Anticataractogenesis activity of *Polyalthia longifolia* leaves extracts against glucose-induced cataractogenesis using goat lenses *in vitro*

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**ABSTRACT**

Cataracts are the leading cause of blindness worldwide but safe and effective pharmacological intervention is lacking to prevent cataractogenesis. Glucose-induced cataractogenesis in vitro model serves as a good model. The objective of the present study was to evaluate the anticataractogenesis and antioxidant effect of the ethanol and chloroform extracts of *Polyalthia longifolia* (Sonner.,) Thw. (Annonaceae) leaves against glucose-induced cataractogenesis using goat lenses. Goat lenses were incubated in artificial aqueous humor containing 55 mM glucose alone, simultaneously with the ethanol and chloroform extracts of *Polyalthia longifolia* leaves and standard drug, vitamin E. Incubation with high concentration glucose-induced lens opacification started in 8-10 h and the lens was completely cataractous by the end of 72 h. Simultaneous treatment with plant extracts or vitamin E reversed this opacification. The lens homogenate of cataractous lenses were found to have significantly (P < 0.01) increased levels of total protein, lipid hydroxides and malondialdehyde, while there was a decrease in Ca$^{2+}$-ATPase and Cu$^{2+}$-induced lipoprotein dienes. There was increase in the levels of both the enzymatic (catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase) and non enzymatic (glutathione) antioxidants. Both the extracts and vitamin E reversed these changes. Based on the results obtained from the present study, it can be understood that the leaves of *Polyalthia longifolia* possess protection against glucose-induced cataractogenesis. Potential leads for clinical use in the prevention of cataract can be identified by isolating and identifying the active phytoconstituents.

**Key words:** Lens opacification, cataractogenesis, antioxidants.

**INTRODUCTION**

The eye lens is a transparent structure which helps to refract light to be focused on the retina and provide vision. Cataract is defined as the partial or total opacity of the crystalline lens of the eye, causing impairment of vision or blindness. The natural lens is a crystalline structure composed of water and proteins that are arranged in a precise structure to create a clear passage for light to pass through it, but with aging, the lens becomes opaque, thus reducing the amount of light reaching to the retina [1, 2, 3]. Cataracts are the leading cause of blindness worldwide. Nearly 20 million people have severely reduced vision due to cataract in the world and account for 47.8% of total blindness in
the world [3]. The current 20 million people with severely reduced vision as a result of cataract will increase to 40 million by the year 2020. The age-adjusted prevalence of cataract in India was three times that of the US, with 82% of Indians of 75 to 83 years old having visually significant cataract, compared to 46% of those aged 75 to 85 years in the US [4].

Diabetes and aging are the major risk factors that accelerate cataract development. In particular, hyperglycaemia or sustained increase of blood glucose contributes to cataract formation in three ways. They are nonenzymatic glycation of eye lens proteins, oxidative stress and activated polyol pathway in glucose disposition. These effects contribute for both age-related and diabetic cataracts [5, 6, 7]. Oxidative stress may also be implicated in the cataract induced by glucose and age-related process. 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 [8].

Although cataracts can be surgically removed, in many countries surgical services are inadequate or do not produce equal outcomes, and cataract remains the leading cause of blindness. Preventive interventions must be identified, perfected and delivered, through research. The challenges are to prevent or delay cataract formation, and cure that which does occur [4]. There are many herbal preparations, vitamin supplementations and drugs used to prevent cataractogenesis and reverse cataract. In 1980s aspirin was thought to prevent cataractogenesis. But further publications have not supported aspirin or vitamin supplementation to prevent cataractogenesis [9, 10]. Aldose reductase inhibitors as a prophylaxis against cataractogenesis are currently being studied in humans and yet to be proven beneficial [11]. Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to treat diseases of the eye [12].

As described above implications resulting from mechanisms involving high glucose levels form the basis for research to identify possible pharmacological interventions to prevent diabetic cataract. Glucose-induced cataractogenesis in vitro model uses high concentrations of glucose to induce cataractogenesis and serves as a good model for studying drugs for anticataractogenesis activity [13, 14].

*Polyalthia longifolia* (Sonner.) Thw. (Annonaceae) is a lofty evergreen tree, native to India, commonly planted due to its effectiveness in alleviating noise pollution. The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis [15]. The plant was reported to possess antibacterial, cytotoxic, antiulcer and antifungal activities [16, 17, 18, 19].

The objective of the present study is to evaluate the anticataractogenesis and antioxidant effect of the ethanol and chloroform extracts of *Polyalthia longifolia* leaves against glucose-induced cataractogenesis using goat lenses.

**MATERIALS AND METHODS**

**Plant collection and authentication**
The plant material consists of dried powdered leaves of *Polyalthia longifolia* belonging to the family Annonaceae. The leaves were collected in Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India during the month of July 2010 and was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore (Ref No BSI/SRC/5/23/10-11/Tech.709).

**Preparation of the extracts**
The air-dried powdered leaves of *P. longifolia* (100 g) were extracted with ethanol (500 ml) by maceration process for 72 h [19]. The resultant extract was concentrated under reduced pressure to obtain a residue of *P. longifolia* leaf ethanol extract (PLEE). The same procedure was followed replacing ethanol with chloroform to prepare *P. longifolia* leaf chloroform extract (PLCE).

**Drugs and chemicals**
Glucose and vitamin E were obtained from SD Fine Chemicals, Mumbai. Nitro blue tetrazolium chloride (NBT), 1-amino-2-naphthol-4-sulfonic acid, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), nicotinamide adenine dinucleotide
reduced salt (NADH), nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), oxidised glutathione, reduced glutathione and adenosine-5’-triphosphate (ATP) were obtained from HiMedia Laboratories Ltd., Mumbai. Fresh goat lenses were purchased locally from the Corporation slaughter house in Coimbatore. All other chemicals used in the study were obtained commercially and were of analytical grade.

Glucose-induced cataract
Fresh goat eyeballs were obtained from a local slaughterhouse within two hours after killing of the animals and the lenses were isolated. They are preserved and carried to the laboratory at 0-4°C. The isolated lens were incubated in artificial aqueous humor at 37°C and pH 7.8 for 72 h. Glucose at a concentration of 55 mM was used to induce cataract [20, 14]. A total of 42 goat lenses were used and divided into seven experimental groups consisting of 6 in each group.

Group I : Artificial aqueous humor alone (Normal control)
Group II : Glucose 55 mM alone (Negative control)
Group III-A: Glucose 55 mM + PLEE (100 µg/ml)
Group III-B: Glucose 55 mM + PLEE (200 µg/ml)
Group IV-A: Glucose 55 mM + PLCE (100 µg/ml)
Group IV-B: Glucose 55 mM + PLCE (200 µg/ml)
Group V : Glucose 55 mM + Vitamin E (100 µg/ml, Positive control)

At the end of the experiment, the lenses were removed from the medium and rolled on filter paper to remove medium, adhering non lens tissue, and vitreous humor.

Examination of lens opacity
Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity [21, 14].

Preparation of lens homogenate
After incubation, lenses were homogenized with 10 volumes of 0.1M potassium phosphate buffer, pH 7.0 [22]. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of biochemical parameters.

Biochemical parameters
Estimation of total protein content
To 0.1 ml of lens homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min [23]. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min 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.

Estimation of malondialdehyde (MDA)
Lenses were homogenized in10% (w/v) 0.1 M Tris-HCl buffer (pH 7.5) (24). One ml 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 levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles of MDA/ min/ mg lens protein.

Estimation of lipid hydroperoxides (LH)
About 0.1ml of lens homogenate was treated with 0.9 ml of Fox reagent (49 mg of ferrous ammonium sulfate in 50 mL of 250 mM H₂SO₄, 0.397 g of butylated hydroxytoluen, and 38 mg of xylene orange in 950 mL of methanol) and incubated for 3 min [25]. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/ mg lens protein.
Inhibition of Cu^{2+} induced lipoprotein diene formation

Lens homogenate was diluted to 0.67% in phosphate buffered saline [24]. 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 CuSO_{4}.5H_{2}O 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 min at 37°C. The lipoprotein diene formation was measured from the absorbance at a time. The absorbance provides an indication of protection of tissue lipoprotein against oxidation.

Assay of Ca^{2+}-ATPase activity

To 0.1 ml of the lens homogenate (10% w/v in 0.25 M sucrose) add 0.2 ml of the substrate, ATP [26]. The tubes were incubated for 30 min in a water bath at 37°C. The enzyme activity is stopped by adding 2 ml of 10% TCA and the same kept in ice for 20 min. All the tubes were then centrifuged at 2500g 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 and three parallel experiments were conducted.

Determination of enzymatic antioxidants

Assay of catalase

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) [27]. 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 seconds at 25°C. 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 25°C.

Estimation of glutathione peroxidase (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 0.2 ml of lens homogenate supernatant incubated at 37°C for 10 min [28]. 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.

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 in 10% w/v 0.25 M sucrose buffer) and distilled water to a final volume of 3.0 ml [29]. Reaction was started by the addition of NADH and incubated at 30°C 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.

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm [30]. 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. 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 reductase activity was expressed as nmoles NADPH oxidized/min/ mg lens protein at 30°C.

Determination of non enzymatic antioxidant:

Estimation of reduced glutathione (GSH)

Lenses were homogenized in10% (w/v) cold 20 mM EDTA solution on ice [31]. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150 µM DTNB. 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.

Statistical analysis
Results are expressed as mean ± SEM of six lenses in each group. The groups were compared using one-way analysis of variance (ANOVA) with post-hoc Dunnett’s test using glucose 55 mM group as control. P values < 0.05 were considered significant.

RESULTS

Percentage yield of the extracts
The percentage yield of the ethanol extract of \textit{P. longifolia} leaves (PLEE) was found to be 14.82% w/w and that of the chloroform extract (PLCE) was found to be 18.76% w/w.

Cataractogenesis
Incubation of goat lenses with 55 mM of glucose showed opacification starting at the periphery, at the end of 8 h, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification resulting at the end of 72 h.

Examination of lens opacity
After 72 h of incubation in glucose 55 mM, lens becomes completely opaque (Fig. 1b) as against lenses in normal control (Fig. 1a). Incubation of lenses with PLEE (Figs. 1c and 1d) and PLCE (Figs. 1e and 1f), at both the concentrations used, seem to retard the progression of lens opacification, compared with lenses incubated in glucose 55 mM (negative control, Fig. 1b). The effect of vitamin E, the positive control groups is showing considerable retardation in the progression of lens opacification (Fig. 1g) which is near normalcy when compared to negative control (Fig. 1b).

<p>| Table 1: Effect of the ethanol and chloroform extracts of the leaves of \textit{Polyalthia longifolia} on lens protein, MDA, LH, Ca$^{2+}$-ATPase and Cu$^{2+}$-induced lipoprotein diene in control and experimental groups |</p>
<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Protein (mmoles/min/mg lens tissue)</th>
<th>MDA (µmoles/min/mg protein)</th>
<th>LH (nmoles/min/mg protein)</th>
<th>Ca$^{2+}$-ATPase (µmoles/min/mg inorganic phosphate)</th>
<th>Cu$^{2+}$-induced Lipoprotein diene absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>97.83±2.90</td>
<td>0.198±0.016</td>
<td>6.29±0.32</td>
<td>4.64±0.18</td>
<td>0.6862±0.05</td>
</tr>
<tr>
<td>Glucose control (55 mM)</td>
<td>54.34±2.70$^a$</td>
<td>0.690±0.061$^a$</td>
<td>13.87±1.31$^a$</td>
<td>1.19±0.10$^a$</td>
<td>0.1565±0.02$^a$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLEE (100 µg/ml)</td>
<td>71.97±1.26$^a$</td>
<td>0.316±0.022$^b$</td>
<td>9.06±1.63$^b$</td>
<td>3.63±0.21$^b$</td>
<td>0.4890±0.04$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLCE (200 µg/ml)</td>
<td>77.00±3.75$^a$</td>
<td>0.303±0.20$^a$</td>
<td>8.21±1.58$^c$</td>
<td>3.96±0.26$^b$</td>
<td>0.5870±0.02$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLCE (100 µg/ml)</td>
<td>70.37±1.83$^b$</td>
<td>0.433±0.017$^a$</td>
<td>9.28±1.58$^b$</td>
<td>2.04±0.18$^c$</td>
<td>0.3791±0.02$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLCE (200 µg/ml)</td>
<td>73.32±2.53$^b$</td>
<td>0.412±0.023$^b$</td>
<td>8.79±0.73$^a$</td>
<td>2.40±0.21$^b$</td>
<td>0.5218±0.03$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + Vit-E (100 µg/ml)</td>
<td>84.41±3.17$^a$</td>
<td>0.243±0.018$^b$</td>
<td>7.82±1.03$^a$</td>
<td>4.13±0.18$^b$</td>
<td>0.6244±0.05$^b$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=6 in each group; $^a$P<0.01 when compared to normal control; $^b$P<0.01, $^c$P<0.05 when compared using one-way ANOVA with post-hoc Dunnett’s test using glucose 55 mM group as control.

Biochemical parameters
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 lenses incubated with 55 mM glucose when compared to normal control. Lenses incubated with 55 mM glucose and simultaneously with PLEE, PLCE or standard drug, vitamin E caused a reversal of the above effects. PLEE and PLCE each used at two different concentrations (100 & 200 µg/ml) showed a dose dependent increase in the effect. PLEE (P<0.01), PLCE (P<0.05) and Vitamin E (100 µg/ml, P<0.05) showed 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 1).
Enzymatic and non enzymatic antioxidants
Incubation with 55 mM glucose for 72 h produced a significant (P<0.01) decrease in the levels of 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 PLEE and PLCE at doses of 100 & 200 µg/ml and Vitamin E (100 µg/ml) simultaneously with 55 mM 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 2).
Table 2: Effect of the ethanol and chloroform extracts of the leaves of Polyalthia longifolia on lens enzymatic and non enzymatic antioxidants in control and experimental groups

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Catalase (µmole/min/mg protein)</th>
<th>GPx (µmole/min/mg protein)</th>
<th>SOD (µmole/min/mg protein)</th>
<th>GSSH (µmole/min/mg protein)</th>
<th>GSH (µmole/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (55 mM)</td>
<td>2.68±0.08</td>
<td>4.05±0.19</td>
<td>4.95±0.15</td>
<td>3.02±0.38</td>
<td>3.76±0.38</td>
</tr>
<tr>
<td>Glucose control (55 mM)</td>
<td>0.76±0.06$^a$</td>
<td>1.78±0.05$^a$</td>
<td>2.24±0.25$^a$</td>
<td>1.21±0.06$^a$</td>
<td>1.38±0.15$^a$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLEE (100 µg/ml)</td>
<td>1.85±0.10$^b$</td>
<td>2.83±0.09$^b$</td>
<td>3.75±0.24$^b$</td>
<td>2.20±0.06$^b$</td>
<td>2.71±0.19$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLEE (200 µg/ml)</td>
<td>2.47±0.16$^b$</td>
<td>3.87±0.15$^b$</td>
<td>4.07±0.16$^b$</td>
<td>3.08±0.21$^b$</td>
<td>3.00±0.16$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLCE (100 µg/ml)</td>
<td>1.22±0.06$^c$</td>
<td>2.50±0.16$^c$</td>
<td>3.01±0.14$^c$</td>
<td>1.71±0.01$^c$</td>
<td>2.19±0.06$^c$</td>
</tr>
<tr>
<td>Glucose (55mM) + PLCE (200 µg/ml)</td>
<td>1.54±0.08$^b$</td>
<td>3.25±0.25$^b$</td>
<td>3.14±0.18$^c$</td>
<td>2.09±0.14$^c$</td>
<td>2.23±0.17$^b$</td>
</tr>
<tr>
<td>Glucose (55mM) + Vit-E (100 µg/ml)</td>
<td>2.30±0.15$^b$</td>
<td>3.88±0.24$^b$</td>
<td>4.85±0.21$^b$</td>
<td>2.92±0.15$^b$</td>
<td>3.39±0.12$^c$</td>
</tr>
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</table>

One-way ANOVA:

<table>
<thead>
<tr>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.992</td>
<td>6, 35</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>26.199</td>
<td>6, 35</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>26.795</td>
<td>6, 35</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>33.695</td>
<td>6, 35</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>15.718</td>
<td>6, 35</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=6 in each group; $^a$P < 0.01 when compared to normal control; $^b$P < 0.01, $^c$P < 0.05 when compared using one-way ANOVA with post-hoc Dunnett’s test using glucose 55 mM group as control.

DISCUSSION

For cataract, presently there is no definitive pharmacological therapy available and the only solution for the patient with advanced cataract is surgery [8]. The limitations of cataract surgery have stimulated experimental cataract research mainly focusing on the prevention of cataract formation [32]. Research using plants and plant products are on the rise owing to their natural origin and comparative safety [12].

Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. Non enzymatic glycation, oxidative stress and polyol pathway are the possible mechanisms by which high glucose concentrations induce and accelerate lens opacification leading to cataract formation. High concentrations of glucose contribute to oxidative stress by generating more reactive oxygen species at the mitochondrial level owing to increased intracellular glucose metabolism. Increased reactive oxygen species initiates polyol pathway by stimulating aldose reductase resulting in high levels of sorbitol. Sorbitol does not easily cross cell membranes and accumulates in cells causing damage by disturbing osmotic homeostasis. This intralenticular accumulation of polyols is a major factor in acute models of sugar cataract [8].

In the present study investigation of lens opacity was carried out to differentiate the control and experimental lenses. Incubation with glucose in high concentration results in various biochemical changes leading to formation of cataract. However the groups incubated with PLEE and PLCE have reversed the lens opacity which is almost similar to the standard drug, vitamin E.

High glucose concentration causes accumulation of Na$^+$ and loss of K$^+$ altering the Na$^+$-K$^+$ ratio which in turn alters the protein content of the lens, leading to a decrease in the content of water soluble proteins [33]. Extracts of P. longifolia and vitamin E restored the levels of proteins, which may be due to direct action on the Na$^+$-K$^+$ ratio or indirectly by their free radical scavenging activity.

Lipid peroxidation is an autocatalytic process, which is a common cause of cell death. The by-products of lipid peroxidation are toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability [34]. In this study, glucose-induced cataractous goat lenses showed an increase in MDA and LH levels. Incubation of extract simultaneously with glucose reversed these effects. This effect was almost similar to the vitamin E treated group.

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The Ca\(^{2+}\)-ATPase is a transport protein in the cells that serves to maintain the Ca\(^{2+}\) levels in the cell. This protein is sensitive to oxidative damage [35]. In glucose induced cataract there was a significant (P<0.01) decrease in the levels of Ca\(^{2+}\) ATPase. The extracts and vitamin E increased the levels considerably.

The Cu\(^{2+}\)-induced lipoprotein diene formation was used to determine the antioxidant potential [36]. The results provide an indication of protection of lipoprotein by the \textit{P. longifolia} extracts against oxidation. The effect was similar to the standard drug vitamin E.

Several varieties of toxic species of oxygen are formed in the lens including the superoxide anion, hydrogen peroxide, hydroxyl radical and lipid hydroperoxides. This increase has been proposed as a central mechanism of oxidative injury [37]. Thus the determination of the lens \textit{in vitro} antioxidant enzymes like SOD, CAT, GPx, GSSH and non enzymatic antioxidant enzyme, GSH were carried out.

Catalase catalyses the decomposition of H\(_2\)O\(_2\) to water and oxygen and thus protects the cell from oxidative damage by H\(_2\)O\(_2\) and hydroxyl radical. The first enzyme involved in the antioxidant defence is superoxide dismutase. The oxygen radicals are strongly inhibited by superoxide dismutase. Glutathione peroxidase has a major role in degrading the levels of H\(_2\)O\(_2\) in cells and plays a vital role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration The enzyme GSSH catalyses the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), thereby maintaining a constant level of GSH in normal lens [38]. The nonenzymatic antioxidant GSH protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects [39]. The incubation with 55 mM of glucose severely reduced the levels of both enzymatic and non enzymatic antioxidants in the goat eye lens. Simultaneous incubation with the extracts of \textit{P. longifolia} and vitamin E considerably increased the levels of all the antioxidants studied.

CONCLUSION

Based on the results obtained from the present study, it can be concluded that the leaves of \textit{Polyalthia longifolia} possess protection against glucose-induced cataractogenesis. This anticataractogenesis and antioxidant effect might be helpful in preventing or slowing the progression of cataract. Further \textit{in vivo} studies and investigations on the isolation and identification of active components may lead to complete understanding of the mechanism of protection against and prevention of cataract formation. Also, leads with potential for clinical use in the prevention of cataract may emanate.

REFERENCES