Effect of standardized fruit extract of *Luffa cylindrica* on oxidative stress markers in hydrogen peroxide induced cataract

Suchita Dubey, Sudipta Saha¹, Gaurav Kaithwas¹, Shubhini A. Saraf¹

ABSTRACT

Objective: The ability of *Luffa cylindrica* Roem fruit extract (LCE) to modulate biochemical parameters was investigated by *in vitro* studies for its role in hydrogen peroxide induced cataract on isolated goat lenses which were incubated for 72 h at 37°C.

Materials and Methods: Test groups contained 5, 10, 15, 20, 25, and 30 µg/ml of LCE along with 1 ml of H₂O₂ (0.5 mM) as cataract inducer. Lenses were examined for morphological variation and transparency periodically during the incubation. Biochemical parameters such as superoxide dismutase (SOD), reduced glutathione (GSH), total protein content (TPC), and malondialdehyde (MDA) were estimated.

Results: SOD, GSH, and TPC levels were found to increase proportionally with the concentration of LCE. However, MDA levels were found to be inversely proportional to the concentration of LCE. Opacity was graded as per “lens opacities classification system III.” Morphological examination suggested that LCE (25 µg/ml) maintained a vision for 44 h. No lens in LCE dose groups developed dense nuclear opacity after 24 h as opposed to 80% in negative control.

Conclusion: The results suggest that LCE can delay the onset and/or prevent the progression of cataract which can be attributed to the presence of adequate phenolics, flavonoids, and Vitamin A and its high nutritional value. This preliminary study can be further synergized by testing LCE against other *in vivo* and *in vitro* models of cataract.

KEY WORDS: Anticataract, antioxidant, hydrogen peroxide, *Luffa cylindrica*, sponge gourd

Introduction

*Luffa cylindrica* (Sponge gourd) belonging to family *Cucurbitaceae* is widely used across the globe as a vegetable. *L. cylindrica* Roem fruit extract (LCE) has been found to be an excellent antidiabetic and antioxidant.[1,2] Oxidative stress has been identified as an initiating factor in the development of cataract.[3] It is a complex disease, characterized by opacification of lenses leading to blindness. Intraocular lens implantation is the most effective method to treat cataract, though rare, but it involves risks such as irreversible loss of vision, retinal detachment, and endophthalmitis.[14,35] Reduction of oxidative stress is considered as one of the targeting strategies for prevention or treatment of cataract. Hence, the present study was designed to assess the anticataract potential of LCE in H₂O₂ induced cataract in isolated goat lens through determination of lens morphology and estimation of some biochemical parameters such as superoxide dismutase (SOD), reduced glutathione (GSH), total protein content (TPC), and malondialdehyde (MDA) content in order to further potentiate a substantial preliminary correlation.
between antioxidant and anticataract activity in context with LCE.

Materials and Methods

Plant Extract and Other Materials

LCE (Batch No. HNLC110850) was obtained from Herbo Nutra™ New Delhi along with the certificate of analysis which stated that the extract complies with all the morphological specification of color, odor, taste, loss on drying, ash value, and microbial load of yeast, mold and *Escherichia coli* by total plate count method. Preliminary phytochemical tests such as carbohydrates, starch, gums and mucilages, proteins and amino acids, fixed oils and fats, alkaloids, glycosides and flavonoids were performed, and the results were in conformity with the previously reported literature. LCE was extracted by the cold maceration method and concentrated by vacuum distillation to reduce the volume to 1/10. Hydrogen peroxide was purchased from Loba Chemie (Mumbai, India). Streptomycin and penicillin were obtained from Hindustan Antibiotics Ltd., (Pune, India). Marketed formulation (Catalin eye drops) containing pirenoxine which is a preparation of 1-hydroxy-5-oxo-5H-pyrido (3,2-c)-phenoxazine-3-carboxylic acid, a compound having a chemical structure similar to xanthommatin, an eye pigment of the insect, with a pyridophenoxazone nucleus dissolved in 0.02% methylparaben, 0.01% propylparaben, and 0.0001% thimerosal as preservatives, available for treatment and prevention of cataract, was purchased from a medical store in Lucknow, India. Hydrogen peroxide was procured from Loba Chemie, Navi Mumbai, India. All the chemicals used during the study were of analytical grade.

Preparation of Lens Culture

A total of 72 lenses, isolated from a group of 6–8 years old goats through extracapsular extraction, were used for the study. Age of goats was determined using teething method. These lenses were further divided into 9 groups containing 8 lenses each. Freshly extracted transparent lenses were incubated in tyrode physiological salt solution (PSS) containing sodium bicarbonate (0.9 g/ml), streptomycin (100 µg/ml) and penicillin (100 IU/ml) at 37°C in an incubator with 95% air and 5% CO₂. The lenses were incubated initially for 2 h to discard any lens that had opacified due to damage during the extraction procedure. 1 ml of H₂O₂ (0.5 mM) was used as cataract inducer. The quantity of catalin used was 1 ml. LCE was added in varying concentration of 5, 10, 15, 20, 25, and 30 µg/ml of lens culture, respectively.

Control Group
- Normal control: Lens + PSS + antibiotic solution
- Negative control: Lens + PSS + antibiotic solution + H₂O₂ solution
- Positive control: Lens + PSS + antibiotic solution + H₂O₂ solution + catalin.

Experimental Group
- Lens + PSS + antibiotic solution + H₂O₂ solution + LCE (varying concentrations of 5, 10, 15, 20, 25, 30 µg/ml of lens culture)

In order to measure the degree of opacity of lenses, photographic evaluation was performed during the entire period of incubation at 0, 6th, 24th, 48th, and 72nd h. A grade of opacity was adopted which was based on the “lens opacities classification system III.”

Grading of Opacity
- 0: Transparency
- 1: Slight cortical opacity
- 2: Diffuse cortical opacity
- 3: Dense nuclear cataract.

Total Phenolic Content of Luffa cylindrica Roem Fruit Extract

Total phenol content was estimated in APE by Folin-Ciocalteu’s reagent (FCR) based assay. To the aliquot (50 µl) taken from stock solution (1 mg/ml) of the extracts, 3.5 ml distilled water and 250 µl of FCR was added, the mixtures were kept at room temperature for 1–8 min and 750 µl of 20% sodium carbonate solution was added to the extract. Mixtures were kept at room temperature for 2 h and absorbance of the color developed was recorded at 765 nm with the help of ultraviolet-visible spectrophotometer against blank. Total phenolic content was determined using gallic acid standard curve (R² = 0.996) and expressed in mg/g as gallic acid equivalents (GAE).

Total Flavonoid Content of Luffa cylindrica Roem Fruit Extract

The total flavonoid content was estimated by the method of Zhishen et al., 1999. The reaction mixture contained 0.5 ml of extract in di-methyl Sulfoxide or standard solutions of quercetin, diluted with 2 ml distilled water and 0.15 ml of 5% sodium nitrite. After 5 min, 0.3 ml of 10% aluminum chloride was added. After 6 min, 1 ml of 1 M sodium hydroxide was added and the total volume was made up to 5 ml with water. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm. The flavonoids content was expressed as mg of quercetin equivalents (QE) per g of dried extract, by using a standard graph (R² = 0.9997).

Biochemical Estimation

Total protein estimation

Total protein estimation was done by Lowry’s method. It was evaluated as mg/g of fresh weight of the lens. 5 ml of alkaline solution was added to 1 ml of the solution after centrifugation of lens homogenate at 10,000 rpm and allowed to stand for 10 min. 0.5 ml of diluted folin's reagent was added, and the tube was shaken to mix the solution. After 30 min, the extinction against appropriate blank at 750 nm was recorded.

Malondialdehyde Levels

MDA levels were estimated by the method of Ohkawa et al., 1979. Lenses were weighed and homogenized in 1 ml of 0.15 M potassium chloride. To the supernatant, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% w/v acetic acid (pH-3.5) and 1.5 ml of 0.81% w/v thiobarbituric acid aqueous solution were added in succession. The mixture was then heated in boiling water for 30 min. After cooling to room temperature, 5 ml of butanol-pyridine (15:1 v/v) solution was added. The mixture was then centrifuged at 5000 rpm for 10 min. The upper organic layer was separated, and the intensity of resulting pink color was read spectrophotometrically at 532 nm. Standard prepared from 1,1′,3,3′-tetramethoxypropane was used as a reference.

Superoxide Dismutase Estimation

The supernatant was assayed for SOD activity by assessing the inhibition of pyrogallol auto-oxidation. 100 µl of the supernatant
obtained by homogenizing the lenses in 10 ml of 0.1 M potassium phosphate buffer further subjected to centrifugation at 10,000 rpm for 45 min was added to tris-HCl buffer, pH 8.5. The final volume was adjusted to 3 ml with the same buffer. At last, 25 µl of pyrogallol was added. Absorbance was recorded at 420 nm at 1 min interval for 3 min. The increase in the absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD. One unit of SOD is described as the amount of enzyme required causing 50% inhibition of pyrogallol auto-oxidation per 3 ml of assay mixture and is given by the formula: Unit of SOD/mg of protein = \((100 \times (A - B)/(A \times 50))/mg \text{ of protein, where } A = \text{Change in absorbance per minute in control and } B = \text{Change in absorbance per minute in test sample}\).\[10\]

**Glutathione Levels**

The lenses from each group were weighed and homogenized in 1 ml of 5% v/v trichloroacetic acid, and a clear supernatant was obtained by centrifugation at 5000 rpm for 10 min. To 0.5 ml of this supernatant, 4.0 ml of 0.3 M disodium hydrogen phosphate and 0.5 ml of 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid in 1% w/v trisodium citrate were added in succession. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. Reduced GSH was used as a standard.\[11\]

**Statistical Analysis**

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). All results were expressed as mean ± standard deviation. The data was analyzed by one-way ANOVA followed by Bonferroni multiple comparison test and statistically significant data were accepted when \(P < 0.05\). Paired t-test was calculated between treated groups, and data were considered to be statistically significant when values were found to be \(P < 0.05\).

**Results**

**Total Phenolic and Flavonoid Content**

Total phenol content was found to be 508 ± 1.698 mg GAE/g of LCE. The total flavonoid content was found to be 410.26 ± 2.512 mg QE/g of LCE.

**Effect on Lens Morphology**

Morphological observations confirmed the reduction in opacity when the lenses were incubated in LCE [Figure 1]. 85% of the lenses incubated in LCE (25 µg/ml) maintained visibility for 44.0 h whereas nearly all lenses in the standard group could maintain the transparency only for 33 h approximately [Table 1]. Only 11.42% of the lenses in the treated group showed cortical opacity \((P < 0.01)\).

**Effect on Total Protein Content, Malondialdehyde, Superoxide Dismutase and Glutathione (Oxidative Stress Markers)**

LCE protected the lens proteins from oxidative damage. The toxic group had significantly lower concentrations of total proteins in the lens homogenate \((P < 0.01)\) compared with a normal group [Table 2]. Test groups had higher concentrations of TPC \((P < 0.01)\), compared to the toxic group which increased in direct proportion with the concentration of LCE, 25 µg/ml of LCE maintained the highest concentration of TPC. It suggests that LCE can help in protecting the structure and arrangement of proteins in the cells, which is responsible for maintenance of visibility and prevents the proteins from getting oxidized.

Change in MDA level was evaluated in lenses cultured in the presence of \(H_2O_2\) (0.5 mM) using varying concentrations of LCE. In normal control, MDA level was estimated to be 0.39 ± 0.07 µM/g of TPC whereas that of toxic control was found to be 1.76 ± 0.05 µM which was far more than normal group. However, LCE (25 µg/ml) significantly brought down MDA levels to 0.76 ± 0.02 µM/g of TPC.

**Discussion**

The \(H_2O_2\) insult of isolated adult goat lenses was responded very interestingly by LCE. Free radicals are known since long to significantly contribute to the pathogenesis of many disorders including cataract, and these free radicals are neutralized by the presence of endogenous antioxidants in the eye. It has been confirmed that free radicals mediate the formation of cataract which is mostly brought about by age.\[12\] Oxidative stress is the main marker of cataract and is responsible for its pathogenesis.

In eye lenses, reactive oxygen species attack biological molecules, including DNA, protein and phospholipids, leading to lipid peroxidation and depletion of the antioxidant enzymes SOD and GSH, resulting in further increase in oxidative stress.\[13\] A significant correlation exists between phenols and antioxidants.\[14\] Balakrishnan and Sharma, 2013 reported that LCE is rich in phenolics in a preliminary phytochemical...
Table 1:
**Time of maintenance of vision in test groups**

| Groups                      | 0 h | 6 h | 24 h | 48 h | 72 h | Time in hours (maintenance of vision in test groups) |
|-----------------------------|-----|-----|------|------|------|---------------------------------------------------|
| Normal (positive control)   | 0   | 0   | 2    | 2    | 2    | 59                                                |
| Toxic control (negative control) | 0   | 1   | 3    | 3    | 3    | 15                                                |
| Positive control (catalin)  | 0   | 0   | 1    | 1    | 2    | 36                                                |
| LCE (5 µg/ml)               | 0   | 0   | 2    | 3    | 3    | 24                                                |
| LCE (10 µg/ml)              | 0   | 0   | 2    | 3    | 3    | 28                                                |
| LCE (15 µg/ml)              | 0   | 0   | 1    | 2    | 3    | 32                                                |
| LCE (20 µg/ml)              | 0   | 0   | 1    | 2    | 3    | 38                                                |
| LCE (25 µg/ml)              | 0   | 0   | 1    | 1    | 3    | 44                                                |
| LCE (30 µg/ml)              | 0   | 0   | 1    | 1    | 3    | 44                                                |

*Opacity scale in hours=0 for transparency, 1 for slight cortical opacity, 2 for partial cortical opacity, 3 for dense nuclear cataract. LCE=Luffa cylindrica Roem fruit extract

Table 2:
**Effect of LCE on total protein content, MDA, SOD and GSH levels in different groups**

| Groups                      | TPC (mg/g of fresh weight of lens) | MDA levels (micromoles of MDA/g of protein) | SOD levels (unit/mg of protein) | GSH (µmoles/g of protein) |
|-----------------------------|-----------------------------------|---------------------------------------------|---------------------------------|---------------------------|
| Normal group                | 268.26±1.56                       | 0.39±0.07                                   | 1.65±0.09                       | 2.95±0.23                 |
| Toxic control               | 198.39±1.48                       | 1.76±0.05                                   | 0.9±0.05                        | 1.12±0.03                 |
| Positive control (catalin)  | 264.21±1.87                       | 0.58±0.04                                   | 1.39±0.09                       | 2.76±0.05                 |
| LCE (5 µg/ml)               | 219.65±1.42                       | 1.32±0.02                                   | 0.46±0.05                       | 2.15±0.03                 |
| LCE (10 µg/ml)              | 226.05±2.29                       | 1.15±0.02                                   | 0.61±0.07                       | 2.190±0.05                |
| LCE (15 µg/ml)              | 242.89±1.24                       | 0.84±0.05                                   | 0.84±0.03                       | 2.35±0.03                 |
| LCE (20 µg/ml)              | 246.89±1.78                       | 0.51±0.01                                   | 0.91±0.02                       | 2.41±0.04                 |
| LCE (25 µg/ml)              | 259.67±1.96                       | 0.76±0.02                                   | 1.26±0.01                       | 2.89±0.05                 |
| LCE (30 µg/ml)              | 260.09±1.42                       | 0.78±0.01                                   | 1.32±0.03                       | 2.84±0.04                 |

*Various biochemical parameters were estimated in the lens. Results of biochemical parameters are reported as mean±SD. Comparisons were made on the basis of one-way ANOVA and Bonferroni test was also performed between test samples and data was considered to be statistically significant when \( P<0.05 \). MDA=Malondialdehyde, SOD=Superoxide dismutase, GSH=Reduced glutathione, TPC=Total protein content, SD=Standard deviation, LCE=Luffa cylindrica Roem fruit extract

[15] Our results are in conformity with the previously reported literature. Many dietary polyphenolics exhibit more potency than Vitamin C or E during in vitro studies. Hence, the value suggests the possible role of phenolics in the prevention of cataract. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants in vitro is well documented. Therefore, it can also be asserted that flavonoids of the LCE are imparting antiacataract property to LCE via its antioxidative action.

The study demonstrated that LCE is effective against H₂O₂ induced cataractogenesis in isolated goat eye lens. Significant prevention of cataract was observed during the study. SOD, GSH and TPC formation was found to increase proportionally with the concentration whereas MDA (a product of membrane lipid peroxidation) significantly decreased as the concentration of LCE increased [Table 2].

Reactive oxygen species are also responsible for protein oxidation which decreases the free protein concentration of tissues during pathophysiological conditions. It is, however, noteworthy that TPC is decreased during damage of the lens. Elevated oxidative stress can alter the function and cause irreversible damage to macromolecules such as proteins and DNA. The perfect physicochemical arrangement of the lens proteins gives transparency to the lens. Protein aggregation increases with age. The crystallins, which constitute approximately 90% of the TPC of the lens, accumulate, and show many age-related oxidative changes. These include the formation of disulfide and other inter/intramolecular cross-links and methionine oxidation, all of which result in the aggregation of high molecular weight molecules. Therefore, the protein redox status seems to be fundamental to maintain the lens function and transparency. It is observed that in general, there is a decrease in levels of protein in cataractous lens due to leakage of high molecular weight proteins, which result in the aggregation of these proteins in aqueous humor. Hence, we evaluated it as a preliminary parameter. The antioxidant effect of LCE, as hypothesized, might help in preventing the oxidation of proteins.

The increase in SOD level of LCE groups shows protection of cells against toxicity of active oxygen species by the virtue of their capability to scavenge superoxide free radicals. Furthermore, LCE (25 µg/ml) was found to be the concentration at which optimized response was observed, photographic evaluation as well as biochemical estimation. The photographic evaluation based on the opacity scale [Table 1] showed that highest concentrations of LCE (25 µg/ml) maintained the vision for 44 h. Any further increase in the concentration of LCE did not affect the time of maintenance of vision. The absence of significant difference in time of maintaining vision in the
The results suggest that LCE can delay the onset and/or prevent the progression of cataract which can be attributed to the presence of adequate phenolics, flavonoids, and Vitamin A and its high nutritional value. This preliminary study can be further synergized by testing LCE against other in vivo and in vitro models of cataract. Isolation of constituents from the extract and their development into a suitable formulation could produce significant prevention/termination of cataract. Further studies on other cataract models are needed to provide a better correlation between the anticataract and antioxidative activity of LCE.

**Financial Support and Sponsorship**

Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**References**

1. Du Q, Xu Y, Li L, Zhao Y, Jerz G, Winterhalter P. Antioxidant constituents in the fruits of *Luffa cylindrica* (L.) Roem. J Agric Food Chem 2006;54:4186-90.
2. Hazra M, KunduSen S, Bhattacharya S, Haldar PK, Gupta M, Majumdar UK. Evaluation of hypoglycemic and antihyperglycemic effects of *Luffa cylindrica* fruit extract in rats. J Adv Pharm Educ Res 2011;2:138-46.
3. Spector A. Oxidative stress-induced cataract: Mechanism of action. FASEB J 1995;9:1173-82.
4. Greenberg PB, Tseng VL, Wu WC, Liu J, Jiang L, Chen CK, et al. Prevalence and predictors of ocular complications associated with cataract surgery in United States veterans. Ophthalmology 2011;118:507-14.
5. Ottonello S, Foroni C, Carta A, Petrucco S, Maraini G. Oxidative stress and age-related cataract. Ophthalmologica 2000;214:78-85.
6. Singleton VL, Rossi JA. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-56.
7. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-9.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-73.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thioarbituric acid reaction. Anal Biochem 1979;95:351-6.
10. Elman GL. Tissue sulphhydril groups. Arch Biochem Biophys 1959;82:70-7.
11. Moren MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
12. Thiagarajan R, Manikandan R. Antioxidants and cataract. Free Radic Res 2013;47:337-45.
13. Sulochana KN, Punitham R, Ramakrishna S. Effect of cigarette smoking on cataract: Antioxidant enzymes. Indian J Pharmacol 2002;34:428-31.
14. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997;2:152-60.
15. Balakrishnan N, Sharma A. Preliminary phytochemical and pharmacological activities of *Luffa cylindrica* Fruit. Asian J Pharm Clin Res 2013;6:113-6.
16. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63:1035-42.
17. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 2011;25:267-99.
18. El-Shafey AF, Armstrong AE, Terrill JR, Grounds MD, Arthur PG. Screening for increased protein thiol oxidation in oxidatively stressed muscle tissue. Free Radic Res 2011;45:459-68.
19. Siddique MA, Tiwary BK, Paul SB. Phospholipid and protein contents of lens proteolipids in human senile cataract. Eye (Lond) 2010;24:720-7.
20. Boscia F, Grattagliano I, Vendemiale G, Micelli-Ferrari T, Altomare E. Protein oxidation and lens opacity in humans. Invest Ophthalmol Vis Sci 2000;41:2461-5.
21. Suryanarayana P, Saraswat M, Mrudula T, Krishna TP, Krishnaswamy K, Reddy GB. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 2005;46:2092-9.
22. Scandalios JG. Oxygen stress and superoxide dismutases. Plant Physiol 1993;101:7-12.