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Original Research Article (Experimental)

Anticataract effects of *S. cumini* and *A. marmelos* on goat lenses in an experimental diabetic cataract model

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A R T I C L E I N F O

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A B S T R A C T

Background: Cataractogenesis in diabetes mellitus is mainly due to generation of free radicals causing oxidative stress. Antioxidants are known to delay cataractogenesis. Indigenous plants are potential promising sources of antioxidants.
Objectives: The present study was done in goat lenses for exploring local antioxidant and anticataract potential of *Syzygium cumini* (Jamun) and *Aegle marmelos* (Bael) and comparing their activities.
Material and methods: "Lens organ culture technique" was employed using "tissue culture medium 199" (TC 199). Lenses were divided into four groups of 30 each. Group 1 was "Normal Control". In remaining 3 groups, experimental diabetic cataract was produced using dextrose (110 mM). Group 2: "Toxic Control" (untreated experimental diabetic cataract lenses). Group 3: *S. cumini* seed extract (0.25%) treated lenses. Group 4: *A. marmelos* leaf extract (0.25%) treated lenses. Biochemical parameters measured in lens homogenates included total soluble lens proteins (index of cataractogenesis), malondialdehyde (index of lipid peroxidation), and superoxide dismutase, glutathione reductase, and glutathione peroxidase (indices of antioxidant enzyme activity). Lens morphology was compared in all groups.
Results: *S. cumini* and *A. marmelos* showed significantly increased activity of all three antioxidant enzymes, preserved total soluble proteins and decreased malondialdehyde (MDA). Lens morphology was well preserved with these extracts. *S. cumini* aqueous seed extract scored better over *A. marmelos*.
Conclusion: In goat lenses with dextrose-induced experimental diabetic cataract, *S. cumini* and *A. marmelos* showed antioxidant and anticataract properties and preservation of lens morphology ($p < 0.0001$ to 0.05). *S. cumini* showed better anticataract activity than *A. marmelos*.

1. Introduction

Cataract is the leading cause of blindness world over. Diabetes mellitus has been considered to be a major risk factor for cataractogenesis. It is known that in diabetics, the cataracts occur at comparatively an earlier age and 2–5 times more frequently. *It is reported that about 20% cataract surgeries are performed for diabetics alone* [1]. Lens opacification in cataract as a complication of diabetes mellitus is associated with increased oxidative and hyperosmolar stress.

Oxidative damage to the lens has been linked with development of cataract, and decrease in antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and subsequent development of cataract [2]. A wide range of drugs like aldose reductase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) are being tried for their anticataract activity [3].

There has been a growing interest in the various activities of indigenous plants. Many indigenous plants have been explored as potential promising sources of antioxidants [4–6].

Syzygium cumini (*S. cumini*) (L.) Skeels (jambolan), called "Jambhul" in Marathi and "Jamun" in Hindi, has been one of the most widely used plants in the management of various diseases. Ayurvedic system of medicine is known to be using jamun seeds in the management "madhumeha" (diabetes mellitus) [7]. Leaves of *Aegle marmelos* (*A. marmelos*) (Bael) are valued in Ayurveda in the

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management of diabetes mellitus, and also some ophthalmic and inflammatory conditions. The antioxidant and hypoglycemic effects of leaves of *A. marmelos* have been explored in alloxan-induced diabetic rats [8]. *S. cumini* has been studied as having antioxidant activity [9,10]. Isolation studies of seed extract of *S. cumini* demonstrated significant antioxidant activity in the form of caffeic acid [11].

The present study was done to specifically test the local antioxidant and anticataract effects of extracts of *S. cumini* seeds and *A. marmelos* leaves on dextrose-induced experimental models of diabetic cataract by lens organ culture technique in isolated goat lenses. The study also extrapolates their antioxidant and cataract preventing properties. The lens morphology, lipid peroxidation, cataractogenesis, and specific antioxidant activity were the areas of interest.

2. Material and methods

The study was approved by Institutional Ethics Committee. The study was done in 120 fresh isolated goat lenses. Goat eyeballs were obtained from the slaughter house and were transported to the laboratory in an ice box. Lenses were removed from the eyeballs by intracapsular lens extraction method. Lenses were carefully placed on sterile petri dishes with a dark colored nylon net. The lenses were incubated in "Tissue Culture Medium" (TC 199) by "Lens Organ Culture Technique" for 72 h [12]. The study was carried out in 4 groups of 30 lenses each.

Group 1 served as "Normal Control" (subjected only to TC 199). Group 2 served as "Toxic Control" consisting of lenses in which experimental diabetic cataract was produced by using 110 mM dextrose ("Toxic Control") [13]. Group 3 consisted of goat lenses treated with aqueous extract of seeds of *S. cumini* (Jamun) along with dextrose and TC 199. Group 4 consisted of goat lenses treated with aqueous extract of *A. marmelos* (Bael) leaves, dextrose, and TC 199.

2.1. Preparation of plant water extracts

Dry powders of *S. cumini* (Jamun) seeds and *A. marmelos* (Bael) leaves were taken and 25% w/v water extracts were prepared. The extracts were analyzed for their purity and "total dissolved solids" at Indian Drugs Research Association & Laboratory, Pune. The concentration of solution of each extract used for the study was 0.25%.

2.2. Preparation of lens homogenate

At the end of 72 h of incubation, lenses from each group were removed and 10% homogenate of whole lens was prepared in 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 $\times g$ for 30 min at -4 °C in a refrigerated centrifuge. The supernatant was collected and stored at -20 °C until further use.

2.3. Estimation of biochemical parameters

The supernatant was subjected to the estimation of biochemical parameters that included total soluble lens proteins, malondialdehyde (MDA), superoxide dismutase, glutathione peroxidase, and glutathione reductase.

Lowry's method was used for estimation of total soluble lens proteins and the method by Kei Satoh was used to estimate MDA, as an index of lipid peroxidation by thiobarbituric acid reacting substances (TBARS) quantification [14]. Superoxide dismutase activity was spectrophotometrically measured by monitoring pyrogallol reduction rate [14]. Randox kits were used for estimation of glutathione peroxidase and glutathione reductase. For glutathoine peroxidase estimation, the substrates used were hydroperoxide and GSH [15]. Glutathoine reductase was assayed by measuring decreased absorbance at 340 nm due to oxidation of NADPH to NADP during GSSG getting converted to GSH [16].

2.4. Statistical analysis

Percent difference was calculated (increase in percentage for the activity of the three antioxidant enzymes and decrease in percentage for amounts of total soluble lens proteins and malondialdehyde), and the values were subjected to statistical analysis.

Biochemical parameters were subjected to student's "t" test to compare between the Group 1 and Group 2 (Normal control and Toxic control). For comparing the biochemical parameters between Group 2, Group 3, and Group 4 (Dextrose-induced cataract lenses, *S. cumini* treated lenses, and *A. marmelos* treated lenses respectively), "One-way Analysis of variance" (ANOVA) was performed.

2.5. Lens morphology

For studying the lens morphology, the lenses were placed on a grid/net and changes in lens transparency were observed by noting the number and characteristics of the squares of the grid/net seen through the lens. Generalized haziness or opacity, intumescence, swelling, disruption and other morphological changes were also noted. The grades of cataract changes were classified based on modification of the criteria used in past for grading the cataract changes [17]. They are shown in Table 1.

3. Results

3.1. Changes in experimental dextrose induced cataract lenses

Changes in Experimental dextrose induced cataract lenses were observed by estimating the changes in total soluble lens proteins, malondialdehyde (MDA), superoxide dismutase, glutathione peroxidase, and glutathione reductase etc.

3.1.1. Expression of lipid peroxidation

The values of expression of lipid peroxidation is listed in Table 2.

Table 1
Grades of cataract changes.

Grade	Description	Details
0	No changes	Visible grid lines, lens outline and shape preserved
1	Mild	Visible grid lines, minimal lens swelling, lens outline and shape preserved
2	Moderate	Faintly visible grid lines, lens swelling present
3	Moderate to severe	Almost obstructed grid lines, lens outline and shape damaged
4	Severe	Invisible grid lines, distorted lens shape and outline, mature cataract about to rupture

Table 2
Expression of lipid peroxidation and oxidative stress in dextrose-induced lenses.

No	Parameter	Group 1 (n = 30) ("Normal control")	Group 2 (n = 30) ("Toxic control") (cataract lenses)
1	Total soluble lens proteins (mg/dL) (Mean ± SD)	344 ± 62.56	255 ± 62.46 ($p < 0.0001$)
2	Malondialdehyde (MDA) (nmoles/ml) (Mean ± SD)	9.56 ± 3.43	13.45 ± 3.0 ($p < 0.0001$)
No	Antioxidant Enzyme	Group 1 (n = 30) ("Normal control") (units/mg lens) (Mean ± SD)	Group 2 (n = 30) ("Toxic control") (cataract lenses) (units/mg lens) (Mean ± SD)
1	Superoxide dismutase	0.41 ± 0.14	0.30 ± 0.15 ($p < 0.01$)
2	Glutathoine peroxidise	36.16 ± 9.90	29.71 ± 13.92 ($p < 0.05$)
3	Glutathione reductase	13.32 ± 3.55	10.49 ± 2.11 ($p < 0.001$)

3.1.1.1. Total soluble lens proteins. The total soluble lens protein content in Group 2 ("Toxic control") (dextrose-induced cataract lenses) decreased by 25.8% as compared to Group 1 (normal control) lenses. The decrease was statistically highly significant ($p < 0.0001$).

3.1.1.2. Malondialdehyde. Lipid peroxidation measured in terms of malondialdehyde (MDA) levels showed an increase in MDA levels by 28.9% in Group 2 ("Toxic control") (dextrose induced cataract lenses) as compared to Group 1 (normal control lenses). The increase in MDA was statistically highly significant ($p < 0.0001$).

3.1.2. Expression of oxidative stress

3.1.2.1. Superoxide dismutase. Group 2 (Dextrose induced cataract lenses) showed a decrease in the specific activity of enzyme superoxide dismutase by 26.8% as compared to Group 1(Normal control lenses) (Table 2). The decrease was statistically significant ($p < 0.01$).

3.1.2.2. Glutathione peroxidase. Glutathione peroxidase specific activity was reduced by 17.7% in Group 2 (Dextrose induced cataract lenses) as compared to Group 1 (Normal control lenses). The decrease was statistically significant ($p < 0.05$).

3.1.2.3. Glutathione reductase. A decrease of 21.2% in the specific activity of Glutathione reductase was noted in Group 2 (Dextrose induced cataract lenses) as compared to control lenses with a significance of $p < 0.001$.

3.1.3. Lens morphology (Comparison Group 1 and Group 2)

The Group 1 ("normal control" lenses) had the transparency and clarity maintained as evidenced by the clearly visible grids. As compared to the lenses in Group 1 ("Normal control"), the lenses in Group 2 ("Toxic control") showed total loss of transparency with development of mature cataract nearing rupture as evidenced by the invisibility of grids, thus showing Grade 4 changes (Fig. 1).

3.2. Effect of *S. cumini* aqueous seed extract and *A. marmelos* aqueous leaf extract on dextrose-induced cataract lenses

3.2.1. Expression of lipid peroxidation

3.2.1.1. Total soluble lens proteins. The total soluble lens protein content in Group 3 (*S. Cumini*) increased by 31.4% ($p < 0.01$) (Table 3) whereas that in Group 4 (*A. marmelos*) increased by 13.3% (not significant) as compared to Group 2 (toxic control) lenses.

3.2.1.2. Malondialdehyde. Lipid peroxidation measured in terms of malondialdehyde (MDA) levels showed a significant decrease in MDA levels by 30.2% ($p < 0.001$) in Group 3 (*S. cumini*), whereas in the *A. marmelos* group the decrease in MDA levels was by 13.3%, which was not significant.

Table 3
Effect of aqueous extracts of medicinal plants on lipid peroxidation and antioxidant enzymes.

No	Parameter	Group 2 (n = 30) ("Toxic control") (cataract lenses)	Group 3 (n = 30) (*S. cumini* seed extract treated lenses)	Group 4 (n = 30) (*A. marmelos* leaf extract lenses)
1	Total soluble lens proteins (mg/dL) (Mean ± SD)	255 ± 62.4 ($p < 0.0001$)	335.3 ± 74.03 Increase by 31.4% $p < 0.01$	294.33 ± 34.9 Increased by 13.3% Not significant
2	Malondialdehyde (MDA) (nmoles/ml) (Mean ± SD)	13.45 ± 3.0 ($p < 0.0001$)	9.48 ± 2.4 Decreased by 30.2% $p < 0.001$	10.34 ± 3.2 Decreased by 13.3% Not significant
1	Superoxide dismutase (units/mg lens) (Mean ± SD)	0.30 ± 0.15	1.01 ± 0.28 Increased by 69.3% $P < 0.01$	0.37 ± 0.12 Increased by 16.1% Not significant
2	Glutathione peroxidase (units/mg lens) (Mean ± SD)	29.71 ± 13.92	45.14 ± 14.2 Increased by 51.8% $P < 0.001$	49.07 ± 22.4 Increased by 39.43% $p < 0.01$
3	Glutathione reductase (units/mg lens) (Mean ± SD)	10.49 ± 2.11	31.33 ± 6.7 Increased by 66.4% $p < 0.001$	23.06 ± 6.8 Increased by 54.46% $p < 0.001$

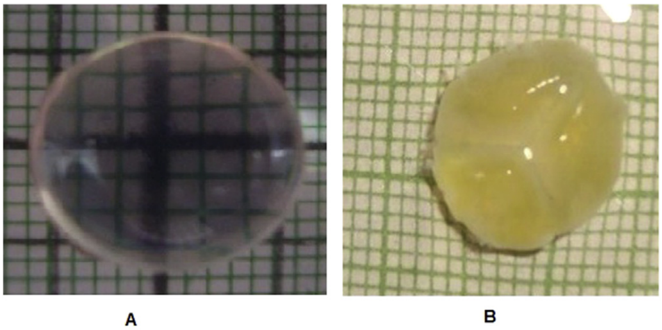

Fig. 1. **A**- Normal control goat lens (Grids visible) – Grade 0. **B**- Dextrose-treated lenses (Group 2) – Grade 4 changes.

3.2.2. Expression of oxidative stress

3.2.2.1. Superoxide dismutase. As compared to the Group 2 (cataract lenses), the Group 3 (*S.cumini*) showed significant increase in the specific activity of the enzyme superoxide dismutase by 69.3% ($P < 0.01$), whereas in Group 4 (*A. marmelos*) the increase was by 16.1%, which was not statistically significant (Table 3).

3.2.2.2. Glutathione peroxidase. As compared to the Group 2 (cataract lenses), the glutathione peroxidase specific activity was increased significantly in both Group 3 (*S.cumini*) and Group 4 (*A. marmelos*). With *S. cumini*, it increased by 51.8% ($p < 0.001$), whereas in *A. marmelos* it increased by 39.43% ($p < 0.01$).

3.2.2.3. Glutathione reductase. Similarly, statistically significant increase in the specific activity of glutathione reductase by 66.4% ($p < 0.001$) was recorded with *S. cumini* group (Group 3), and the increase was by 54.46% ($p < 0.001$) with *A. marmelos* group (Group 4) when compared to the Group 2 (cataract lenses).

3.2.3. Lens morphology

As compared to the cataract lenses, the lenses treated with *S. cumini* seed extract (Group 3) and *A. marmelos* extract (Group 4) showed mild Grade 1 changes with visible grid lines and maintenance of lens outline and shape, with minimal lens swelling (Fig. 2).

4. Discussion

S. cumini (Jamun) and *A. marmelos* have been mentioned in ayurvedic literature for their antidiabetic properties. However, there was a need to assess the *in vitro* effect of these plant extracts on the lens morphology and on the oxidative stress related biochemical changes happening in cataract. Streptozotocin-induced diabetic rats and galoctosemic rats have been used as animal models of diabetic cataract, and mice have been used for *in vivo* studies [18]. The present study was done by developing an *in vitro* cataract model in goat lenses by use of a higher concentration of dextrose than that used in the previous known studies [17]. The lens organ culture technique was employed by using tissue culture medium (TC 199) and dextrose was used as a toxicant for induction of experimental cataract in the concentration as high as 110 mM dextrose, based on the hyperglycemic model for fish retina developed by Alvarez et al. [13].

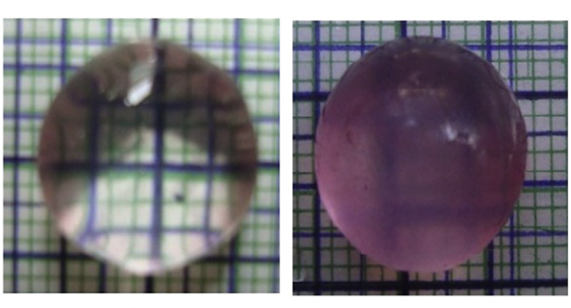

Fig. 2. **A**- Effect of *S. cumini* seed extract (Grade 1 changes). **B**- Effect of *A. marmelos* leaf extract (Grade 2 changes).

The purpose of the present work was to specifically evaluate the local effects of the extracts of seeds of *S. cumini* and leaves of *A. marmelos* for the antioxidant and anticataract properties tested by biochemical and physical parameters.

Oxidative stress is known to be participating in development of diabetes and its complications like cataract. On exposure to dextrose in a high concentration, the glucose in the lens starts getting utilized through sorbitol pathway. Accumulation of polyols (sugar alcohols) causes overhydration and oxidative stress leading to generation of cataract [13]. Hyperglycemia induces oxidative stress through various pathways [19]. Further oxidation of the lens crystallins as well as membrane proteins results in the formation of insoluble protein aggregates. Loss of soluble proteins from lens by their conversion into insoluble ones due to lens protein oxidation reflected as decrease in total lens protein content as evidenced in the present study.

Lipid peroxidation was assessed by the total soluble lens protein content and the malondialdehyde levels. The oxidative stress was assessed by measurement of the three antioxidant enzymes namely superoxide dismutase, glutathione peroxidase, and glutathione reductase [19].

Increased oxidative stress impairs the enzymatic defenses against reactive oxygen species. This is evidenced by the significant decrease of various antioxidant enzymes in experimental models of cataract in lenses. Phytochemical antioxidants such as flavonoids, tannoids, gallic acid, and phenols can scavenge free radicals. *S. cumini* seeds and *A. marmelos* leaves are rich in these phytochemicals [9]. Addition of *S. cumini* aqueous seed extract exhibited significant antioxidant activity evident by increase in the levels of all the three antioxidant enzymes. *A. marmelos* aqueous leaf extract exhibited significant antioxidant activity pertaining to the enzymes glutathione peroxidase and glutathione reductase. This is brought about by quenching free radicals. Richness of phytochemical antioxidants in the extracts of these two plants directs to their probable role in prevention of lipid peroxidation and thereby cataract formation. The free radical scavenging activity of *S. cumini* aqueous extract is evident in the present study by the marked increase in activities of the antioxidant lens enzymes. *A. marmelos* aqueous leaf extract is also known to enhance the cellular glutathione levels [20]. A significant increase in the activity of glutathione peroxidase and glutathione reductase in lenses treated with *A. marmelos aqueous* leaf extract in the present study can be thus explained.

The goat lens morphology was studied based on various parameters like lens shape, outline, swelling, and transparency; and the cataract changes were graded according to severity. It was found that exposure to 110 mM dextrose produced grade 4 changes in the goat lenses. There was total loss of transparency with osmotic swelling and development of mature cataract nearing rupture. This was evident by the invisibility of grids. Gradation of cataract severity was useful to compare the effects of the two plants extracts.

Both aqueous extracts demonstrated an increase in the specific activity of all the three antioxidant enzymes and a significant decrease in malondialdehyde levels as well as significant preservation of the levels of total soluble proteins. Both extracts were able to demonstrate preservation of lens morphology against the cataract changes. However, aqueous seed extract of *S. cumini* demonstrated a consistently better effect as compared to the aqueous leaf extract of *A. marmelos* with regards to all the parameters including preservation of lens morphology. The effects found in the present study reinforce the findings in past related to *S. cumini* [9,10,21].

The experimental evidence in past has demonstrated the involvement of lipid peroxidation in the pathogenesis of cataract. Malondialdehyde (MDA) is known as a predominant breakdown product of lipid peroxides. In human cortical cataract as well as nuclear cataract, MDA has been found to be increased by 3.5 times and 2.5 times respectively than the normal levels in various studies, and is identified as a 2-thiobarbituric acid reactive substance (TBARS) [22,23]. Increased MDA levels in the experimental cataract model developed in the present study mimicked these effects seen in humans. Impaired defense mechanism against reactive oxygen metabolites has been demonstrated in human cataractous lenses as expressed by significantly decreased activities of various antioxidant enzymes [22]. The experimental model developed in the present study mimicked these effects as reflected in the decrease in all the three antioxidant enzymes.

5. Limitations of the study

There is further need for assessing the antioxidant activity of these plant extracts in a different cataract model. Also potency studies of both extracts need to be done to explore the possible future steps for *in vivo* studies.

6. Conclusion

In isolated goat lenses with experimental dextrose induced diabetic cataract, *S. cumini* seed extract showed significant antioxidant and anticataract properties. *A. marmelos* leaf extract also showed significant anticataract properties and antioxidant properties on all the parameters tested except for the enzyme superoxide dismutase for which the effects were comparatively less.

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None declared.

Conflict of interest

None.

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