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Anticataractic and antioxidant activities of *Abrus precatorius* Linn. against calcium-induced cataractogenesis using goat lenses

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ABSTRACT

The present study evaluated the in vitro anticataract and antioxidant activities of ethanolic seed extract of Abrus precatorius Linn. (Fabaceae) against calcium-induced cataractogenesis using goat lenses. Transparent isolated goat lenes were incubated in artificial aqueous humor and divided into seven experimental groups. The extracts at a dose of 100 µg/ml and 200 µg/ml were incubated simultaneously with calcium chloride (10 mM) for a period of 16 h. Vitamin E (100 µg/ml) was used as the standard drug. At the end of the incubation, levels of various biochemical parameters such as protein content, malondialdehyde (MDA), lipid hydroperoxides (LH), Cu^{2+}-induced lipoprotein diene formation, Ca^{2+}ATPase and enzymatic antioxidants like catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GSSH) and non-enzymatic antioxidant like reduced glutathione (GSH) were measured in the lens homogenate. Incubation with calcium produced a mature cataract and there was a significant increase in LH and MDA and a decrease in protein content, Ca^{2+}ATPase, Cu^{2+}-induced lipoprotein diene formation and enzymatic and non-enzymatic antioxidants when compared to normal control. Our results indicated that simultaneous incubation of the plant extracts prevented the preoxidative damage caused by calcium, which is evidenced from the improved antioxidant potential. The ethanolic seeds extract of Abrus precatorius protected the lens against calcium-induced oxidative damage which might be helpful in delaying the progression of cataract.

Key words: antioxidant; cataract; calcium; *Abrus precatorius.*

INTRODUCTION

Cataract is the opacification or optical dysfunction of the crystalline lens, associated with the breakdown of the eye lens micro-architecture, which interferes with transmission of light onto the retina. Several biochemical processes such as oxidative stress, altered epithelial metabolism, phase transition, calpain-induced proteolysis, calcium accumulation, and cytoskeletal loss occur during the development of cataract [1].

Cataract is a major cause of blindness worldwide and more so in developing countries. Surgery is the only effective treatment for cataract and the exact mechanism is not clear. Pharmacological interventions to inhibit or delay lens opacification is yet at experimental stage. Studies are being conducted to explore the mechanism of cataractogenesis using various models of cataract and to target crucial steps to stop this process. Limitations in acceptability, accessibility and affordability of cataract surgical services make it more relevant and important to look into alternative pharmacological measures for treatment of this disorder Thus, much eagerness is being laid on identification of natural compounds that will help to prevent cataractogenesis [2].

Increased levels of lenticular calcium activate calcium dependent proteases. The activated proteases hydrolyze cytoskeletal proteins and lens crystalline. Crystalline cleavage would result from lower molecular weight peptides that could, in turn, aggregate to form higher molecular weight proteins [3].

Oxidative stress may also be implicated in the cataract induced by calcium and age related process due to the formation of superoxide ($O_2^{\cdot-}$) radicals and H_2O_2 because of these free radicals are readily react with the biomolecules [4, 5]. The toxic effects of the reactive oxygen species are neutralized in the lens by antioxidants such as ascorbic acid, vitamin E, the glutathione system (GSH peroxidase, GSH reductase), superoxide dismutase and catalase. The enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic (ascorbate, glutathione, cysteine) antioxidant system activities are decreased in the lens and aqueous humor during aging and in the development of cataract [6].

Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants [7, 8]. Researchers have examined the effect of plants used traditionally by indigenous people to treat diseases of the eye [9]. *Abrus precatorius* Linn. belonging to the family Fabaceae is a tropical climber widely distributed in southern region of India. The seeds are reported to possess alkaloids, tannins, and flavonoids. The seeds are used in Indian traditional medicine for the treatment of ophthalmic and other eye infections, it also possess anti-oxidant, anti-hepatitis, anti-microbial, diuretic, aphrodisiac, purgative and anti-fertility activities [10,11]. Literature suggest that the seeds of *Abrus precatorius* Linn. possess antidiabetic [12], antiallergic [13] activities and also to treat renal damage [14].

The main objective of the study was to evaluate the ethanolic extract of seeds of *Abrus precatorius* Linn. for its *in vitro* anticataract and antioxidant activities against calcium-induced cataractogenesis using goat lenses.

MATERIALS AND METHODS

Drugs and chemicals

Calcium chloride and vitamin E were obtained from SD fine chemicals, Mumbai. 1-amino 2-naphthol-4-sulfonic acid, 5, 5-dithiobis-(-2-nitrobenzoic acid) (DTNB), Nitro blue tetrazolium chloride (NBT), Nicotinamide adenine dinucleotide reduced salt (NADH), Nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), Oxidised glutathione, Reduced glutathione, Adenosine-5'-triphophate (ATP) were obtained from Himedia Laboratories Ltd., Mumbai. Fresh goat lenses were obtained from the slaughterhouse, Coimbatore. All other chemicals used in the study were obtained commercially and were of analytical grade.

Plant collection and authentication

The seeds of *Abrus precatorius* Linn. were collected from Pachai hills, Salem, Tamilnadu, India during the month of June 2010. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Director, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SRC/5/23/10-11/Tech-708.

Plant extract

Abrus precatorius ethanolic seed extract

The fresh seeds were shade dried, broken into small pieces and powered coarsely. About 600 gm of air dried powdered material was extracted with 2.5 l of 99.9% of ethanol in a Soxhlet apparatus for 7 days. The extract was dried at controlled temperature (40-50ºC) [15]. The *Abrus precatorius* Linn. seed extract was abbreviated as ASE.

Calcium-induced cataract
Fresh goat eyeballs were obtained from a local slaughterhouse within two hours after killing of the animals and the lenses were isolated. Fresh goat lenses were incubated for 16 hours at 37ºC in 10 mM $CaCl_2$ solution made in Tris-HCL buffer (0.01 mM) pH-7.4 [16].

A total of 30 goat lenses were used and divided into five experimental groups consisting of 6 in each group.

Group I : Tris-HCL (solvent control)
Group II : $CaCl_2$ 10 mM alone (negative control)
Group III: ASE (100 µg/ml) + $CaCl_2$ 10 mM
Group IV: ASE (200 µg/ml) + $CaCl_2$ 10 mM
Group V : Vitamin E (100 µg/ml) + $CaCl_2$ 10 mM

Examination of lens opacity
To study the opacity of the lens, the lenses from the control and experimental groups were placed on a wire mesh and photographed [17].

Preparation of lens homogenate
After incubation, lenses were homogenized in 10 volumes of 0.1M potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 10,000 rpm for 1 h and the supernatant was used for estimation of biochemical parameters [18].

Biochemical parameters
Estimation of total protein content
To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue [19].

Estimation of lipid hydroperoxides (LH)
About 0.1ml of lens homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 ammonium ion sulphate were added to 90 ml of methanol and 10ml 250 mM sulphuric acid) and incubated for 3 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/ mg lens protein [20].

Estimation of malondialdehyde (MDA)
Lenses were homogenized in10% (w/v) 0.1 M Tris–HCl buffer(pH 7.5). One milliliter of the homogenate was combined with 2 ml of TCA–TBA–HCl reagent 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl and boiled for 15 min. Precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as nmoles of MDA/ min/ mg lens protein [21].

Inhibition of Cu^{2+} induced lipoprotein diene formation
Lens homogenate was diluted to 0.67% in phosphate buffered saline. Control experiments consisted of identical assay conditions but without the sample. Oxidation was initiated immediately after addition of sample by the addition of 12 µM final concentration of Cu^{2+} added as CuSO4_5H2O dissolved in deionized distilled water. Oxidation was determined by measuring the absorbance at 234 nm using a UV–Visible Spectrophotometer. Absorbance was taken after 120 mins at 37ºC.The lipoprotein diene formation was measured from the absorbance at a time. The absorbance provides an indication of protection of tissue lipoprotein against oxidation [21].

Assay of Ca^{2+}-ATPase activity
To added 0.1 ml of the lens homogenate (10% (w/ v) in 0.25 M sucrose) and add 0.2 ml of the substrate, ATP. The tubes were incubated for 30 min in a water bath at 37ºC. The enzyme activity is stopped by adding 2 ml of 10% TCA. Then 0.2 ml of ATP is added and the same kept in ice for 20 min. All the tubes were then centrifuged at 2500 rpm for 10 min and the supernatant collected. The protein free supernatant was analyzed for inorganic phosphate.

For that 3 ml of the supernatant was treated with 1 ml of ammonium molybdate and 0.4 ml 1-amino-2-naphthol-4-sulfonic acid (ANSA). The colour developed was read at 680 nm after 20 min and the inorganic phosphate value expressed as nm three parallel experiments were conducted [22].

Determination of enzymatic antioxidants

Assay of superoxide dismutase (SOD)

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 µM Phenazonium methosulphate (PMS), 0.3 ml of 300 µM NBT, 0.2 ml of 780 µM NADH, 1.0 ml homogenate (lens were homogenized in10% (w/v) 0.25 M sucrose buffer) and distilled water to a final volume of 3.0 ml. Reaction was started by the addition of NADH and incubated at 30ºC for 1 min. The reaction was stopped by the addition of 1.0 ml glacial acetic acid and the mixture stirred vigorously. 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer taken out and the absorbance was measured at 560 nm against a butanol blank. A system devoid of enzyme served as the control and three parallel experiments were conducted [23].

Assay of catalase (CAT)

The reaction mixture contained 2.0 ml of homogenate (lens were homogenized in 10% (w/v) 50 mM phosphate buffer, pH 7.0) and 1.0 ml of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0). A system devoid of the substrate (hydrogen peroxide) served as the control. Reaction was started by the addition of the substrate and decrease in absorbance monitored at 240 nm for 30 s at 25ºC. The difference in absorbance per unit time was expressed as the activity and three parallel experiments were conducted. One unit is defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25ºC [24].

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidised glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of lens homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min against a blank. Glutathione activity was expressed as nmoles NADPH oxidized/min/ mg lens protein at 30ºC [25].

Estimation of glutathione peroxidise (GPx)

The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2ml of lens homogenate supernatant incubated at 37ºC for 10 min. The reaction was arrested by the addition of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as nmoles/min/mg lens protein [26].

Determination of non enzymatic antioxidant:

Estimation of glutathione (GSH)

Lenses were homogenized in10% (w/v) cold 20 mM EDTA solution on ice. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150 µM DTNB [5, 5-dithiobis-(-2-nitrobenzoic acid)]. The product was detected and quantified spectrophotometrically at 416 nm. Pure GSH was used as standard for establishing the calibration curve and three parallel experiments were conducted [27].

Statistical analysis

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

RESULTS AND DISCUSSION

Phytochemical screening

The ethanolic seed extract of *Abrus precatorius* Linn. was subjected to the phytochemical screening and for the presence of various phytochemical constituents.

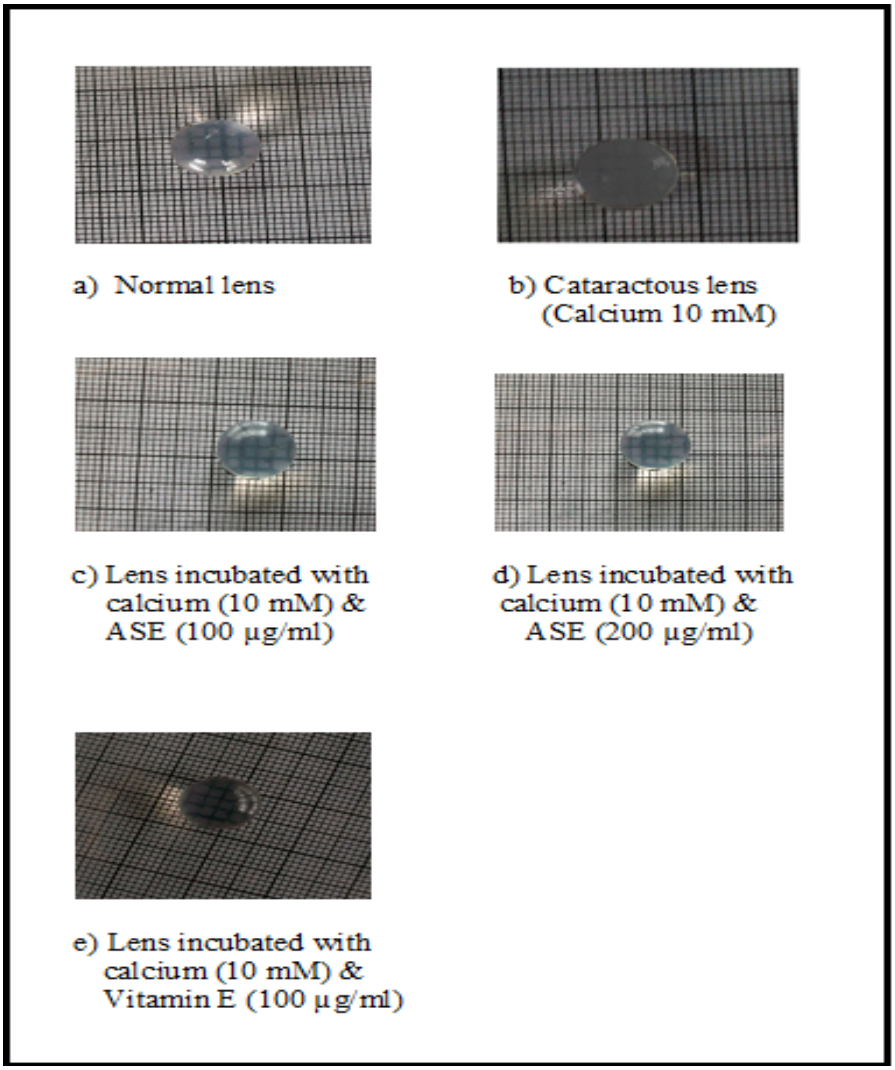
Table 1. Phytochemical screening

Phytochemicals	*Abrus precatorius* L.
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins and phenolics	+
Terpenoids	+

Photographs of lenses in normal and experimental groups incubated with calcium chloride are shown in Fig 1(a-e).

Fig 1a shows the normal lens **Fig 1b** is the lens incubated with calcium chloride (10 mM) for a period of 16 hrs showing complete opacification of the lens fibres compared to normal control. **Fig 1c** are the lenses incubated simultaneously with calcium chloride (10 mM) and ASE at a concentration of 100 µg/ml showing a decrease in opacity compared to cataractous lenses. **Fig 1d** are the lenses incubated simultaneously with calcium chloride (10 mM) and ALE at a concentration of 200 µg/ml showing a decrease in opacity compared to cataractous lenses. **Fig 1e** is the lens incubated with calcium chloride (10 mM) and vitamin E (100 µg/ml) showing almost normal transparency when compared to catatactous lenses.

Fig 1. Photographs of lenses in normal and experimental groups incubated with Calcium



Effect of the ethanolic seed extract of *Abrus precatorius* Linn. on lens protein and lipid peroxidation in control and experimental groups

There was a significant (P<0.01) decrease in the level of total protein, Ca^{2+}ATPase, Cu^{2+}induced lipoprotein diene formation and an increase in the level of malondialdehyde and lipid hydroperoxides in calcium-induced cataractous lenses when compared to normal control. Incubation with the ethanolic seed extract of *Abrus precatorius* at doses of 100 & 200 µg/ml and Vitamin E (100 µg/ml) simultaneously with calcium chloride for 16 h caused a significant (P<0.01) increase in the total protein, Ca^{2+}ATPase, Cu^{2+}induced lipoprotein diene formation and a decrease in the level of malondialdehyde and lipid hydroperoxides (Table 2).

Table 2. Effect of the ethanolic seed extract of *Abrus precatorius* Linn. on lens protein, MDA, LH, Ca^{2+}ATPase and Cu^{2+} induced lipoprotein diene in control and experimental groups

GROUP	Protein (mmoles/min/ mg lens tissue)	MDA (µmoles/min/mg protein)	LH (nmoles/min/mg protein)	Ca^{2+}ATPase (µmoles/min/mg inorganic phosphate)	Cu^{2+}induced Lipoprotein diene absorbance
Normal control	116.29±4.96	0.117±0.003	2.75±0.22	4.65±0.18	0.8522±0.07
Calcium control	64.23±4.28 [a]	0.66±0.05 [a]	8.675±1.17 [a]	1.19±0.11 [a]	0.1983±0.03 [a]
ASE (100 µg/ml)	89.32±5.56 [b]	0.324±0.03 [b]	4.43±0.49 [b]	3.75±0.36 [b]	0.5644±0.07 [b]
ASE (200 µg/ml)	94.03±4.77 [b]	0.28±0.01 [b]	3.78±0.47 [b]	3.95±0.29 [b]	0.6275±0.07 [b]
Vitamin-E (100 µg/ml)	108.52±4.77 [b]	0.19 [b]	3.08±0.2 [b]	4.14±0.18 [b]	0.7738±0.07 [b]

Values are mean ± SEM; n=6 in each; [a]P <0.01 when compared to normal control;
[b]P<0.01 when compared to calcium control (one way ANOVA followed by Dunnett's test).

Effect of the ethanolic seed extract of *Abrus precatorius* Linn. *on* lens enzymatic and non enzymatic antioxidants in control and experimental groups

Incubation with calcium chloride 10 mM for 16 h produced a significant (P<0.01) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens homogenate when compared to normal control. Incubation with the ethanolic seed extract of *Abrus precatorius* and Vitamin E simultaneously with glucose significantly (P<0.01) restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group (Table 3).

Table 3. Effect of the ethanolic seed extract of *Abrus precatorius* Linn. on lens enzymatic and non enzymatic antioxidants in control and experimental groups

GROUP	Catalase (mmoles/min/ mg protein)	GPx (µmoles/min/ mg protein)	SOD (µmoles/min/ mg protein)	GSSH (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
Normal control	1.61±0.05	3.18±0.11	5.37±0.3	2.42±0.27	3.81±0.25
Calcium control	0.302±0.02 [a]	0.64±0.08 [a]	1.87±0.24 [a]	0.42±0.04 [a]	1.07±0.07 [a]
ASE (100 µg/ml)	1.05±0.12 [b]	2.59±0.26 [b]	3.62±0.46 [b]	1.95±0.38 [b]	2.93±0.34 [b]
ASE (200 µg/ml)	1.12±0.12 [b]	2.99±0.31 [b]	4.14±0.45 [b]	2.02±0.19 [b]	3.08±0.28 [b]
Vitamin-E (100 µg/ml)	1.45±0.15 [b]	3.07±0.19 [b]	5.07±0.26 [b]	2.18±0.29 [b]	3.45±0.21 [b]

Values are mean ± SEM; n=6 in each; [a]P <0.01 when compared to normal control;
[b]P<0.01 when compared to calcium control (one way ANOVA followed by Dunnett's test).
The ethanolic seeds extract of Abrus precatorius protected the lens against calcium-induced oxidative damage which might be helpful in delaying the progression of cataract.

CONCLUSION

To conclude, the study suggested that the extract of *Abrus precatorius* L. seeds possess anticataract and antioxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further *in vivo* studies and

investigations on the isolation and identification of active components in the seeds may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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