Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Effect of *Plectranthus Vettiveroides* areal Parts on Oxidative Stress Markers in Hydrogen Peroxide Induced cataract

Sujatha palatheeya[1], Babu Rao Bhukya[2] and Anil kumar Boda[3]

[1]Department of pharmacy, Krishna university, Machilipatnam Andhra Pradesh, India
[2]Department of Pharmacognosy and ethnopharmacology, university college of pharmaceutical sciences, kakatiya university, warrangal, india-506009
[3]Department of Pharmacology, Nethaji institute of pharmaceutical sciences, somidi kazipet, warrangal urban, Telangana-506003

ABSTRACT

The ability of *Plectranthus vettiveroides* areal part extract (PVE) 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.Test groups contained 5, 10, 15, 20, 25, and 30 µg/ml of PVE along with 1 ml of H_2O_2 (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. SOD, GSH, and TPC levels were found to increase proportionally with the concentration of PVE. However, MDA levels were found to be inversely proportional to the concentration of PVE. Opacity was graded as per "lens opacities classification system III." Morphological examination suggested that PVE (25 µg/ml) maintained a vision for 44 h. No lens in PVE dose groups developed dense nuclear opacity after 24 h as opposed to 80% in negative control.The results suggest that PVE 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 PVE against other *in vivo* and *in vitro* models of cataract.

Keywords: Anticataract, antioxidant, hydrogen peroxide, *Plectranthus vettiveroides*.

INTRODUCTION

Oxidative stress has been identified as an initiating factor in the development of cataract.[1] 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

Sujatha palatheeya
Department of pharmacy, Krishna university, Machilipatnam
Andhra Pradesh, India

endophthalmitis.[2],[3] Reduction of oxidative stress is considered as one of the targeting strategies for prevention or treatment of cataract is also known as *Coleus vettiveroides*, *Coleus zeylanicus*, *Plectranthus zeynanicus* (Lamiaceae). The main phytochemical components of Iris are diterpenoids, essential oils and phenols. About 140 diterpenes were identified from the colored leaf glands of Platycladus species. The main components of Jerusalem artichoke essential oil are mono and sesquiterpenes. Flavonoids seem to be rare in *Platycladus orientalis*, only two flavonoids have been identified, 4',7-dimethoxy-5,6-cone in *Platycladus orientalis*, thus obtaining viologen from *P.marruboides* And golden chicken essence. Traditionally, it has been used as an antibacterial, deodorant, and cooling agent. It has also been used to prevent headaches and fever from burning eyes. The purpose of this research is to study the antibacterial activity of the stem bark of Phoenix tail. The present study was designed to assess the anticataract potential of PVE in H_2O_2 induced cataract in isolated goat lens through determination of lens morphology and estimation of some biochemical *Plectranthus vettiveroides* 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 PVE.

MATERIALS AND METHODS

Plant Identification and Collection

The plant was collected from Namakkal, Tamil Nadu, India in January 2019. The herbarium specimens of plants are stored in the Pharmacognosy Department. The plant was identified by Dr. G.V.S. Murthy, co-director of the Indian Botanical Survey in the South Ring of Coimbatore TNAU campus, who identified the plant with information he obtained from the literature.

Extraction Procedure

The powdered plant material (bark) weighing 300 gm was extracted by the cold maceration method and concentrated by vacuum distillation to reduce the volume to 1/10. The extract is transferred to a closed tank for further processing. 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.

Other Materials

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-α)-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 LobaChemie, 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 extra capsular 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% CO_2. 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 H_2O_2 (0.5 mM) was used as cataract inducer. The quantity of catalin used was 1 ml. PVE 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 + H_2O_2 solution
- Positive control: Lens + PSS + antibiotic solution + H_2O_2 solution + catalin.

Experimental Group

Lens + PSS + antibiotic solution + H_2O_2 solution + PVE solution (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."

0: ***Grading of Opacity***

- Transparency
- 1: Slight cortical opacity
- 2: Diffuse cortical opacity
- 3: Dense nuclear cataract.

Total Phenolic Content of Plectranthus vettiveroides Areal part Extract

Total phenol content was estimated in APE by Folin-Ciocalteu's reagent (FCR) based assay.[4] 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 ($R^2 = 0.996$) and expressed in mg/g as gallic acid equivalents (GAE).

Total Flavonoid Content of Plectranthus vettiveroides Areal part Extract:

The total flavonoid content was estimated by the method of Zhishen *et al.,* 1999.[5] 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 ($R^2 = 0.9907$).

Biochemical Estimation

Total protein estimation

Total protein estimation was done by Lowry's method. [6] 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.[7] 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 of protein, where A = Change in absorbance per minute in control and B = Change in absorbance per minute in test sample.[8]

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.[9]

STATISTICAL ANALYSIS

Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad 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 PVE. The total flavonoid content was found to be 410.26 ± 2.512 mg QE/g of PVE.

Effect on Lens Morphology

Morphological observations confirmed the reduction in opacity when the lenses were incubated in PVE. 85% of the lenses incubated in PVE (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 ($P < 0.01$).

Table 1: Time of maintenance of vision in test groups

Groups	0 h	1 h	2 4h	4 8h	7 2h	Time in hours (Maintenance of vision in test group)
Normal	0	0	2	2	2	59
Toxic control	0	1	3	3	3	15
Positive control	0	0	1	1	2	36
PVE (5µg/ml)	0	0	2	3	3	24
PVE (10µg/ml)	0	0	2	3	3	28
PVE (15µg/ml)	0	0	1	2	3	32
PVE (20µg/ml)	0	0	1	2	3	38
PVE (25µg/ml)	0	0	1	1	3	44
PVE (30µg/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, PVE: *Plectranthus vettiveroides* Extract.

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

PVE 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 PVE. 25 µg/ml of PVE maintained the highest concentration of TPC. It suggests that PVE 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

.

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 (micromoles of MDA/g of protein	SOD (unit/mg of protein)	GSH (µmoles/g of protein)
Normal	267.25±1.55	0.38±0.06	1.64±0.08	2.94±0.22
Toxic control	197.38±1.47	1.75±0.04	0.36±0.04	1.11±0.02
Positive control	263.20±1.86	0.57±0.03	1.38±0.08	2.75±0.02
PVE (5µg/ml)	218.64±1.41	1.31±0.01	0.45±0.04	2.14±0.02
PVE (10µg/ml)	225.04±2.28	1.14±0.01	0.60±0.06	2.18±0.04
PVE (15µg/ml)	241.88±1.23	0.83±0.04	0.83±0.02	2.34±0.02

PVE (20µg/ml)	245.85±1.77	0.50±0.01	0.90±0.01	2.40±0.03
PVE (25µg/ml)	258.66±1.95	0.75±0.01	1.25±0.01	2.88±0.04
PVE (30µg/ml)	259.08±1.41	0.77±0.01	1.32±0.03	2.83±0.03

Various biochemical parameters were estimated in this lens. Results expressed as mean±SD. Comparisons were made on the basis of one-way ANOVA and benferroni test was also performed between test sample 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, PVE: *Plectranthus vettiveroides* Extract. Change in MDA level was evaluated in lenses cultured in the presence of H_2O_2 (0.5 mM) using varying concentrations of PVE. In normal control, MDA level was estimated to be 0.38 ± 0.06 µM/g of TPC whereas that of toxic control was found to be 1.75 ± 0.04 µM which was far more than normal group. However, PVE (25 µg/ml) significantly brought down MDA levels to 0.75 ± 0.01 µM/g of TPC. [Figure:1].

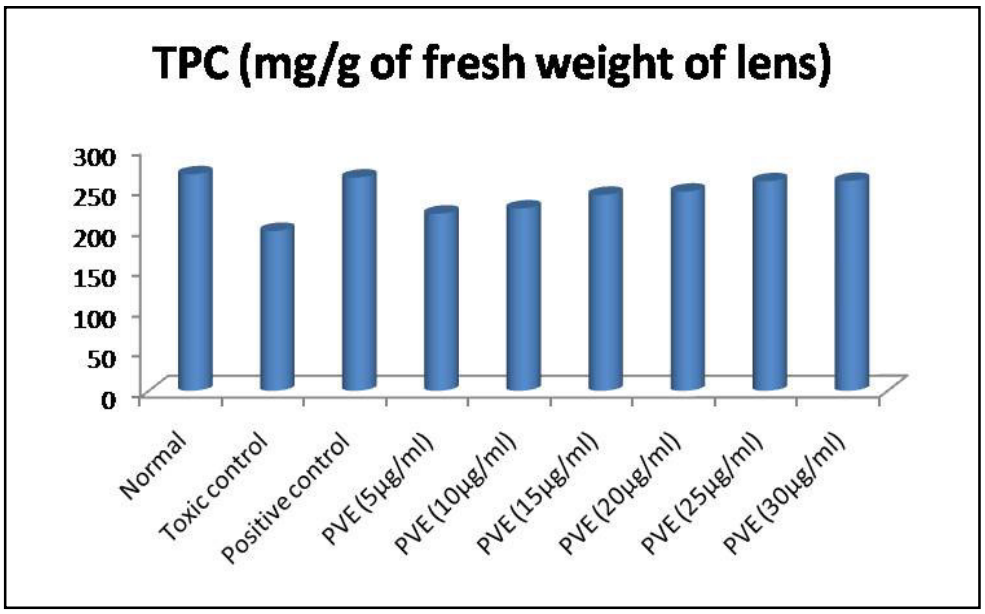

Fig 1: Effect of PVE on TPC (mg/g of fresh weight of lens)

SOD level as compared to the toxic group (0.36 ± 0.05 unit/mg of TPC) was found to be higher in normal group (1.64 ± 0.08 unit/mg of TPC). PVE (25 µg/ml) increased the level of SOD in the presence of H_2O_2 to 1.25 ± 0.01 unit/mg of TPC. Significant ($P < 0.05$) difference has been found in toxic, and PVE treated groups. The mean GSH value in normal lenses was found to be 2.94 ± 0.22 µg/mg, which decreased to 1.11 ± 0.02 µg/mg of fresh weight of the lens in toxic control. In the presence of PVE (25 µg/ml), there was the significant restoration of the levels of GSH to 2.88 ± 0.04 µg/mg ($P < 0.05$). [Figure:2].

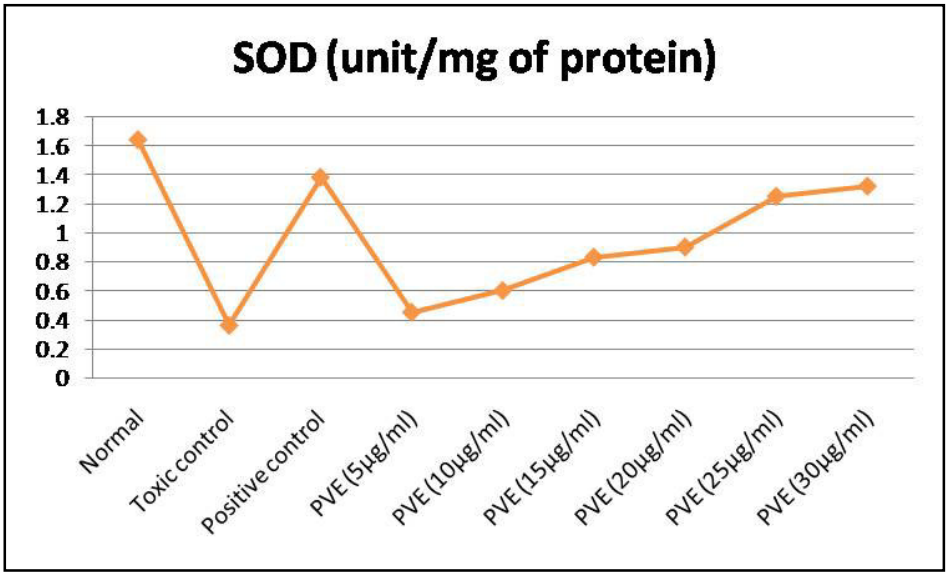

Fig 2: Effect of PVE on SOD (unit/mg of protein)

DISCUSSION

The H2O2 insult of isolated adult goat lenses was responded very interestingly by PVE. 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.[10] 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.[11]. A significant correlation exists between phenols and antioxidants.[12] Balakrishnan and Sharma, 2013 reported that PVE is rich in phenolics in a preliminary phytochemical analysis.[153 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.[14] Therefore, it can also be asserted that flavonoids of the PVE are imparting anticataract property to PVE via its antioxidative action. The study demonstrated that PVE 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 PVE increased [Table 2]. Reactive oxygen species are also responsible for protein oxidation which decreases the final protein concentration of tissues during pathophysiological conditions.[15] 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.[16] The perfect physicochemical arrangement of the lens proteins gives transparency to the lens.[17] 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.[18] It

is observed that in general, there is a decrease in levels of protein in cataractous lens due to leakage of these proteins in aqueous humor.[19] Hence, we evaluated it as a preliminary parameter. The antioxidant effect of PVE, as hypothesized, might help in preventing the oxidation of proteins.

The increase in SOD level of PVE groups shows protection of cells against toxicity of active oxygen species by the virtue of their capability to scavenge superoxide free radicals.[20] Furthermore, PVE (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 PVE (25 µg/ml) maintained the vision for 44 h. Any further increase in the concentration of PVE did not affect the time of maintenance of vision. The absence of significant difference in time of maintaining vision in the aforesaid concentration reconfirms the fact that 25 µg/ml of PVE is the optimum concentration at which the anticataract effect is likely to be produced. Oxidative stress may result in the emergence of inflammatory reactions. During the present investigation also, it was evident that the lenses of the toxic control group swelled and imbibed more solution which can be a possible outcome of inflammation while the lenses of the experimental groups were less swollen which further confirms the anti-inflammatory activities of PVE. Universally accepted pharmacological agents are not readily available to either inhibit or reverse the progression of cataract. Experimentations directed at increasing the antioxidative defenses of the lens by gene amplification could be a new strategy to prevent cataract. Genetic mouse models available for cataract generally suffer from congenital cataract, and very few develop a cataract in their older ages. Therefore, the mouse cataract models can only contribute to the understanding of lens development than to the ageing process taking place in the lens. Hence, we tried to propose a possible prophylactic approach of delaying/terminating the progression of cataract as PVE.

CONCLUSION

The results suggest that PVE 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 PVE 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 PVE.

REFERENCES

1. Spector A. Oxidative stress-induced cataract: Mechanism of action. FASEB J 1995;9:1173-82.
2. 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.
3. Ottonello S, Foroni C, Carta A, Petrucco S, Maraini G. Oxidative stress and age-related cataract. Ophthalmologica 2000;214:78-85.
4. Singleton VL, Rossi JA. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
5. 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.
6. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
7. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
8. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
9. Moron 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.
10. Thiagarajan R, Manikandan R. Antioxidants and cataract. Free Radic Res 2013;47:337-45.
11. Sulochana KN, Punitham R, Ramakrishna S. Effect of cigarette smoking on cataract: Antioxidant enzymes. Indian J Pharmacol 2002;34:428-31.

12. Rice-Evans CA, Miller NJ, Paganga G. Antioxidants properties of phenolic compounds. Trends Plant Sci 1997;2:152-60.
13. Balakrishnan N, Sharma A. Preliminary phytochemical and pharmacological activities of *Plectranthus vettiveroides Areal part*. Asian J Pharm Clin Res 2013;6:113-6.
14. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63:1035-42.
15. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 2011;25:287-99.
16. 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;45:991-9.
17. Siddique MA, Tiwary BK, Paul SB. Phospholipid and protein contents of lens proteolipids in human senile cataract. Eye (Lond) 2010;24:720-7.
18. 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.
19. 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.
20. Scandalios JG. Oxygen stress and superoxide dismutases. Plant Physiol 1993;101:7-12