



ANTICATARACT ACTIVITY OF ETHANOLIC EXTRACT OF *FICUS RELIGIOSA* (L.) LEAF ON *IN VITRO* GLUCOSE- INDUCED CATARACT IN GOAT LENS MODEL

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ABSTRACT

Cataracts are one of the largest causes of blindness and vision impairment globally. They occur when the natural lens of the eye becomes partially or totally cloudy. Lens is made of proteins. When proteins in the lens aggregate together, create opaque areas in the lens that limit the light from passing through them, leading to the development of a cataract. This degenerative condition can affect one or both eyes and is not contagious. The primary treatment method is cataract surgery. However, it has some kind of limitations, including post-operative inflammation, posterior capsular opacification, and intraocular lens dislocation. Hence, the objective of the present study is to discover a Non-surgical therapeutic agent for the prevention of cataractogenesis. The *in vitro* glucose-induced cataract goat lens model is used. The 55 mM glucose induces cataract in 72 hours of incubation and is treated with the ethanolic extract of *F. religiosa* (L.). The result suggests that

the *F. religiosa* (L.) potentially prevents the onset and progress of glucose-induced cataract, and it is likely due to the antioxidant activity of the extract. The present study suggests the use of *F. religiosa* (L.) extract as a non-surgical therapeutic agent and warrants further in vivo studies followed by clinical trials to fully establish its efficacy and safety in ocular health care.

KEYWORDS: Cataract, *Ficus religiosa* (L.), Goat lens, 55mM Glucose, Diabetic mellitus,

Diabetic cataract.

INTRODUCTION

A major public health issue that affects millions of individuals worldwide is visual impairment. Cataracts are one of the largest causes of blindness and vision impairment worldwide. They occur when the natural lens of the eye becomes partially or totally cloudy. The lens of the eye, situated behind the iris and pupil, is crucial for focusing light onto the retina, enabling clear vision. When proteins in the lens aggregate, they create opaque areas that limit the light from passing through them, leading to the development of a cataract. The primary cause is attributed to advanced aging, the secondary cause including genetic mutation and systemic disease, especially diabetic mellitus.^[1] Diabetic cataract (DC) is characterized by a higher incidence, earlier development, and more complications. The pathological mechanisms of DC are unknown, and the study indicates it is mainly due to oxidative stress, autoimmunity, epithelial-mesenchymal transition (EMT), and lens epithelial cells (LECs) apoptosis.^[2]

Age, sex, race, and genes are significant but non-modifiable factors for cataract development.^[3] Hence, the current research mainly focuses on the modifiable risk factors, like diet and systemic diseases.^[4] Diabetes is the second leading risk factor for cataracts after advanced age, and also the hyperglycemia and the duration of diabetes increase the risk of developing cataracts at a younger age.^[5] In diabetes, there is a high glucose level in the aqueous humor that is passively transported into the lens. The enzyme Aldose Reductase (AR) catalyses the conversion of excess glucose to sorbitol through the polyol pathway and resulting in intracellular accumulation of sorbitol that further leads to osmotic changes, resulting in degeneration of hydropic lens fibers and formation of cataract. In addition, the intracellular sorbitol cannot be removed through diffusion because of its polar character. Hence, accumulation induces apoptosis in Lens Epithelial Cells (LEC), leading to the collapse and liquefaction of the lens and development of diabetic cataract.^[6]

Currently, cataract surgery is the god standard strategy for cataract where in natural cloudy lens replace with artificial intraocular lens. However, this method has several drawbacks, such as post-operative inflammation, posterior capsular opacification, xerophthalmia, macular edema. Additionally, it causes a social and economic burden on health system.^[7] The non-surgical therapies could potentially reduce the need for surgery, minimize surgical complications, and improve the overall quality of life of cataract patients.

F. religiosa (L.) is popular plants that exhibit numerous religious and health benefits, including in eye care. *Ficus religiosa* (L.) Linn (Moraceae) known as 'Peepal tree' in India, is a branched tree with heart-shaped, long-tipped leaves on long slender petioles and purple fruits. Many traditional herbals. In Ayurveda, *F. religiosa* (L.) belongs to a class of drugs called rasayana. Rasayana are rejuvenators, antioxidants and relieve stress in the body.^[11,12]

Due to the anti-hyperglycemic effects^[13], antioxidants and anti-inflammatory action,^[14] *Ficus religiosa* (L.) may offer promising results in these fields, making it a potential candidate for further research and development. In this context, present study intends to evaluate the anticataract activity of *Ficus religiosa* (L.) on glucose-induced cataracts in isolated goat lenses as a non-surgical therapeutic agent.

MATERIAL AND METHODS

1. PLANT AND CHEMICAL PROCUREMENT

Ficus religiosa (L.) was collected from the campus of Government Medical College, Thiruvananthapuram. The plant materials were identified by Prof. Dr. E.A. Siril, HOD of the Department of Botany, University of Kerala, Kariavattom, Kerala, India. All chemicals used in the study were of analytical grade and were purchased from established vendors.

2. PLANT EXTRACT PREPARATION

The leaves were shade dried for 10 days and coarsely powdered, and 100g of dry leaf powder were taken and defatted using petroleum ether by Soxhlet apparatus, and the marc was collected and subsequently extracted with absolute ethanol by Soxhlet apparatus. Then the solvent evaporates using a rotary evaporator under vacuum. The dried extract was stored at 4 °C for use.

3. PHYTOCHEMICAL ANALYSIS

The ethanolic extract was subjected to preliminary qualitative phytochemical screening to identify the presence of phytochemicals in the extract according to the standard procedure.^[15]

4. PREPARATION OF GOAT LENS

In the present study, 30 goat eyeballs were collected from a nearby licensed slaughterhouse and transported to the laboratory, and were stored at 4 °C. Then the lenses were separated by the extracapsular extraction technique and lenses are incubated in freshly prepared artificial aqueous humor (sodium chloride: 140 mM, potassium chloride: 5 mM, magnesium chloride: 2

mM, sodium bicarbonate: 0.5 mM, sodium dihydrogen phosphate: 0.5 mM, calcium chloride: 0.4 mM, and glucose: 5.5 mM) at room temperature. The pH of the medium was 7.8. To prevent the growth of microorganisms, 100 µg/ml streptomycin and 100 IU/ml penicillin were added to the medium.^[16]

5. INDUCTION OF CATARACT

For the induction of cataracts on Goat Lenses, incubated in artificial aqueous humor with glucose at a high concentration of 55 mM for 72 hrs. at 37°C.^[17] Glucose in the artificial aqueous humor get incorporated into the lens and in the lens, glucose metabolized at high concentrations through the polyol pathway and sorbitol accumulation, causing overhydration and oxidative stress. As a result, cataractogenesis begins.

Experimental design

The lenses are divided into 4 groups and treated as follows,

Table 1: Grouping lens for in-vitro goat lens model.

Treatment groups	Treatment
Group I (n = 5) Normal Control group	Lens incubated for 72 hrs. in artificial aqueous humor containing 5 mM glucose
Group II (n = 5) Disease control group	Lens incubated for 72 hrs in artificial aqueous humor containing 55 mM glucose
Group III (n = 5) Low treatment group	Lens incubated for 72 hrs in artificial aqueous humor containing 55 mM glucose treated with 100 µg/mL of extract
Group IV (n = 5) High treatment group	Lens incubated in artificial aqueous humor containing 55 mM glucose treated with 200 µg/mL of extract

6. LENS MORPHOLOGY

After incubation, each lens was placed on grid paper (1 cm²) and the photographs were taken and the opacity were graded according to the opacification grading scheme.^[18]

Table 2: Grading of lens opacification.

Grade 0	Absence of opacification (clearly visible gridlines)
Grade 1	slight degree of opacification (minimal clouding of gridlines, which are still visible)
Grade 2	Diffuse opacification of almost the entire lens (moderate clouding of faintly visible grid lines)
Grade 3	Dense opacification of the entire lens (total clouding of gridlines).

7. BIOCHEMICAL ANALYSIS

7.1 Preparation of lens homogenate sample

After 72 hours of incubation, 10% of lens homogenates of each group were prepared in 0.1 M sodium phosphate buffer of pH 7.4. Then, centrifugate the homogenate by Remi instruments refrigeration centrifuge at 1000 g for 30 min at 4 °C, and the supernatant was collected for the estimation of various biochemical parameters.^[19]

7.2 Estimation of Total Protein Concentration

The total protein concentration of the lens homogenates were determined according to the Lowry method, To 0.1 ml of lens homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. 0.4 ml of phenol reagent was added very rapidly and mixed, and incubated at room temperature for 30 min. Reading was taken against a blank prepared with distilled water at 660 nm in UV–visible spectrometer. The protein content was calculated from std. curve prepared with BSA and expressed as µg/ml homogenate.^[20,21]

7.3 Estimation of Malondialdehyde (MDA)

1.0ml of lens homogenate was mixed with 0.4 ml of aqueous sodium dodecyl sulphate and vortexed, and allowed to stand for 10 minutes. 1.0 ml of 0.8% TBA aqueous solution and acetic acid glacial (1:1, v/v) were added. This mixture was incubated at 95 °C in a water bath, with gentle shaking, for one hour. After cooling, 5.0 ml of n-butanol-pyridine solution (15:1, v/v) was added, and the mixture was vortexed vigorously. The mixture was then centrifuged for 15 minutes (4,000 x g) to extract the pink reaction product. The absorbance of this reaction product was measured at 532 nm, and the readings were compared to a standard curve of known malondialdehyde concentrations.^[22,23]

7.4 Estimation of Catalase (CAT)

The assay mixture contained 0.5 ml of 0.2 M H₂O₂. 1.0 ml of 0.01 M Phosphate buffer (pH 7.0) and 0.4 ml of water. 0.2 ml of lens homogenate was added to initiate the reaction. 2.0 ml of the 5 % dichromate / acetic acid solution (mixture diluted to 1:5 with dist. water) reagent was added after 0,30,60,90 seconds of incubation. To the control tube, the enzyme was added after the addition of the acid reagent. The tubes were then heated for 10 min, and the colour developed was read at 610 nm. The activity of catalase of lens homogenate was expressed as unit of H₂O₂ decomposed / min/g protein.^[24]

7.5 Estimation of Inhibition of Cu²⁺-induced lipoprotein diene formation

The lens homogenate was diluted to 0.67% in phosphate-buffered saline. Copper sulphate was then added to the sample as CuSO₄·5H₂O, resulting in the formation of Cu²⁺ as a result of oxidation. Absorbance at 234 nm was measured after 120 minutes of incubation at 37°C using a UV-Visible spectrophotometer. The lipoprotein diene formation was measured to determine the level of tissue protection against any oxidative stress and free radicals.^[24,26]

RESULTS AND DISCUSSION

1. PHYTOCHEMICAL SCREENING

Qualitative phytochemical screening of extract revealed the presence of following phytoconstituents as listed in Table 3

Table 3: Result of Preliminary phytochemical screening of ethanol extract. + indicates presence and - Indicates absence of the phytoconstituent.

Secondary Metabolites	Name Of the Test	Inference
Alkaloids	Mayer's Test	+
	Hager's Test	-
	Wagner's Test	+
Glycosides	General Test	+
Cardiac Glycoside	Legal's Test	+
	Keller–Killiani Test:	-
Anthraquinone Glycoside	Brontrager's Test	+
	Modified Brontrager's Test	+
Proteins	Biuret Test	+
Terpenoids	Salkowski Test	+
Flavonoids	Shinoda Test	+
Saponins	Froth Formation Test	+
Tannins	Ferric Chloride Test	+

2. LENS MORPHOLOGY AND CATARACT GRADING

The effect of *Ficus religiosa* (L.) extract on lens transparency was evaluated after 72 hours of incubation. In the normal control group, the lens remained completely transparent, indicating normal lens morphology and absence of cataract formation. In contrast, the disease control group showed a marked increase in opacity, with the lenses appearing dense and whitish, confirming the successful induction of cataract due to oxidative stress and accumulation of polyols within the lens fibers.

The low treatment group exhibited a moderate degree of opacity, suggesting partial protection of the lens by the lower dose of *F. religiosa* (L.) extract. However, some cortical opacities were

still visible, indicating that the dose was not sufficient to completely inhibit the cataractogenic changes. In the high treatment group, the lenses retained near-normal transparency with minimal or no visible opacities. This indicates a strong protective effect of the higher concentration of *F. religiosa* (L.) extract, possibly due to its antioxidant and free radical scavenging activity, which helps maintain lens protein integrity and prevent oxidative damage.

The cataract grading data also support these observations. The disease control group showed the highest grade of opacity (grade 3), whereas the high treatment group showed minimal or no opacity (grade 0–1), similar to the normal control group. The low treatment group showed an intermediate grade (grade 1–2). These results suggest a dose-dependent protective effect of *F. religiosa* (L.) against cataract formation, likely through inhibition of oxidative stress and preservation of lens transparency.

Table 4: Effect of *F. religiosa* (L.) on lens morphology after 72 hrs of incubation.

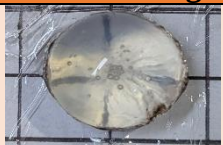
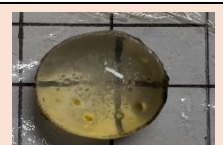
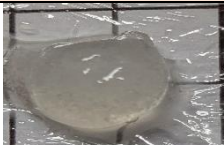
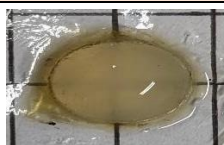
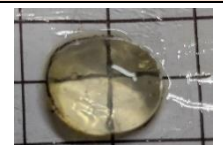
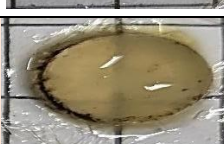
GROUP I Normal Control group	GROUP II Disease Control group	GROUP III Low treatment group	GROUP IV High treatment group
			
			
			
			
			

Table 5: Grading of lens opacification after 72 hrs of incubation.

GROUP I Normal Control group	GROUP II Disease Control group	GROUP III Low treatment group	GROUP IV High treatment group
0	2	3	0
0	3	2	0
1	3	2	1
0	3	1	0
0	3	1	0

3. BIOCHEMICAL ANALYSIS

3.1 Estimation of Total Protein concentration

The cataract group showed a significantly (p value < 0.001) reduced protein level (4.40 ± 0.415 mg/mL) compared to the normal group, which is attributed to excessive protein aggregation and denaturation in the cataractous lens. Treatment with both low (p value < 0.01) and high doses (p value < 0.001) of the extract improved the total protein concentration to 7.42 ± 0.03 mg/mL and 8.26 ± 0.134 mg/mL, respectively, approaching the normal lens value of 10.63 ± 0.73 mg/mL. This suggests that the extract possesses the ability to inhibit protein aggregation under cataract conditions.

3.2 Estimation of Malondialdehyde (MDA) level

Malondialdehyde (MDA) serves as a key marker of oxidative stress within cells. The results revealed that the cataract group exhibited a significantly (p value < 0.001) elevated MDA level (9.90 ± 0.54 nmol/mg protein) compared to the normal group (2.86 ± 0.48 nmol/mg protein). Treatment with low and high doses of the extract significantly (p value < 0.01) reduced MDA levels to 4.12 ± 0.26 and 3.040 ± 0.28 nmol/mg protein, respectively, indicating its potential to mitigate oxidative damage.

3.3 Estimation of Catalase (CAT) level

Catalase, a major antioxidant enzyme found in nearly all cells, plays a crucial role in neutralizing excess reactive oxygen species. The results showed that the cataract group had significant (p value < 0.001) reduced catalase activity (0.62 ± 0.037 U/mg protein) compared to the normal group (2.61 ± 0.1631 U/mg protein). Treatment with low (p value < 0.001) and high doses (p value < 0.01) of the extract significantly improved catalase activity to 2.03 ± 0.105 and 1.39 ± 0.16 U/mg protein, respectively, suggesting its ability to restore antioxidant defence mechanisms.

3.4 Estimation of Inhibition of Cu²⁺-induced lipoprotein diene formation

Copper from CuSO₄·5H₂O reacts with the sample, generating Cu²⁺ ions through oxidation. The extent of Cu²⁺-induced lipoprotein diene formation was assessed after 120 minutes of incubation. The disease group showed a significant (p value < 0.01) higher absorbance value (2.03 ± 1.31) compared with the normal group. In contrast, the low-dose (1.52 ± 0.01), highdose (1.65 ± 0.03), and normal (1.94 ± 0.075) groups exhibited lower absorbance values. The reduction in absorbance suggests that the extract confers a protective effect against oxidative stress.

4. Statistical analysis

The all experimental data were shown as mean ± SEM. The data were statistically evaluated by analysis of variance (ANOVA) Bonferroni's test using Graph Pad Prism software version 10.

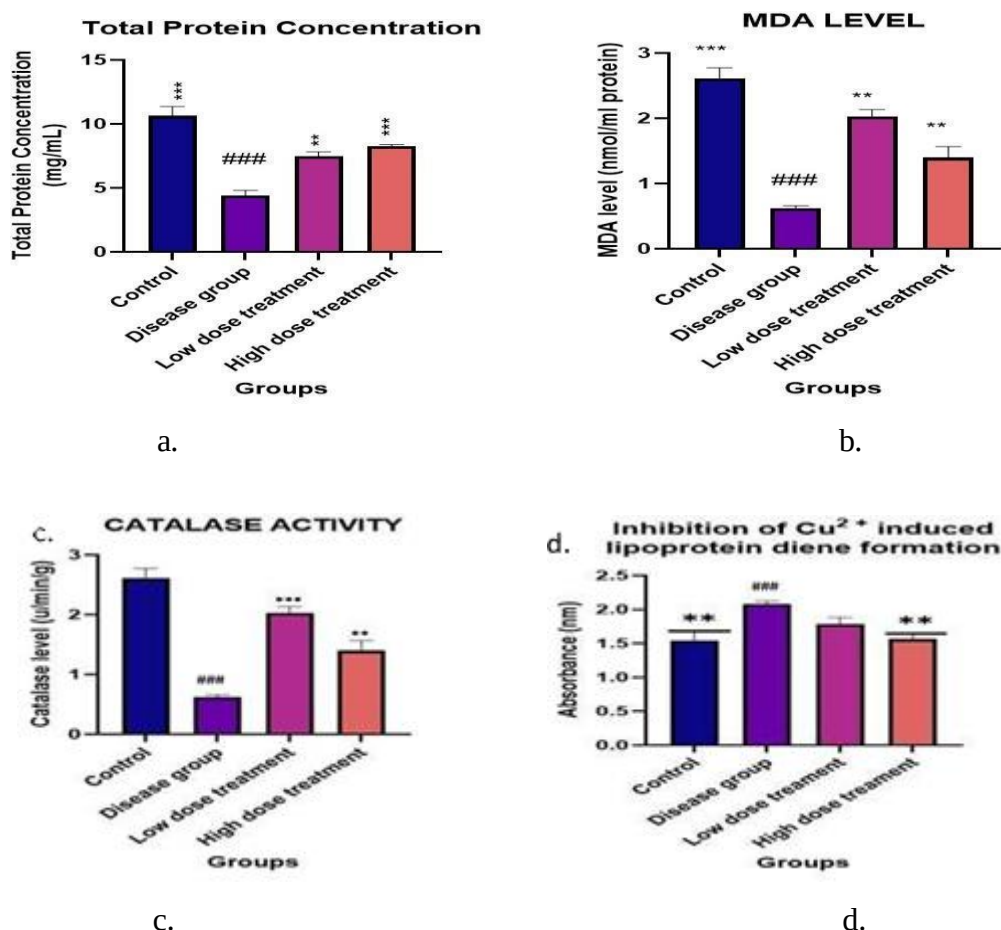


Fig.1: Effect of *F. religiosa* (L.) on a) Total protein concentration, b) MDA level, c) Catalase level, and d) Inhibition of Cu²⁺-induced lipoprotein diene formation. N=5, values are expressed as Mean ± SEM. Comparison were made as following, ### p < 0.001 when compared with normal group. * p < 0.001 when compared with disease**

group.

Table 6: Biochemical estimation of lens homogenate.

Group	Total protein concentration (mg / mL)	MDA level (nanomoles/ mg protein)	Catalase level (U/min/g protein)	Inhibition of cu 2+ induced lipoprotein diene formation
Normal group	10.63±0.739	2.86±0.480	2.61±0.1631	1.94 ± 0.075
Disease group	4.408±0.415	9.90±0.545	0.62±0.037	2.03 ± 1.31
Low-dose treatment	7.482±0.034	4.12 ±0.261	2.03±0.105	1.52 ± 0.01
High-dose treatment	8.262±0.1343	3.040±0.283	1.39±0.16	1.65 ± 0.03

CONCLUSION

The present study demonstrates that the ethanolic extract of *Ficus religiosa* (L.) leaves exerts significant anticataract activity in a glucose-induced goat lens model. The extract effectively preserved lens morphology and restored biochemical parameters, including total protein concentration, catalase activity, and inhibition of lipid peroxidation, while reducing malondialdehyde levels. These findings suggest that the protective effects are primarily attributed to the antioxidant potential of the extract, which prevents protein aggregation and mitigates oxidative stress, the key mechanisms underlying cataractogenesis. Therefore, *F. religiosa* (L.) holds promise as a potential non-surgical therapeutic agent for the prevention or delay of cataract progression. However, further in vivo studies and clinical trials are warranted to validate its efficacy, establish safe dosage levels, and confirm its clinical applicability in human cataract management.

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