

EVALUATION OF *EX VIVO* ANTICATARACT ACTIVITY OF ETHANOLIC EXTRACT OF *ALSTONIA SCHOLARIS* LEAVES ON DEXAMETHASONE-INDUCED CATARACT BY USING ISOLATED GOAT LENS

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ABSTRACT

Objective: To investigate the *ex vivo* anticataract activity of ethanolic extract of *Alstonia scholaris* (EEAS) leaves on dexamethasone-induced cataract using isolated goat lens.

Methods: Anticataract activity is done using isolated goat lens. Goat lens was divided into four groups. Group I: Lens was incubated in artificial aqueous humor (normal control). Group II: Lens was incubated with dexamethasone 10 mg (toxic control). Group III and IV: Lens was incubated with dexamethasone and EEAS (50 µg and 100 µg) and subjected to photographic evaluation for opacity; lens was homogenized using Tris-phosphate buffer, and sodium, potassium, total protein, and catalase concentrations were determined.

Results: The grades of opacity were 0, 3, 1, and 1 in group I, II, III, and IV, respectively. The present study showed higher total proteins ($p < 0.05$ at all concentration) and K^+ ions ($p < 0.05$ at all concentration), whereas lower concentrations of Na^+ ions ($p < 0.05$ at all concentration) with EEAS-treated groups. The level of catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with EEAS showed a significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.

Conclusion: The present investigation suggests that EEAS leaves effectively prevent the cataractogenic condition. Thus, the goat lens model and dexamethasone-induced cataract model could be used for testing of various anticataract agents.

Keywords: Cataract, Artificial aqueous humor, Lens, Dexamethasone, *Alstonia scholaris*.

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INTRODUCTION

Cataract (lens opacification) is a major contributing factor of blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for focusing and producing a clear sharp image. It is called as a "peril of sight" because cataracts have blinded more people throughout the ages than any other affliction of the eye. It is also called as "Senile cataract." Cataract is derived from the Latin word "cataracta" meaning waterfall. Age-related nuclear cataract is the most common form of cataract which is found in ages more than 45 years old and opacity forms in the center of the lens [1]. Cataract is nothing but visual impairment as a result of a disturbance of lens transparency. It is one of the leading causes of blindness worldwide; it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28,000 new cases are reported daily worldwide. Approximately, 25% of the populations over 65 and about 50% over 80 have a serious loss of vision because of cataract [2,3]. Cataractogenesis is influenced by multiple risk factors such as aging, diabetes mellitus, drugs, trauma, toxins, genetics, smoking, and other ocular diseases. Multiple mechanisms such as osmotic graduation, protein aggregates, oxidative stress, post-translational protein changes, and phase separation are proposed for cataract formation. Combined factors of heritage, ultraviolet light exposure, diet, some metabolic disorders, quality of life, cationic pump malfunction, and lens metabolism disorder are believed to have a role in cataract formation. The increased incidence of cataracts in diabetic patients is also well known [4]. Presently, surgery is the only approach for the treatment of cataract, and while favorable outcomes are quite predictable, the limited number of surgeons is underdeveloped countries, and the high cost of surgery have made cataract a major health problem. Drugs developed to delay or prevent lens opacification have failed to give

convincing positive results in clinical trials. This stimulates the research toward the experimental work on cataract to understand the all possible pathway and mechanism which is responsible for the generation of cataract. While the main treatment for cataract is surgical intervention, it is associated with certain risks and subsequent suboptimal outcomes [5].

Prolonged use of glucocorticoids is a significant risk factor for the development of posterior subcapsular cataract. This places a restriction on the use of glucocorticoids in the treatment of systemic and ocular inflammatory conditions as well as organ transplantation. Glucocorticoids induce subcapsular cataract by cause the metabolic disturbances, protein modifications, oxidative damage, and inactivation of Na, K-ATPase system [6].

The prophylactic and therapeutic effect of many herbal extracts has been reported, such as *Adhatoda vasica*, *Allium cepa*, *Cassia fistula*, *Citrus aurantium*, *Cochlospermum religiosum*, *Curcuma longa*, *Ginkgo biloba*, *Momordica charantia*, *Ocimum sanctum*, and *Vitex negundo* having anticataract activity [7,8]. *Alstonia scholaris* leaves extract having a pharmacological action such as antimicrobial, hepatoprotective, and antioxidant. Antiulcer, antidepressant, and antineoplastic activity are also reported [8]. With this background, the objective of the current study was to evaluate the anticataract activity of ethanolic extract of *A. scholaris* (EEAS) leaves.

METHODS

Collection and identification

A. scholaris leaves were collected from the local medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur,

Andhra Pradesh, India. Authenticated by the Department of Botany, ANU, Guntur, voucher specimens kept for future reference.

Drying and grinding

The collected plant part (leaves) was separated from undesirable material, and the leaves were dried under shade at room temperature for 2 week. The dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in airtight container until the analysis was commenced.

Extraction

Coarsely powdered leaves (500 g) were successively extracted with petroleum ether (60-80°C) for 7 days to remove fatty matter. The defatted marc was then subjected to Soxhlet extraction with 75% ethanol to obtain ethanolic extract. The ethanolic extract was evaporated under reduced pressure at low temperature (30°C) to dryness and brownish yellow color extract was obtained [9].

Preliminary phytochemical screening

EEAS was subjected to preliminary phytochemical for the detection of various constituents [10].

Ex vivo evaluation of anticataract activity

In this study, goat lens was used as they were easily available. Fresh goat lens was collected from slaughterhouse from Guntur.

Lens culture

Fresh goat eyeballs were obtained from slaughterhouse were immediately transported to the laboratory at 0-40 C. The lens was removed by extracapsular extraction and incubated in artificial aqueous humor (sodium chloride: 140 mM, hydrochloric acid: 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 and PH 7.8. Cefixime 500 mg was added to the culture media to prevent bacterial contamination [11].

Induction of ex vivo cataract

Dexamethasone 10 mg was used to induce cataract. Dexamethasone-induced posterior subcapsular cataract by oxidative stress, osmotic change, hydration, and conformational change of proteins. A total of 16 lenses were used for the study. These lenses were incubated in artificial aqueous humor with dexamethasone 10 mg/kg served as toxic control for 5 days [12].

Study group

A total 16 lenses were divided into following groups (n=4 in each group).

- Group I: Aqueous humor (normal control)
- Group II: Aqueous humor + dexamethasone 10 mg (toxic/model control)
- Group III: Aqueous humor + dexamethasone 10 mg + EEAS 50 µg/ml
- Group IV: Aqueous humor + dexamethasone 10 mg + EEAS 100 µg/ml.

Photographic evaluation

After 5 days of incubation, lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of squares clearly visible through the lens) was observed through the lens as a measure of opacity.

The degree of opacity was graded as follows:

- 0 - Absence of opacity
- 1 - Slight degree of opacity
- 2 - Presence of diffuse opacity
- 3 - Presence of extensive thick opacity.

Preparation of lens homogenate

After 5 days of incubation, homogenate of lenses was prepared in Tris-buffer (0.23 M, pH 7.8) containing 0.25×10⁻³ M EDTA, and homogenate was adjusted to 10% w/v which was centrifuged at

10,000 G at 4°C for 1 hr and the supernatant was used for the estimation of biochemical parameters [12].

Biochemical parameters

Electrolyte (Na⁺) and potassium (K⁺) estimation was done by flame photometry method, and protein estimation was done by modified biuret end point assay method. Estimation of catalase in lens homogenate was done by Aeibe *et al.* [13-19].

Statistical analysis

Results were expressed as mean±standard error of the mean. The statistical significance of the difference between groups for the various treatments was determined by one-way analysis of variance followed by Dunnett's test. p<0.05 was considered statistically significant.

RESULTS

Phytochemical analysis was performed for various phytochemical constituents present EEAS and results were shown in Table 1.

The grades of opacity were 0, 3, 1, and 1 in Group I, II, III, and IV, respectively, and results were shown in Table 2. The present study showed higher total proteins (p<0.05 at all concentration) and K⁺ ions (p<0.05 at all concentration), whereas lower concentrations of Na⁺ ions (p<0.05 at all concentration) with EEAS-treated groups. The level of catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with EEAS showed a significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity and results were shown in Table 3. The obtained results were plotted in Graphs 1-4.

Photographic evaluation

Incubation of lenses with dexamethasone 10 mg showed moderate opacification starting after 2 days at the periphery, on the posterior surface of the lens. This progressively increased toward the center, with complete opacification at the end of 5 days as compared to lenses incubated in normal aqueous humor where transparency maintained, and squares were clearly visible. Incubation of lenses with EEAS at (50 µg/ml, 100 µg/ml) concentrations seems to retard the progression of lens opacification (Fig. 1).

DISCUSSION

Cataract is a major cause of blindness worldwide. It is an age-related phenomenon, over and above oxidative stress also plays its role. Surgical treatment has remained the only remedy till now. Hence, if a drug is sought which can either reverse or prevent lenticular opacity,

Table 1: Phytochemical analysis of ethanolic extract of *A. scholaris*

Name of the test	Results
Carbohydrates	++
Steroids	--
Alkaloids	++
Glycosides	++
Tannins	++
Flavonoids	--
Saponins	++
Gums	--

A. scholaris: *Alstonia scholaris*

Table 2: Grades for lens

Study groups	Grade
Group I (normal control)	0
Group II (model control)	3
Group III (measurement 50 µg/ml)	1
Group IV (measurement 100 µg/ml)	1

Table: 3 Effect of EEAS on sodium, potassium, total protein and catalase levels in dexamethasone-induced cataract

Groups	Sodium levels $\mu\text{g/ml}$	Mean \pm SEM		
		Potassium levels $\mu\text{g/ml}$	TPC level g/dl	Catalase levels $\mu\text{m of H}_2\text{O}_2/\text{minutes}$
Group I	105.5 \pm 2.10	10.8 \pm 0.44	3.25 \pm 0.01	228.3 \pm 0.85
Group II	227.3 \pm 3.30	6.17 \pm 0.11	1.87 \pm 0.03	143 \pm 0.91
Group III	165.8 \pm 1.93	8.95 \pm 0.12	2.52 \pm 0.04	267.5 \pm 1.04
Group IV	116 \pm 1.29	10.18 \pm 0.15	2.86 \pm 0.04	304 \pm 1.86

Data presented as mean \pm SEM (n=4). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet test. *p<0.05 significant. When compared to model control. EEAS: Ethanolic extract of *Alstonia scholaris*, SEM: Standard error of the mean

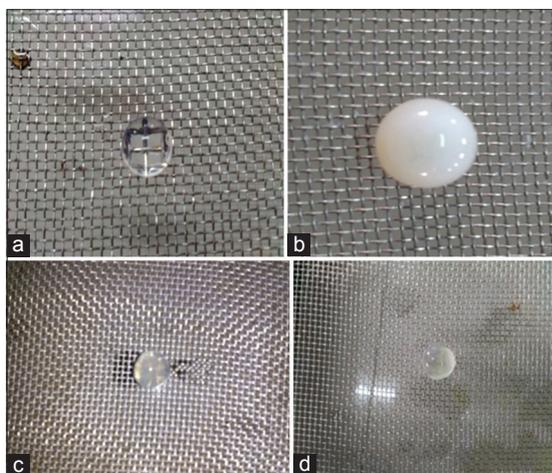
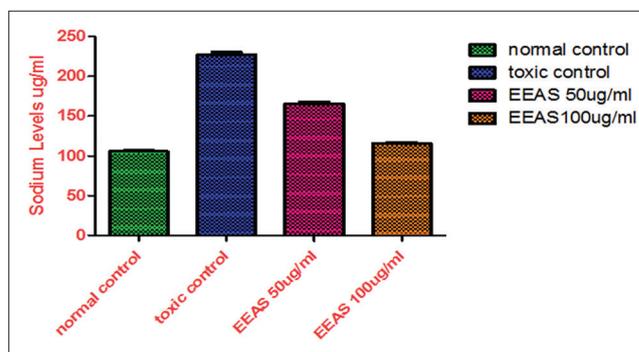


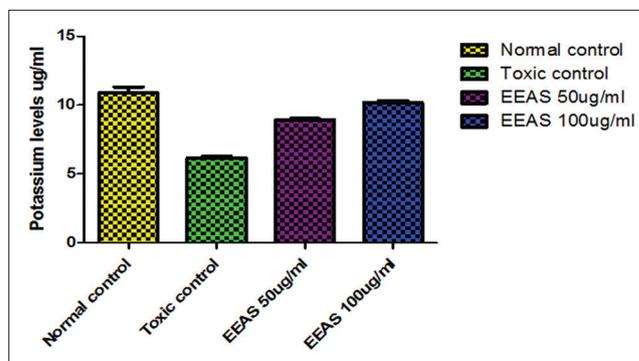
Fig. 1: (a) - Group I (Normal), (b) - Group II (Toxic control), (c) - Group III (Ethanolic extract of *Alstonia scholaris* [EEAS] 50 $\mu\text{g/ml}$), (d) Group IV (EEAS 100 $\mu\text{g/ml}$)

it will be a great advance in the treatment of this disorder. A number of drugs have been shown to interfere with the process of cataract formation such as aldose reductase inhibitors, restatin, sulindac, and aspirin. Cataract is one of the universal processes of aging and is a consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species plays an important role in the process leading to lens opacification. This oxidative crisis is one of the reasons for generation of cataract.

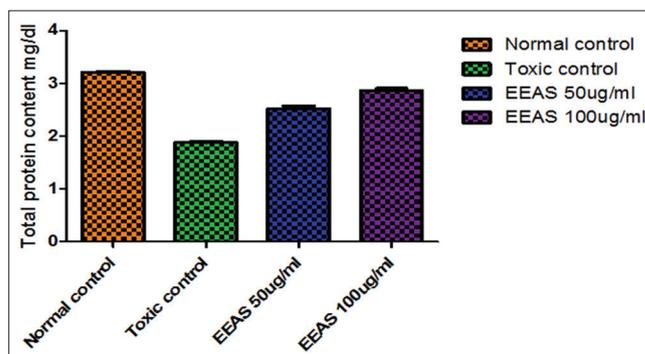
Ex vivo model for inducing cataract using dexamethasone 10 mg provides an effective model on isolated lenses of goat. Incubation of goat lenses in the media containing dexamethasone (10 mg) concentration induces cataract it has shown to cause a considerable drop in $\text{Na}^+/\text{K}^+ - \text{ATPase}$ activity, with the progression of opacity. The impairment of $\text{Na}^+/\text{K}^+ - \text{ATPase}$ causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+ , K^+ ratio change the protein content of the lens, leading to a decrease in total proteins causing lens opacification. The present study showed higher total proteins (p<0.05 at all concentration) and K^+ ions (p<0.05 at all concentration), whereas lower concentrations of Na^+ ions (p<0.05 at all concentration) with EEAS-treated groups. The imbalance of Na^+ and K^+ is prevented due to an action of EEAS which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentrations. Catalase is an important part of the innate enzymatic defense system of the lens which is responsible for the detoxification of H_2O_2 . Decrease in the activities of this enzyme in tissue has been linked with the buildup of highly reactive free radicals leading to injurious effects such as loss of integrity and the function of the cell membranes. The catalase keeps the level of free radicals below toxic levels. In this study, the level of catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with EEAS showed a significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.



Graph 1: Effect if ethanolic extract of *Alstonia scholaris* on sodium levels in dexamethasone-induced cataract



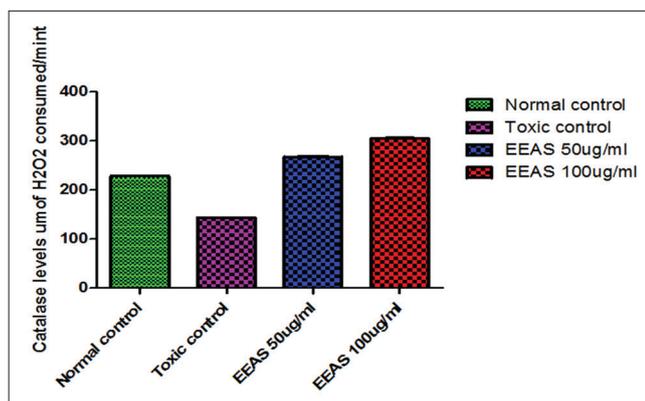
Graph 2: Effect if ethanolic extract of *Alstonia scholaris* on potassium levels in dexamethasone-induced cataract



Graph 3: Effect if ethanolic extract of *Alstonia scholaris* on total protein levels in dexamethasone-induced cataract

CONCLUSION

The present investigation suggests that EEAS leaves effectively prevent the cataractogenic condition which was indicated by increase in the total protein content, potassium level, and decrease in the sodium. However, antioxidant property of EEAS leaves was confirmed by



Graph 4: Effect of ethanolic extract of *Alstonia scholaris* on catalase levels in dexamethasone-induced cataract

increase. Catalase levels in lens. In conclusion, all the above findings lend credence to leaves of *A. scholaris* in the treatment of cataract.

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