

Research article

A study on protective effect of *Piper longum* and *Hybanthus enneaspermus* on glucose induced diabetic cataract in cultured goat lensAshok Katta^{1,2}, Murugan M²¹Department of Biochemistry, SMBT IMS & RC, Maharashtra University of Health Sciences, Dhamangaon, Nashik-422403, Maharashtra, India²Department of Biochemistry, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, 607403, India

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ABSTRACT

Introduction and Aim: The maintenance of protein redox status, thought to be largely responsible for cell survival and function, may be the outcome of oxidative events, antioxidant protection and degradation of damaged proteins. Diabetes has been one of the substantial predisposing factors of cataract. Cataractogenesis in diabetes is mainly due to generation of free radicals causing oxidation of long-lived proteins. phytonutrient isolated from medicinal herbs are now proven to have great antioxidant & other therapeutic properties. The present study was done to understand the pathophysiology of diabetic cataract to formulate and develop novel and effective anticataract agents and to explore role of *Piper longum* and *Hybanthus enneaspermus* in the prevention of oxidative damage of lens proteins in glucose induced cataract.

Material and Methods: A total of 120 goat lenses were distributed into four groups of 30 each viz. Normal control, experimental diabetic cataract, experimental diabetic cataract + extract of *Piper longum* (0.25%), experimental diabetic cataract extract of *H. enneaspermus* (0.25%). Various study parameters measured in lens homogenates comprised malondialdehyde (MDA), total soluble lens proteins, and protein carbonyl level as a measure of lens protein oxidation.

Results: Both extract of *Piper longum* and *H. enneaspermus* showed significantly declined concentrations of protein carbonyl level, preserved total soluble proteins and decreased malondialdehyde (MDA) ($p < 0.001$). *P. longum* aqueous seed extract performed better over *H. enneaspermus*.

Conclusion: It is concluded that aqueous extract of *Piper longum* and *H. enneaspermus* contains potent antioxidant compounds which shown protective role against cataractogenesis in the present.

Keywords: Cataract; diabetes mellitus; protein oxidation; *Piper longum*; *Hybanthus Enneaspermus*; medicinal plants.

INTRODUCTION

In the recent few years, due to increase consciousness about the effectiveness and side effect of synthetic drugs among the population, there is growing curiosity in the natural product remedies. About 75–80% of the world population nevertheless relied on traditional herbal medicine to meet their healthcare needs may be due to more affordable and accessible than conventional medicines, and many people prefer using them because they align with their personal health ideologies. Herbal medicines always played important role when it comes to meeting the global health care needs and are continuing to do so at present and shall play a major role in future as well. Although India having well-recorded and well-practiced knowledge of traditional system medicine, but it has failed to capitalize on this herbal wealth. This can be achieved by identifying the herbal medicine for the rare

diseases for which no medicine or therapy is available (1).

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent hyperglycemia (2). The degree and duration of hyperglycemic state is the key contributing factor in the progression of secondary complication of diabetes. Prolonged exposure to hyperglycemia can lead to complications of several systems of the body, viz. the visual, cardiovascular, renal, and neurological systems such as lens, nerves, retina, and kidney, which are insulin insensitive and are the target organs for complications such as cataracts, retinopathy, neuropathy, and nephropathy (3).

The incidence of DM is increasing daily, the International Diabetes Federation reported that 415 million people have DM, and it may affect over 642 million in 2040 (4). An elderly population and

sustained patient life expectancy also increases the incidence of DM which expected to exceed 33% by 2050 (5). Cataract, which is one of the secondary complications of diabetes affecting more than 20 million people and accounts 51% of all blindness globally (6).

The protein redox status seems to be largely responsible for maintaining the lens function and transparency. It may be possible that systemic conditions like diabetes affecting the protein redox status leading to oxidation of proteins (7). Increased accumulation of oxidized proteins has been reported to be among the main biological alterations leading to cell damage and tissue modifications, such as cataractogenesis (8). Recently, it has been proposed that increased release of free radicals and consumption of antioxidant compounds may be responsible for an increased lipid peroxidation in poorly controlled diabetic subjects and may contribute to the onset of diabetic complications (9).

The assessment of carbonyl and sulfhydryl groups of protein has been proposed as being a valuable index of the protein redox status in the lens. In fact, the amount of carbonyl proteins, formed during metal-catalysed oxidation of proteins either in vitro or in vivo, signifies measure of the oxidative damage to those molecules. The sulfhydryl proteins, well-known for its structural and functional role in the crystallin lens, contain an high number of thiol groups and therefore are decreases because of oxidation. Because of this, the concentration of these compounds is an indirect measure of protein oxidation leading to protein aggregation (10).

Piper longum is commonly known as long pepper. Due to the presence of many alkaloids *P. longum* acts as antidiabetic, hepatoprotective, antihyperlipidemic, cardioprotective, antibacterial, and bio-enhancing agent (11). Significant anti-hyperglycemic, antioxidant and lipid peroxidative lowering effects of *P. longum* have been explored in hyperglycemic rats (12). In another study on alloxan-induced diabetic mice, piperine, an alkaloid present in *P. longum* has been shown for its hypoglycemic effect (13). The antioxidant and antidiabetic effects of *Hybanthus enneaspermus* leaves have been explored from different solvent fractions (14, 15) and are found to have strong antioxidant potential.

With this background, the present experimental study was done with objective to examine mechanism of oxidative alteration of lens proteins caused by hyperglycemia leading to cataractogenesis and to study anticataract effect of aqueous extract of *Piper longum* and *H. enneaspermus* on glucose induced diabetic cataract by technique of lens organ culture in isolated goat lenses.

MATERIALS AND METHODS

Institutional Ethics Committee approval was taken for the present study.

Study design

The study was done by “Technique of Lens Organ Culture” on 120 fresh isolated goat lenses which were distributed into 4 groups (Table 1).

Chemicals

All chemicals used in this study were of analytical grade and were purchased from Himedia Ltd., India.

Preparation of lens culture

Goat eyeballs were procured from the slaughterhouse and were transferred to the laboratory in an box containing ice packs. Once reached laboratory lenses were separated from the eyeballs by method of intracapsular lens extraction. The lenses were incubated in Krebs Ringer Bicarbonate Buffer (KRB buffer) pH 7.8 with Cefixime 500 mg for 72 hrs. (16).

Preparation of plant extract

Dry powders of *P. longum* fruits and *H. enneaspermus* leaves were taken and 25% w/v water extracts were prepared. The concentration of solution of each extract used for the study was 0.25%.

Preparation of lens homogenate

At the end of 72 h of incubation, lenses from each group were removed and homogenized in 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000xg for 30 min at -4 °C in a refrigerated centrifuge. The supernatant was subjected to the estimation of various study parameters.

Table 1: Study design

Group	Name of the group	Composition
Group 1	Normal Control	Lens + KRB buffer + Glucose 5.5mM
Group 2	Experimental diabetic cataract	Lens + KRB buffer + Glucose 55mM
Group 3	Experimental diabetic cataract + Extract of <i>Piper longum</i>	Lens + KRB buffer + Glucose 55mM + Extract of <i>Piper longum</i>
Group 4	Experimental diabetic cataract + Extract of <i>H. enneaspermus</i>	Lens + KRB buffer + Glucose 55mM + Extract of <i>H. enneaspermus</i>

Estimation of biochemical parameters

Lowry's method was used for estimation of total soluble lens proteins (17), content of protein carbonyl in the lens was determined by colorimetric method (18). The content of protein sulfhydryl (P-SH) in the lens was assessed spectrophotometrically with a modification of the Ellman procedure (19) and Kei Satoh method was used to estimate MDA, as an index of lipid peroxidation by thiobarbituric acid reacting substances (TBARS) quantification (20).

Statistical analysis

GraphPad Prism (Version 6) was used for statistical analysis. All results were expressed in mean \pm SD. Student's "t" test was used to compare results of biochemical parameters among the Group 1 and Group 2 (Normal control and Experimental diabetic cataract). "One-way Analysis of variance" (ANOVA) was used for comparing the results of biochemical parameters between Group 2, Group 3, and Group 4. A p value < 0.05 was considered significant.

RESULTS

Effect of high glucose on test parameters of lens

Effect on lens soluble proteins

Lens soluble proteins are essential for maintaining over all transparency of the lens. Reduction in protein solubility rises scattering of light, therefore measurement of soluble fractions of proteins are key aspect for determining of progression of cataractogenesis. The results demonstrated in Table 2, clearly shows that; the total soluble protein concentration of lens incubated in 55 mM glucose (group 2) is significantly decreased as compared with

lens incubated with 5.5 mM glucose (group 1). The decrease was statistically highly significant ($p < 0.001$).

Effect on lens protein carbonyl content

High glucose level by various mechanisms causes oxidation of proteins results into the formation of protein carbonyl groups. Quantification of carbonyl groups gives an idea about the rate and extent of oxidative injury of proteins. Present data about concentration of protein carbonyl groups (Table 2) shows that, significantly high amount of protein carbonyl groups was generated in experimental diabetic cataract (group 2) (3.28 ± 0.68 nmol/mg protein) as compare with normal control lens (group 1) (1.08 ± 0.21 nmol/mg protein) and this increase in protein carbonyl groups was statistically highly significant ($p < 0.001$).

Effect on lens protein sulfhydryl group

The sulfhydryl group plays a very important role in the structure as well as function of the crystallin lens. Present study demonstrates enhanced protein oxidation which can be observed from the Table 2. Statistically significant decrease in protein sulfhydryl group was seen in group 2 when compared with group 1.

Effect on lipid peroxidation (MDA)

Lipid peroxidation measured in the form of malondialdehyde (MDA) was significantly higher in lens incubated with high concentration of glucose (Group 2) compared with control lens (Group 1) ($p < 0.001$) which suggest that hyperglycemia may induce oxidative stress.

Table 2: Expression of protein oxidation and oxidative stress in normal control and experimental diabetic cataract

Parameters \ Groups	Group 1	Group 2
	(n=30) Mean \pm SD	(n=30) Mean \pm SD
Total soluble lens proteins (mg/dL)	345.55 \pm 46.63	253.05 \pm 26.09
Protein carbonyl (nmol/mg protein)	1.08 \pm 0.21	3.28 \pm 0.68
Protein Sulfhydryl (nmol/mg protein)	12.99 \pm 3.85	6.47 \pm 2.33
Malondialdehyde (MDA) (nmol/ml)	9.09 \pm 1.20	15.68 \pm 1.93

Group 1: Normal Control (Lens + KRB buffer + Glucose 5.5mM), Group 2: Experimental diabetic cataract (Lens + KRB buffer + Glucose 55mM)

Table 3: Expression of protein oxidation and oxidative stress in different experimental cataract groups

Parameters \ Groups	Group 2	Group 3	Group 4
	(n=30) Mean \pm SD	(n=30) Mean \pm SD	(n=30) Mean \pm SD
Lens total soluble proteins (mg/dL)	253.05 \pm 26.09	326.74 \pm 35.40	290.85 \pm 24.15
Protein carbonyl (nmol/mg protein)	3.28 \pm 0.68	1.35 \pm 0.32	2.95 \pm 0.67
Protein Sulfhydryl (nmol/mg protein)	6.47 \pm 2.33	12.92 \pm 3.39	9.32 \pm 2.04
Malondialdehyde (MDA) (nmol/ml)	15.68 \pm 1.93	9.81 \pm 0.91	12.39 \pm 1.52

Group 2: Lens + KRB buffer + Glucose 55mM, Group 3: Lens + KRB buffer + Glucose 55mM + Extract of *Piper longum*, Group 4: Lens + KRB buffer + Glucose 55mM + Extract of *H. enneaspermus*.

Effect on *Piper longum* and *H. enneaspermus* extracts on glucose induced cataract lenses

Effect on lens total soluble proteins

The total soluble lens protein content in Group 3 (*P. longum*) and Group 4 (*H. enneaspermus*) increased as compared to Group 2 (Experimental diabetic cataract) lenses (Table 3). This increase was statistically significant in Group 3 ($p < 0.001$) but not in Group 4.

Effect on lens protein carbonyl content

The protective role of *P. longum* (Group 3) in the prevention of protein oxidation is much effective than that of *H. enneaspermus* (Group 4) when compared with experimental diabetic cataract (Group 2) which can be seen in the form of decrease lens protein carbonyl level shown in the Table 3.

Effect on lens protein sulfhydryl group

Similar protective role of *P. longum* and *H. enneaspermus* (Group 4) was seen to prevent the oxidation of sulfhydryl (SH) group of lens protein to bisulfide group. Significant increase levels of protein sulfhydryl (SH) group were noted in Group 3 and Group 4 when compared with Group 2 ($p < 0.001$).

Effect on lipid peroxidation (MDA)

Table 3 also shows, decreased levels of MDA in both Group 3 and Group 4 as compared with Group 2. But statistically significant decrease (p) was observed in lens supplemented with *P. longum* (Group 3) than that of lens supplemented with *H. enneaspermus* (Group 4) ($p < 0.001$).

DISCUSSION

In the present world of modern medicine, excessive and inappropriate use of drugs and antibiotics caused. It was known that during cataractogenesis large amounts of insoluble protein derived from the soluble protein by protein oxidation especially sulfhydryl group of the amino acid and aggregation gets accumulated resulting in decreased soluble protein.

increased risk of adverse effects, resistance of microbes to antibiotics and their expensiveness are of an immense concern and there is a need for development of alternate treatment options which are safe, natural, effective and inexpensive. Herbal plants have been used in traditional medicine for many diseases from ancient time in many parts of the world and these plants are considered as the heart of Indian traditional medicine. Bioactive compounds derived from medicinal plants are the basis for the discovery of new compound leads for the pharmaceutical industry. It has been shown that out of the 5,00,000 plant species found around world, only 1% has been phytochemically researched, this indicates that the medicinal plants have a great potential for discovering new bioactive compounds. (21). Although diabetes is known to speed up the pace of cataractogenesis in humans, the mechanism of such acceleration remains speculative. Knowing the mechanism involved becomes more difficult because of a large number of metabolic changes involving lipids, carbohydrates and proteins associated with this disease.

In the present work, similar emphasis was given to evaluate the mechanism of cataractogenesis and the potential of medicinal plant *P. longum* and *H. enneaspermus* in prevention of oxidation of lens protein in glucose induced diabetic cataract. The cataract development was monitored by using the *in-vitro* organ culture model that mimics the *in-vivo* system. In the present experimental model with high glucose, we found the formation of lens cloudiness suggesting the role of oxidative stress and protein oxidation in pathophysiology of cataract. Our results show the total soluble proteins and protein sulfhydryl content of the lenses exposed to high glucose concentration was reduced significantly at the end of 72 hrs incubation than that of the lenses incubated with normal physiological glucose concentration. However, decrease in soluble protein and protein sulfhydryl were significantly prevented by addition of *P. longum* and *H. enneaspermus* indicating the protective effect of these medicinal plants. Our results were similar with previous studies showing increased

protein carbonyls and MDA in lenses incubated in high glucose concentrations suggest an increased oxidation of protein and lipid molecules. This might be due to the formation of reactive oxygen species like superoxide, H_2O_2 inside the cataractous lens due to hyperglycemia causing lipid peroxidation (22). Our findings are clearly indicating that addition of *P. longum* and *H. enneaspermus* in the respective cultured lenses containing physiological high concentration of glucose prevented the lipid peroxidation and oxidation of lens proteins significantly represented by the decreased levels of MAD and protein carbonyl. However, *P. longum* fruit extract demonstrated a consistently better effect as compared to the *H. enneaspermus* leaf extract with regards to all the parameters. The effects found in the present study reinforce the findings in past related to *P. longum*. *In vitro* studies revealed that piperine an active compound present in *P. longum* inhibits lipid peroxidation and spare the action of antioxidant enzymes protecting the cell against ROS and oxidative injury (23). Present study is strongly suggestive of the possible usefulness of *P. longum* and *H. enneaspermus* to subjects even in early stages of cataract.

CONCLUSION

In the present study, medicinal plants *P. longum* and *H. enneaspermus* were assessed for their protective role in the prevention of glucose-induced cataract in goat lenses maintained in culture medium. Both these medicinal plants exhibited potent anti-cataract activity against glucose-induced cataract formation in organ culture model. This can be attributed to its antioxidant activities which might be helpful in inhibiting peroxidation of lipid and oxidation of lens proteins. However, *P. longum* demonstrated a consistently better effect as compared to the *H. enneaspermus* with regards to all the parameters. From the points discussed above we can conclude that, use of *P. longum* and *H. enneaspermus* has been found to be effective in attenuating diabetic cataract formation, it would be more practical to use eye drops since ocular diseases are known to be treated more effectively by administering the treatment compounds topically.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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