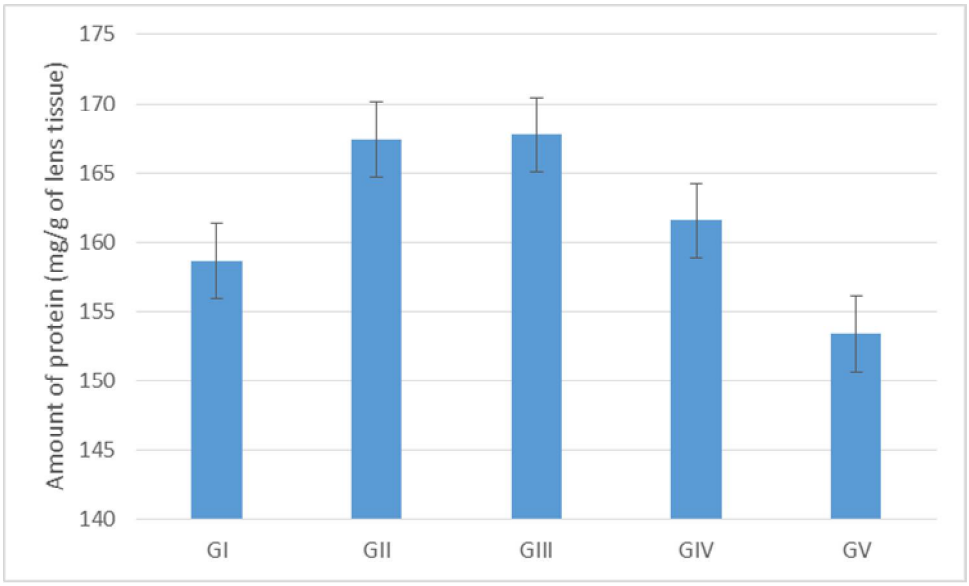

Figure 2: Effects of EGE on amount of total soluble protein of isolated goat lens. Values are represented as mean ± SD (n = 4).

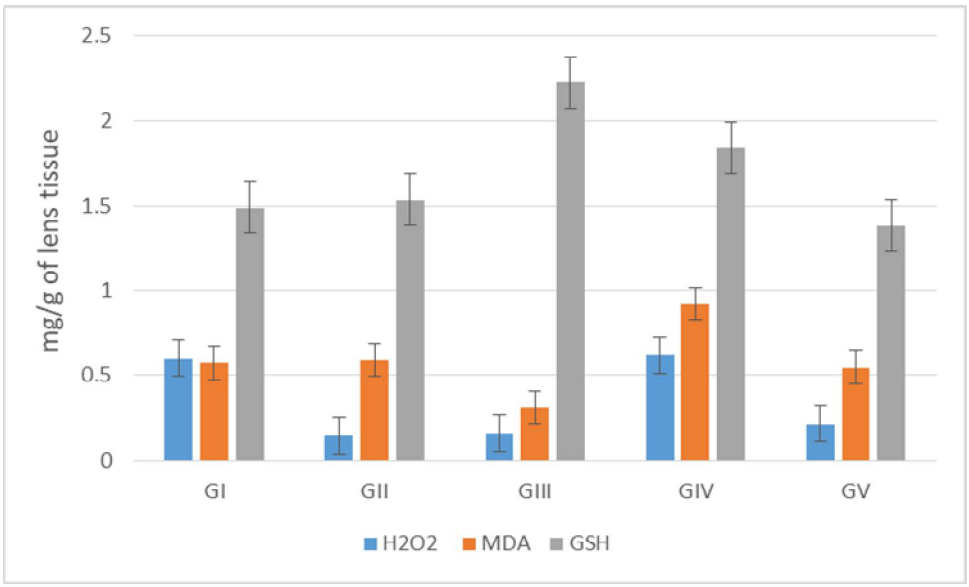

Figure 3: Effects of EGE on H_2O_2, MDA, and reduced glutathione (GSH) levels of isolated goat lens. Values are represented as mean ± SD (n = 4).

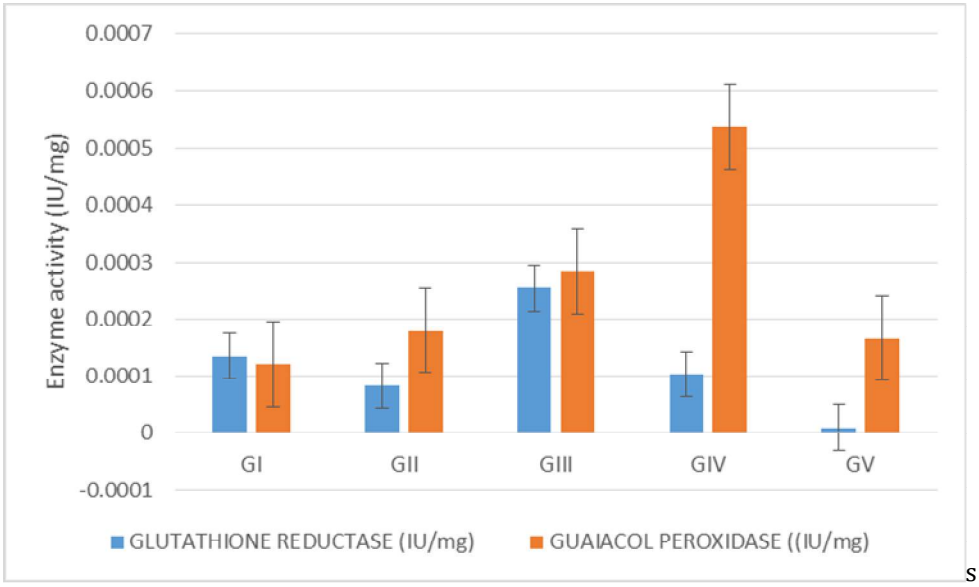

Figure 4: Effects of EGE on enzyme activity of glutathione reductase (GR) and guaiacol peroxidase (Gpx) in isolated goat lens. Values are represented as mean ± SD (n = 4).

Isolation of Rutin: The isolated Rutin sample and standard Rutin was extracted and isolated with chloroform. The HPLC chromatograms are shown in (Fig 5) respectively.

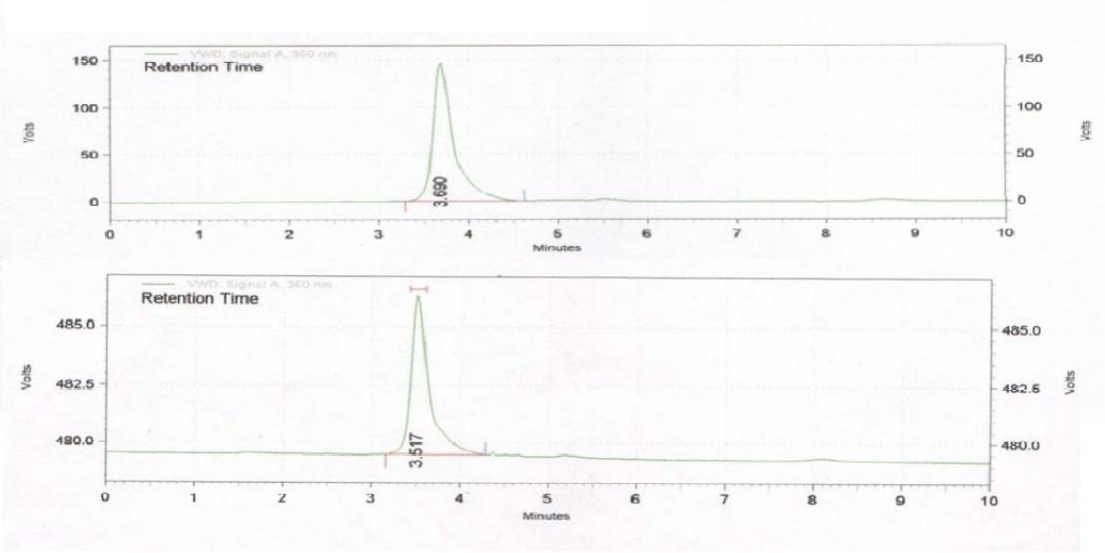

Fig 5: HPLC chromatogram of the standard (upper panel) and isolated Rutin (lower panel).

Determination of *in silico* pharmacokinetics, bioactivity, toxicity properties: The computational calculation for Rutin was executed through online software: Molinspiration (https://www.molinspiration).com/), Pre-ADMET and prediction of ADME/Tox (https://preadmet.bmdrc.kr/), and SwissADME (https://swissadme.ch). Rutin (Fig 6) was analyzed for pharmacokinetic properties and drug likeness following the Lipinski's rule of five. The molecular weight of the selected drug is 610.52 Da which falls in the appropriate range for a drug candidate. This is an important parameter to ensure easy absorption, diffusion and transportation in comparison to high molecular weight compounds. The MLogP (octanol/ water partition coefficient), used to determine lipophilic efficiency was -1.06 indicating that the drug has a higher affinity for the aqueous phase (Fig 6). The logP value of the octanol-water partition coefficient is critical in rational drug design and QSAR investigations. TPSA (Topological Polar Surface Area), a measurement of polarity indicates the drugs transport capability and should ideally be between 20 and 130 Å, however, in the case of Rutin, the value is much higher and hence not favorable.

The bioactivity score provides evidence of the binding of the drug molecule and is an indicator of binding selectivity and probability of side effects. Bioactivity score greater than 0.00 indicates that the drug has

significant biological activity, while scores of 0.5 - 0.00 and less than -0.50 is said to be moderately active and inactive respectively. Rutin was found to have significant biological activity as an enzyme inhibitor (Fig 6).

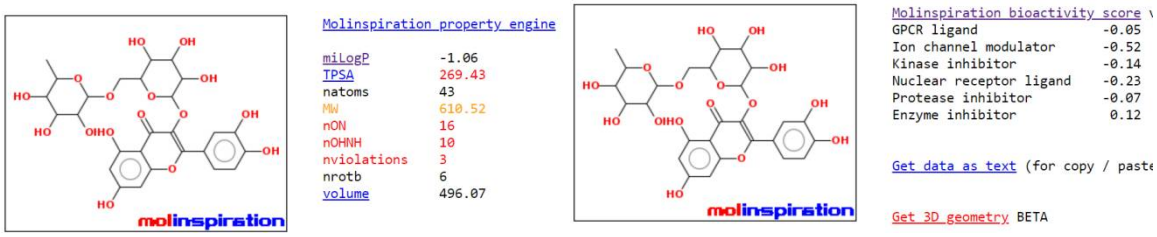

Figure 6:Pharmacokinetic parameters and bioactivity scores of Rutin

Docking analysis: Lead optimization of the selected and purified compound, Rutin(Fig 7) was performed by computation of druglikeness properties. Lipinski's rule was used to analyze the drug candidate for its inhibitory activity on aldose reductase enzyme. AutoDock4.2 was used for docking studies. The docking pose obtained demonstrates the binding positions of the Rutin with aldose reductase. Rutin showed intermolecular binding energy of -8.62 kcal/mol which is better than that of standard Epalrestat possessing -5.59kcal/mol (Table 3). Due to lesser binding energy, Rutin has greater activity. These values were obtained by taking into consideration parameters involved in successful docking such as binding energy, hydrogen bond interactions, RMSD of active site residues and π – π interactions. The potential binding sites of Rutin for aldose reductase was found to be TRP 20, TYR 48, LYS 77, HIS 110, SER 159, ASN 160, GLN 183, GLU 185, TYR 209, SER 210, PRO 211, SER 214, ILE 260 and CYS 298 (Fig 8). The inhibition constant is directly proportional to the binding energy. The AutoDock 4.2 software was used to calculate IC50 value. The standard Epalrestat had a value of 80.08 µM, while Rutin demonstrated a better value of 1.58 µM (Table 3).

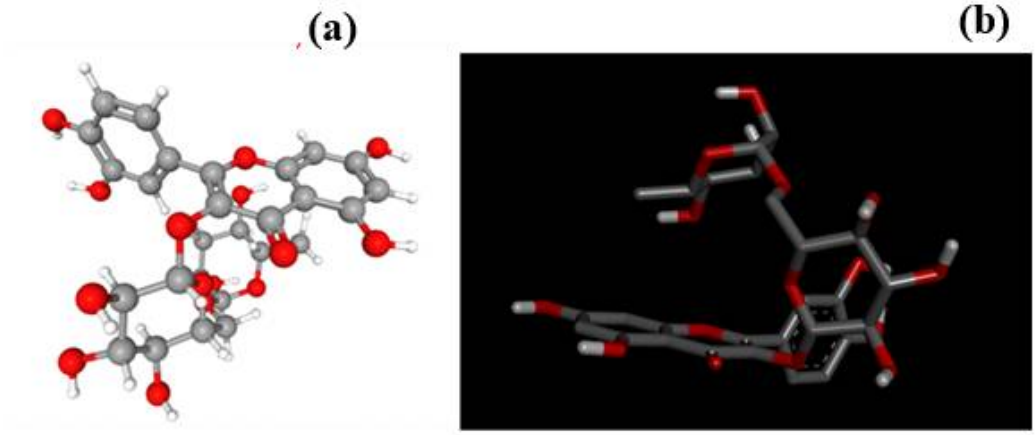

Figure 7: Ball and stick model (a) and wireframe structure (b) of Rutin

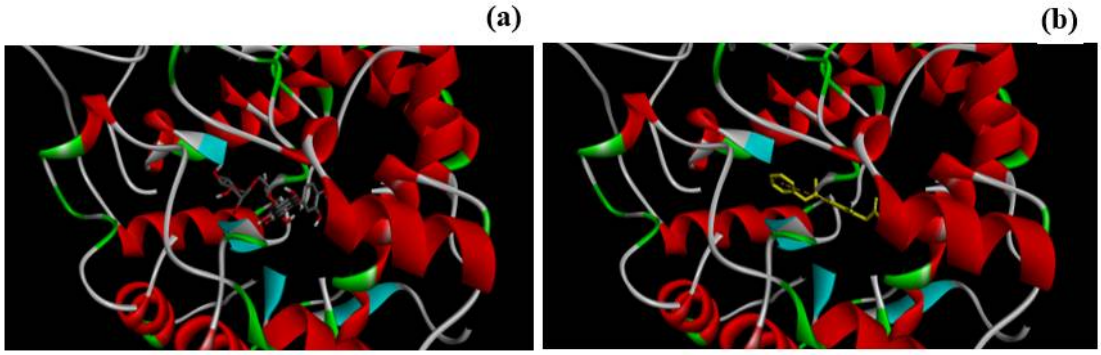

Figure 8: Docked pose of aldose reductase with Rutin (a) and Epalrestat (b)

Table 3: Comparison of inhibition constant (IC50) and intermolecular binding energy of Rutin and Epalrestat

Compound	IC50 (µM)	Intermolecular binding energy (kcal/mol)
Epalrestat	80.08	-5.59
Rutin	1.52	-8.62

CONCLUSION

The purpose of this study was to ascertain the *in vitro*efficacy of Eucalyptus extract in treating glucose-induced cataract in goat lens. Natural substances such as phytochemicals or secondary metabolites, with antioxidant or anti-inflammatory properties, such as phytochemicals or secondary metabolites, can be used in possible anticataract treatments. To determine the efficacy of the extracts, biochemical tests comprising antioxidant and lipid peroxidation assays were conducted. According to the aforementioned results, Group III of methanolic extract can help prevent cataract because it possesses highest GR activity, and GSH levels. From the data obtained, it may be predicted that Rutin demonstrates potential ability to inhibit aldose reductase activity when compared to the standard drug Epalrestat. This can be explained by the variation in the orientation and position of functional groups in both drug molecules. Hence, Rutin or its derivatives can be further developed as potential drug candidates.

REFERENCES

1. Zhu, X., He, W., Zhang, Y., Chen, M., Du, Y., & Lu, Y. (2018). Inferior Decentration of Multifocal Intraocular Lenses in Myopic Eyes. American journal of ophthalmology, 188:1–8.
2. Lin, Z. N., Chen, J., Zhang, Q., Li, Q., Cai, M. Y., Yang, H., & Cui, H. P. (2017). The 100 most influential papers about cataract surgery: a bibliometric analysis. International journal of ophthalmology, 10(10):1586–1591.
3. Heruye, S. H., MaffofouNkenyi, L. N., Singh, N. U., Yalzadeh, D., Ngele, K. K., Njie-Mbye, Y. F., Ohia, S. E., &Opere, C. A. (2020). Current Trends in the Pharmacotherapy of Cataracts. Pharmaceuticals (Basel, Switzerland), 13(1): 15.
4. Hilliard, A., Mendonca, P., Russell, T. D., & Soliman, K. (2020). The Protective Effects of Flavonoids in Cataract Formation through the Activation of Nrf2 and the Inhibition of MMP-9. Nutrients, 12(12): 3651.
5. Azadbakht, M., Asghari, M., NowroozpoorDailami, K., Davoodi, A., & Ahmadi, A. (2020). The Preventive Effects of Asparagus officinalis Extract on Sodium Selenite-Induced Cataractogenesis in Experimental Animal Models. Evidence-based complementary and alternative medicine :eCAM, 2020, 3708730.
6. Thiagarajan, R., & Manikandan, R. (2013). Antioxidants and cataract. Free radical research, 47(5): 337–345.
7. Kinoshita J. H. (1990). A thirty year journey in the polyol pathway. Experimental eye research, 50(6): 567–573.
8. Kovacikova, L., Prnova, M. S., Majekova, M., Bohac, A., Karasu, C., &Stefek, M. (2021). Development of Novel Indole-Based Bifunctional Aldose Reductase Inhibitors/Antioxidants as Promising Drugs for the Treatment of Diabetic Complications. Molecules (Basel, Switzerland), 26(10): 2867.
9. Du, Z. Y., Bao, Y. D., Liu, Z., Qiao, W., Ma, L., Huang, Z. S., Gu, L. Q., & Chan, A. S. (2006). Curcumin analogs as potent aldose reductase inhibitors. Archiv der Pharmazie, 339(3): 123–128.
10. Ramirez, M. A., & Borja, N. L. (2008). Epalrestat: an aldose reductase inhibitor for the treatment of diabetic neuropathy. Pharmacotherapy, 28(5): 646–655.
11. Dixit, A., Rohilla, A., Singh, V., (2012). Eucalyptus globulus: A new perspective in therapeutics. Int J Pharm Chem Sci 2012; 1(4): 23-31.
12. González-Burgos, E., Liaudanskas, M., Viškelis, J., Žvikas, V., Janulis, V., & Gómez-Serranillos, M. P., (2018). Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from Eucalyptus globulus leaves. Journal of food and drug analysis, 26(4):1293-1302.
13. Khandelwal, K.R. Practical Pharmacognosy, 19th Ed., NiraliPrakashan, Pune 2009.
14. Langade, D.G., Rao, G., Girme, R.C., Patki, P.S., Bulakh, P.M. (2006). In vitro prevention by ACE inhibitors of cataract induced by glucose. Ind J Pharmacol 38(2): 107-109.
15. Sanderson, J., Marcantonio, J. M., & Duncan, G. (2000). A human lens model of cortical cataract: Ca2+-induced protein loss, vimentin cleavage and opacification. Investigative ophthalmology & visual science, 41(8): 2255–2261.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, 193(1): 265–275.
17. Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics, 125(1): 189–198.
18. Beutler, E., Duron, O., & Kelly, B. M. (1963). Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine, 61: 882–888.
19. Velikova et al., (2000). Oxidative stress and some antioxidant systems in acid rain treated bean plants: protective role of exogenous polyamines. Plant Science 151: 59-66.
20. Chance, B., &Maehly, A.C., (1955). Assays of catalases and peroxidases. In: Methods in Enzymology, Academic Press, New York, Vol II, 764-775.
21. Mannervik, B., & Carlberg, I., (1985). Glutathione Reductase. Methods in Enzymology 113: 484-490.

22. Ashok, P. K., & Saini, B. (2013). HPLC analysis and isolation of rutin from stem bark of Ginkgo Biloba L. Journal of Pharmacognosy and Phytochemistry, 2(4): 68-71.
23. Marchler-Bauer, A., Derbyshire, M. K., Gonzales, N. R., Lu, S., Chitsaz, F., Geer, L. Y., Geer, R. C., He, J., Gwadz, M., Hurwitz, D. I., Lanczycki, C. J., Lu, F., Marchler, G. H., Song, J. S., Thanki, N., Wang, Z., Yamashita, R. A., Zhang, D., Zheng, C., & Bryant, S. H. (2015). CDD: NCBI's conserved domain database. Nucleic acids research, 43(Database issue), D222–D226.
24. Wiederstein, M., &Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic acids research, 35(Web Server issue), W407–W410.
25. Yamamoto, K., Miyake, H., Kusunoki, M., & Osaki, S. (2010). Crystal structures of isomaltase from Saccharomyces cerevisiae and in complex with its competitive inhibitor maltose. The FEBS journal, 277(20): 4205–4214.
26. Singh, S., Pardhan, S., Kulothungan, V., Swaminathan, G., Ravichandran, J. S., Ganesan, S., Sharma, T., & Raman, R. (2019). The prevalence and risk factors for cataract in rural and urban India. Indian journal of ophthalmology, 67(4): 477–483.
27. Alvarez, Y., Chen, K., Reynolds, A. L., Waghorne, N., O'Connor, J. J., & Kennedy, B. N. (2010). Predominant cone photoreceptor dysfunction in a hyperglycaemic model of non-proliferative diabetic retinopathy. Disease models & mechanisms, 3(3-4): 236–245.
28. Pollreisz, A., & Schmidt-Erfurth, U. (2010). Diabetic cataract-pathogenesis, epidemiology and treatment. Journal of ophthalmology, 2010, 608751.
29. Hajarnavis, A. M., &Bulakh, P. M. (2013). Antioxidant potential of Emblica officinalis (amla) aqueous extract delays cataractogenesis in hyperglycemic goat lenses. IOSR J Pharm Biol Sci, 6(1): 32-35.
30. Kisic, B., Miric, D., Zoric, L., Ilic, A., &Dragojevic, I. (2012). Antioxidant capacity of lenses with age-related cataract. Oxidative medicine and cellular longevity, 467130.