Current Eye Research, 2016; 41(2): 222–231

ISSN: 0271-3683 print / 1460-2202 online
DOI: 10.3109/02713683.2015.1011281

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Preventive Effect of *Tephrosia purpurea* on Selenite-Induced Experimental Cataract

Shraddha V. Bhadada[1], Vikas J. Bhadada[2] and Ramesh K. Goyal[3]

[1]*Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India,* [2]*Vikas Eye Hospital, Ahmedabad, Gujarat, India and* [3]*Institute of Life Sciences, Ahmedabad University, Ahmedabad, Gujarat, India*

ABSTRACT

Purpose: Recent investigations have shown that phytochemical antioxidants can scavenge free radicals and prevent various diseases like cataract. The objective of the present study was to assess the efficacy of the *Tephrosia purpurea* in preventing these changes in the lens of selenite-induced cataract models.

Materials and methods: Cataract was induced by a single injection of sodium selenite (4 mg/kg, s.c.) to 9-day-old Sprague–Dawley rat pups. The treatment with different extracts of *T. purpurea* was started on 10th day and continued for 30 days in pups pretreated with sodium selenite. The animals were treated orally with either quercetin (1 mg/kg), flavonoid rich fraction (40 mg/kg) or alcohol extract (300 mg/kg) of *T. purpurea*. Cataract was visualized after 30 days. Encapsulated lenses were analyzed for reduced glutathione and malondialdehyde. Lenses were also analyzed for total protein, insoluble protein, total nitrite, calcium levels, protein sulfhydryl content as well as for the activities of superoxide dismutase and Ca^{2+}-ATPase.

Results: Morphological examination of the rat lenses revealed normal transparent lens with minimal or partial nuclear opacity in control whereas dense opacity developed in rat lens treated with selenite. Both the extracts of *T. purpurea* produced reduction in nuclear opacity as well as improvement in the insoluble proteins, protein sulfhydryl, total nitrite, calcium levels and Ca^{2+}-ATPase activity in lenses. The extracts decreased malondialdehyde levels but also prevented the loss of reduced glutathione levels.

Conclusion: Our data suggests therapeutic potential of *T. purpurea* for the treatment of cataract.

Keywords: Cataract, quercetin, rutin, selenite-induced cataract, sodium selenite, *Tephrosia purpurea*

INTRODUCTION

Visual impairment is a major global health issue. Cataract is responsible for 51% of blindness worldwide.[1] Cataract-induced blindness is a serious health problem especially in developing countries. Currently, the only available treatment for the disease is surgical removal of the cataract lens and replacing it with an artificial intraocular lens. Even though cataract surgery is an effective cure and improving the quality of life of several patients, the outcome in developing countries is still suboptimal and a large backlog exists for the surgery cases.[2] Further, the postoperative vision results are less acceptable and the complications rates are higher like posterior capsule opacification, double vision, cystoid macular edema and detached retina.[3] Cataract removal has also been known to increase the occurrence of macular changes associated with aging and diabetes.[4] Hence there is a need to develop therapeutic options to prevent or delay the development of cataract. Various experimental[5,6] and epidemiological studies[7,8] indicates that delay in the development of cataract can be achieved by use of antioxidants. Over the last few years, there is a developing interest to explore the protective role of natural products possessing antioxidant potential in cataract formation.

Cataract is considered to be multifactorial disease which is associated with a number of pathogenic mechanisms like oxidative stress, osmotic stress,

Received 10 July 2014; revised 1 January 2015; accepted 16 January 2015; published online 2 April 2015

Correspondence: Prof. Ramesh K. Goyal, Distinguished Professor, V ClinBio Labs, Sri Ramachandra University, Chennai, India. Tel: 04445928529. E-mail: goyalrk@rediffmail.com

abnormal glucose metabolism, UV irradiation, etc.[9–11] Oxidative stress is shown to be an initiating factor in the development of cataract. It may cause direct modification of the inner lens proteins, such as cross-linking, aggregation and precipitation.[12,13] Toxic aldehydes generated by peroxidation of lens epithelium and by oxidative damage of the vulnerable retina may contribute to the final damage of lens proteins yielding opacity.[14] One of the ways to prevent cataractogenesis is to reduce the oxidative stress. Antioxidants may act at different levels, for example, by preventing the formation of reactive oxygen species (ROS), by eliminating already created ROS by scavenging, trapping and quenching them, or by binding metal ions into inactive chelates. Hence, increasing the antioxidant defense of the lens may help in delaying the progression of cataract. Selenite-overdose cataract is an extremely rapid and convenient model of nuclear cataracts.[15] Moreover, it incorporates many of the features present in the senile cataract and diabetic cataract.[16]

Tephrosia purpurea (Linn.) Pers. (Leguminosae) (*T. purpurea*), commonly known in Sanskrit as *Sharapunkha* is a highly branched, sub-erect, herbaceous perennial. According to *Ayurveda*, *T. purpurea* is used as digestible, anthelmintic, alexiteric, antipyretic, astringent, thermogenic, acrid and also used to cure diseases of liver, spleen, heart, blood, tumors, ulcers, leprosy and asthma.[17] This plant is rich in flavonoids like quercetin and rutin which are potent antioxidants. On this basis, *T. purpurea* was selected for the present study. The objective of the study was to assess the effectiveness of alcohol extract (AcTp) and the flavonoid rich fraction of *T. purpurea* (FFTp) in selenite-induced experimental cataract *in vivo*.

MATERIALS AND METHODS

Preparation and Standardization of Plant Extract

The whole plant of *T. purpurea* was collected from the medicinal plant garden of Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India and botanically authenticated. Voucher specimen (No. PL08SVBRKGtp001) was deposited in herbarium of the Institute of Pharmacy, Nirma University. It was shade-dried and powdered. Dried plant of *T. purpurea* (200 g) was soxhlet-extracted with 95% alcohol. After concentration under vacuum, the extract (9.28% w/w) was stored for further use. The same extract was suspended in distilled water and partitioned into ethyl acetate. The residue obtained after evaporation of the ethyl acetate fraction was dissolved in alcohol and treated with neutral lead acetate solution. The precipitate obtained was centrifuged, resuspended in alcohol, treated with hydrogen sulfide and filtered. The filtrate was evaporated under vacuum to yield the flavonoid rich fraction (3.07%w/w), which gave positive Shinoda test. This test was performed by adding 0.2 ml of flavonoid rich fraction to three pieces of magnesium chips, and addition of several drops of concentrated HCl. The appearance of orange/red/purple color after several minutes indicated the presence of flavonoids.[18] An aqueous suspension of the alcohol extract and flavonoid rich fraction was used for experimental studies.

Total phenolic content of the AcTp and FFTp was determined through the method previously described by Singleton and Rossi[19] and calculated from the calibration curve of gallic acid standard solution. Results were expressed as %w/w of gallic acid equivalent in dry extract. Total flavonoid content was measured using a previously described method.[20] Quercetin was used as the standard for the calibration curve. Results were expressed as %w/w of quercetin equivalent in dry extract.

The AcTp and FFTp were analyzed by thin layer chromatography for the presence of rutin using ethyl acetate: *n*-butanol: formic acid: water (5:3:1:1 v/v) and quercetin using toluene: ethyl acetate: formic acid (5:4:1 v/v) as solvent systems. The resulting chromatogram was scanned and quantified using CAMAG TLC scanner III (Switzerland) at 254 and 374 nm, respectively. Further, peak purity spectra of the extracts and the standard (rutin and quercetin) were taken between 200 and 700 nm.

Animals

The protocol of the experiment was approved by our institutional animal ethics committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India (Protocol no. IPS/PCOL/FAC10-11/001 dated 5th August, 2010).

Neonatal rat pups of Sprague–Dawley strain initially weighing 10–12 g on the 8th day were used for the study. The pups were housed along with their mother in polypropylene cages in rooms maintained at 25 ± 1 °C and 12 h/12 h light–dark cycle. The animals were maintained on a standard laboratory animal diet (Pranav Agro Ltd., India) and provided water *ad libitum* throughout the experimental period.

Experimental Design

The rat pups were randomly divided into five groups with 12 rats in each group as follows:

Group I: Control animals.
Group II: Animals treated with sodium selenite.
Group III: Animals treated with sodium selenite and quercetin.
Group IV: Animals treated with sodium selenite and FFTp.
Group V: Animals treated with sodium selenite and AcTp.

Group II to Group V animals were administered a single subcutaneous injection of 4 mg/kg body weight of sodium selenite (Central Drug House (P) Ltd., New Delhi, India) on the 10th day. Quercetin (M.P. Biomedicals LLC, France.) was administered at a dose of 1 mg/kg, p.o., AcTp at a dose of 300 mg/kg, p.o. and FFTp at a dose of 40 mg/kg, p.o. for 30 days. The animals treated with sodium selenite only were given equivalent amount of distilled water, p.o. The control animals were injected 0.9% saline instead of sodium selenite.

Cataract Classification

Upon opening of their eyes on the 14–15th day, 80–85% of the pups treated with sodium selenite only had developed bilateral nuclear cataract and the rest of the animals developed advanced nuclear to mature cataract. On 30th day, eyes were dilated with 1% tropicamide and 10% phenylephrine and examined with an ophthalmoscope by an expert ophthalmologist to observe the frequency of cataractogenesis. The cataract was categorized into following stages:
Stage 0: Normal transparent lens.
Stage 1: Lens with minimal nuclear opacity.
Stage 2: Lens with partial nuclear opacity.
Stage 3: Lens with mature dense opacity.

Preparation of Erythrocyte Hemolysate

At the end of four weeks, blood was collected from retro-orbital plexus and centrifuged for 15 min at 4000*g* in refrigerated centrifuge. After removal of plasma and buffy coats, the red cells were washed twice with two volumes of phosphate buffered saline (PBS) of pH 7.00. Hemolysates were prepared by addition of two volumes of cold distilled deionized water to erythrocytes. Cellular debris was removed by centrifugation at 4000*g* for 30 min.

Preparation of Lens Homogenate

After the examination of eyes for cataract, animals were sacrificed, the eye lenses were dissected by posterior approach. The lenses of both the eyes were rinsed with ice-cold distilled water, blotted, weighed and stored at −20 °C for further analysis. Known weights of lens were homogenized in phosphate buffer saline using a glass motor-pestle to prepare 10% homogenate and centrifuged at 4000*g* in the refrigerated centrifuge for 30 min. This homogenate was used for the estimation of protein sulfhydryl content, anti-oxidant activity, pro-oxidant levels, Ca-levels and Ca-ATPase activity.

Protein Estimation

Soluble and insoluble fractions of the protein were estimated by preparing a separate homogenate of dissected lens in double distilled water and centrifuged at 4000*g* in the refrigerated centrifuge for 30 min. The water soluble supernatant was used for estimation of soluble protein and the residue was dissolved in sodium hydroxide and used for the estimation of insoluble protein. The protein was estimated by the method described by Lowry et al.[21]

Estimation of Protein Sulfhydryl Content of Lens Protein

Protein sulfhydryl content of lens proteins was determined using the Ellman's procedure modified by Grattagliano et al.[22] Content of protein sulfhydryls was calculated using a calibration curve prepared with reduced glutathione (SD Fine Chemicals Ltd., Mumbai, India).

Estimation of Anti-Oxidant Activity

Reduced glutathione was measured by the method described by Beutler et al.[23] Instead of 0.5 ml, 0.3 ml of lens supernatant and 0.5 ml of erythrocyte homogenate was used for the estimation. Superoxide dismutase activity was estimated as per the method described by Misra and Fridovich.[24] The enzyme activity has been expressed in terms of U/min/mg protein.

Estimation of Pro-Oxidant Levels

The whole lens homogenate (0.5 ml) and erythrocyte homogenate (0.5 ml) was used to determine MDA levels as described by Ohkawa et al.[25] The nitrite levels in lens were determined according to the method described by Giustarini et al.[26] It was

calculated using standard curve which was plotted using sodium nitrite solution.

Estimation of Calcium Levels and Ca-ATPase Activity

Calcium levels were estimated in the supernatant using the diagnostic kit (Accucare Ltd., India). Ca-ATPase activity in the lens sample was measured by the method of Hjerten and Pan.[27] The phosphorus liberated was calculated using standard curve that was plotted using standard potassium dihydrogen phosphate. Results were expressed as inorganic phosphorus liberated/min/mg protein.

Statistical Analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at a p value <0.05.

RESULTS

Phytochemical Studies

The preliminary studies showed the presence of flavonoids and phenolics. The level of total phenolic content and flavonoids in the AcTp was found to be 22.67%w/w and 18.09%w/w, respectively, whereas that in FFTp was found to be 3.83%w/w and 26.94%w/w, respectively.

On performing HPTLC analysis of the AcTp and FFTp, the presence of rutin and quercetin was confirmed qualitatively by peak purity spectra between 200 and 700 nm (Figure 1A&B). Quantitaitve evaluation by HPTLC showed rutin to be present as 5.37%w/w in AcTp and 2.37%w/w in FFTp and quercetin to be 1.05%w/w in AcTp and 1.75%w/w in FFTp.

Pharmacological Studies

Morphological Assessment of Cataract Formation

Nine days old suckling rat pups of either sex upon opening their eyes on the 14–15th day, developed bilateral nuclear cataract to mature cataract (Figure 2). Treatment of animals with both the extracts of *T. purpurea* prevented significantly the development of nuclear cataract (Table 1).

Effect on Various Biochemical Parameters Related to Cataract

Selenite-induced cataractous animals exhibited significantly decreased soluble protein levels and increased insoluble protein levels in lens as compared to that of control rats. Treatment with standard quercetin, AcTp and FFTp for four weeks significantly prevented the loss of the soluble proteins (Figure 3A) and decreased the insoluble proteins (Figure 3B) as compared to the selenite treated animals. The effect of treatment with *T. purpurea* was comparable to that of standard antioxidant quercetin.

Protein sulfhydryl content was significantly reduced in lens of selenite treated animals as compared to that of control rats. Treatment with standard quercetin, AcTp and FFTp significantly prevented the loss of the protein sulfhydryl content (Table 2) in lens as compared to the selenite treated animals.

The mean levels of reduced glutathione (GSH) in erythrocytes and eye lens of selenite-induced group were significantly lower than the age-matched control animals while the treatment with standard quercetin, AcTp and FFTp was able to prevent the reduction in the GSH levels (Table 2). Further, SOD levels in eye lens were decreased significantly due to selenite administration in the animals. This loss of SOD levels (Table 2) in the lenticular tissue was significantly prevented by the treatment with standard quercetin, AcTp and FFTp.

Selenite administration in rats exhibited significantly increased MDA levels in the erythrocytes and the lenses as compared to those of control rats. Treatment with standard quercetin, AcTp and FFTp significantly decreased lenticular MDA levels (Table 2).

Nitrite levels were found to be significantly higher in lens of selenite treated animals as compared to those of control rats. Treatment with standard quercetin, AcTp and FFTp significantly prevented the increase in the nitrite levels (Table 2) as compared to selenite-induced cataractous rats.

Calcium Levels and Calcium ATPase Activity in Lens

Increase in calcium levels in the lenticular tissue was found due to selenite administration as compared to those of control rats. The calcium levels were significantly decreased by the treatment with standard quercetin, AcTp and FFTp (Figure 4A) as compared to the selenite-induced cataractous rats.

Selenite-induced cataractous rats exhibited significantly decreased calcium ATPase activity in lens as compared to those of control rats. Treatment with standard quercetin, AcTp and FFTp significantly

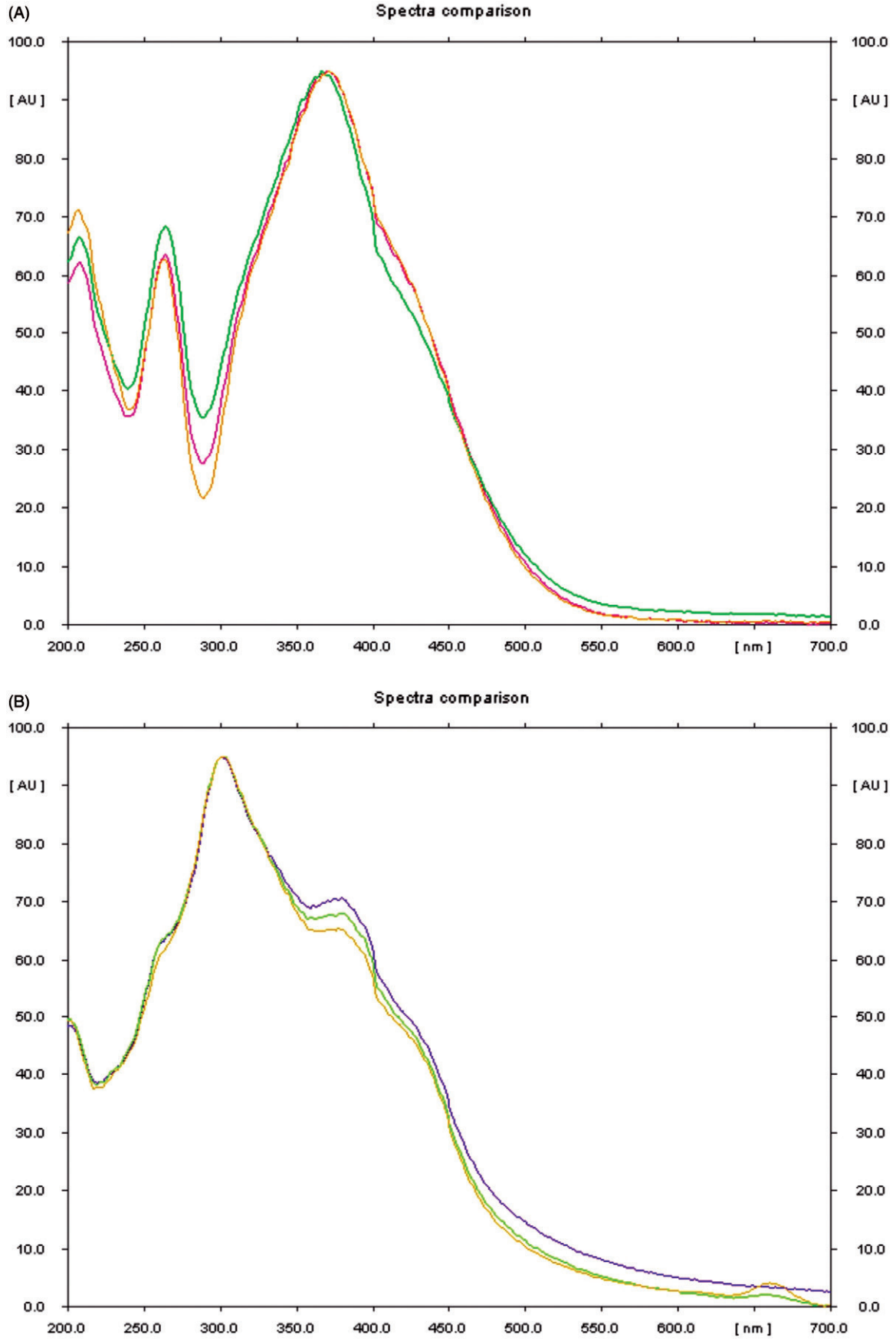

FIGURE 1 Spectral overlay of alcohol extract and flavonoid rich fraction of *T. purpurea* with (A) standard rutin and (B) standard quercetin.

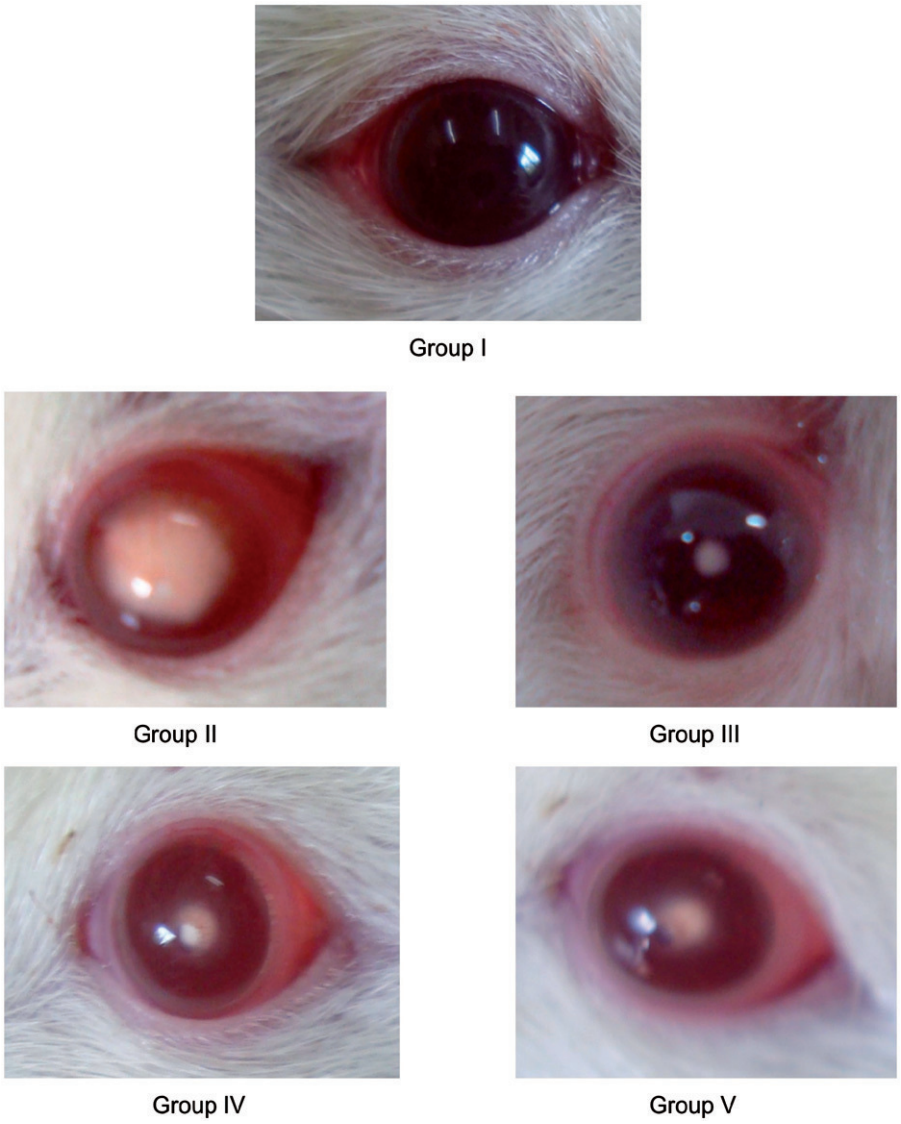

FIGURE 2 Effect of alcohol extract and flavonoid rich fraction of *T. purpurea* on morphological changes in selenite-induced cataractous lens. Group I: control animals, Group II: animals treated with selenite only, Group III: animals treated with selenite and quercetin (1 mg/kg, p.o), Group IV: animals treated with selenite and flavonoid rich fraction of *T. purpurea* (40 mg/kg, p.o), Group V: animals treated with selenite and alcohol extract of *T. purpurea* (300 mg/kg, p.o).

increased calcium ATPase activity in lens (Figure 4B) as compared to selenite-induced cataractous rats.

DISCUSSION

The purpose of the present study was to evaluate the potential anti-cataract effects of *T. purpurea* in selenite-induced cataract. This model incorporates many of the features present in the senile cataract and diabetic cataract.[16] Selenite is strong oxidizing agent and catalyzes the oxidation of critical sulfhydryl groups on proteins and glutathione that results in the formation of protein aggregates which leads to progression of cataract. The biochemical processes occurring during production of cataract by selenite includes ROS generation, oxidative damage of the critical sulfhydryl groups of membrane Ca^{2+}-ATPase, calcium accumulation, calpain mediated proteolysis, precipitation of fragmented lens crystallins and loss of cytoskeletal proteins.[16]

Our observations of the morphological examination of the lenses showed that cataract progression was decreased by the treatment with *T. purpurea* suggesting its anti-cataract potential which may be attributed to its anti-oxidant activity due to presence of flavonoids like rutin and quercetin. Further biochemical estimations were done to support the morphological examination.

Selenite cataract is characterized by marked decline in water soluble protein through protein insolubilization.[16] Similar changes are also observed in human cataract.[28] There was significant change observed in the soluble as well as the insoluble protein in the

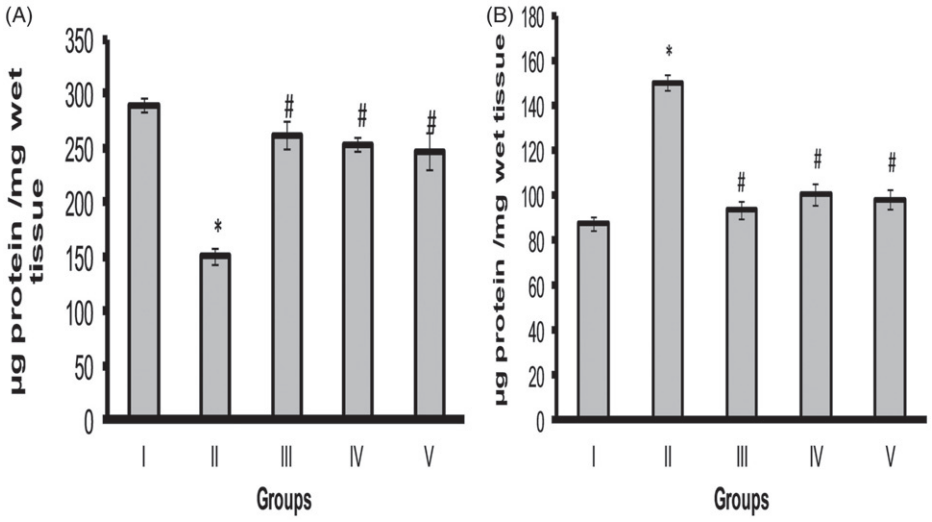

FIGURE 3 Effect of alcohol extract and flavonoid rich fraction of *T. purpurea* on (A) soluble protein levels in lens and (B) Insoluble protein levels in lens. Each bar represents Mean + S.E.M of six experiments. *significantly different from control ($p < 0.05$), #significantly different from selenite control ($p < 0.05$). Group I: control animals, Group II: animals treated with selenite only, Group III: animals treated with selenite and quercetin (1 mg/kg, p.o), Group IV: animals treated with selenite and flavonoid rich fraction of *T. purpurea* (40 mg/kg, p.o), Group V: animals treated with selenite and alcohol extract of *T. purpurea* (300 mg/kg, p.o).

TABLE 1 Morphological examination of cataractous rat lens in selenite-induced cataract.

Groups	Stage 0	Stage 1	Stage 2	Stage 3
I	12	–	–	–
II	–	–	4	8
III	1	6	3	2
IV	–	4	5	3
V	–	4	6	2

Group I: control animals, Group II: animals treated with selenite only, Group III: animals treated with selenite and quercetin (1 mg/kg, p.o), Group IV: animals treated with selenite and flavonoid rich fraction of *T. purpurea* (40 mg/kg, p.o), Group V: animals treated with selenite and alcohol extract of *T. purpurea* (300 mg/kg, p.o). Stage 0: normal transparent lens, Stage 1: lens with minimal nuclear opacity, Stage 2: lens with partial nuclear opacity, Stage 3: lens with mature dense opacity.

TABLE 2 Effect of alcohol extract and flavonoid rich fraction of *T. purpurea* on protein sulfhydryl content, nitrite levels and SOD levels in lens, GSH levels and MDA levels in lens and erythrocytes.

Parameter	Group I	Group 2	Group 3	Group 4	Group 5
Protein sulfhydryl content (µmol/g protein)	17.25 ± 0.42	12.80 ± 0.55[a]	16.61 ± 0.66[b]	16.33 ± 0.37[b]	16.63 ± 0.82[b]
GSH in lens (µmol/g wet tissue)	8.25 ± 0.14	6.06 ± 0.07[a]	8.11 ± 0.12[b]	8.01 ± 0.28[b]	7.88 ± 0.16[b]
GSH in erythrocytes (nmol/g Hb)	803.51 ± 12.54	478.51 ± 18.92[a]	768.74 ± 23.65[b]	759.61 ± 25.27[b]	763.54 ± 28.69[b]
SOD levels in lens (U/mg protein)	3.48 ± 0.36	1.03 ± 0.09[a]	3.08 ± 0.12[b]	2.97 ± 0.08[b]	2.84 ± 0.13[b]
MDA in lens (nmoles/g protein)	3.63 ± 0.23	8.21 ± 0.43[a]	4.02 ± 0.12[b]	4.12 ± 0.31[b]	4.07 ± 0.20[b]
MDA in erythrocytes (nmol/g Hb)	275.26 ± 7.08	390.12 ± 4.44[a]	282.31 ± 6.86[b]	277.30 ± 8.62[b]	275.41 ± 8.75[b]
Nitrite levels in lens (nmole/g wet tissue)	302.75 ± 8.00	437.25 ± 21.63[a]	301.00 ± 7.63[b]	343.25 ± 20.57[b]	344.25 ± 20.34[b]

Group I: control animals, Group II: animals treated with selenite only, Group III: animals treated with selenite and quercetin (1 mg/kg, p.o), Group IV: animals treated with selenite and flavonoid rich fraction of *T. purpurea* (40 mg/kg, p.o), Group V: animals treated with selenite and alcohol extract of *T. purpurea* (300 mg/kg, p.o).
[a]Significantly different from control ($p < 0.05$).
[b]Significantly different from selenite control ($p < 0.05$).

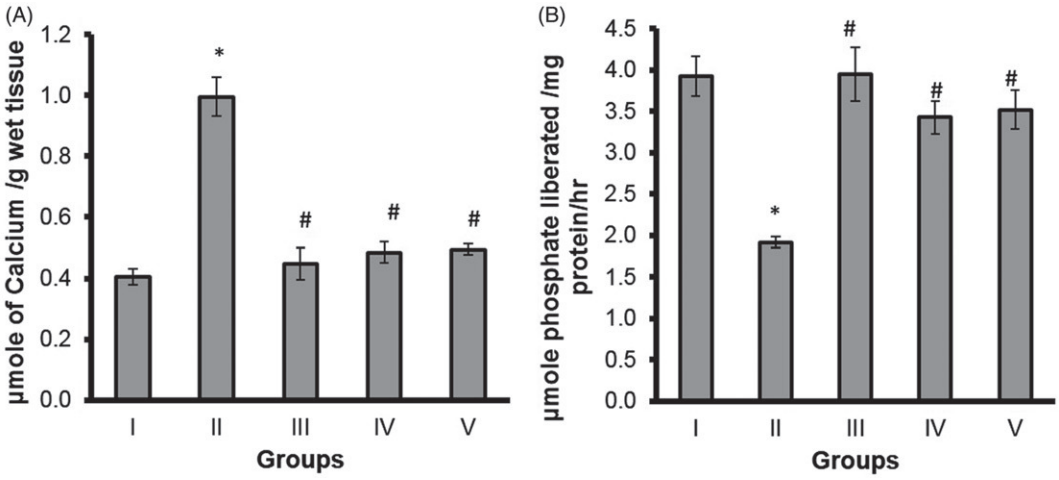

FIGURE 4 Effect of alcoholic extract and flavonoid fraction of *T. purpurea* on (A) calcium levels in lens and (B) Ca-ATPase activity in lens. Each bar represents Mean + S.E.M of six experiments. *significantly different from control ($p < 0.05$), #significantly different from selenite control ($p < 0.05$). Group I: control animals, Group II: animals treated with selenite only, Group III: animals treated with selenite and quercetin (1 mg/kg, p.o), Group IV: animals treated with selenite and flavonoid rich fraction of *T. purpurea* (40 mg/kg, p.o), Group V: animals treated with selenite and alcohol extract of *T. purpurea* (300 mg/kg, p.o).

selenite-induced group compared to that of the age matched control rats. This may be due formation of the insoluble high molecular weight protein aggregates which represent an intermediate stage in conversion of water soluble to water-insoluble proteins.[29] The insoluble protein levels were found to be reduced in the groups treated with standard quercetin, AcTp and FFTp. Pre-treatment with these extracts may delay the oxidation of these proteins and formation of protein aggregates, hence may delay the progression of cataract.

Normal lens contain an unusually high concentration of GSH which decreases in almost all types of cataract. GSH maintains protein thiol groups in the reduced state and prevents the cross linking of soluble crystallins in the lens.[30] Supplementation of GSH or maintenance of its level in lens may help to maintain its protective ability against oxidative stress and lead to slower age-related loss of antioxidant activity of lens and eventually delay the onset of cataract.[31] We found that AcTp and FFTp were able to restore the levels of GSH and thus showing potential anti-oxidant effect. SOD plays a critical role in protecting cells from oxidative stress. Selenite administration was found to decrease the activity of SOD in the lens[30] accompanied by the generation of free radical species in the aqueous humor.[32] Restoration of activity of the antioxidant enzymes in standard quercetin, AcTp and FFTp treated groups could be attributed to their antioxidant effect. Free radical-induced lipid peroxidation indicates oxidative stress which plays a significant role in degradation, oxidation, cross linking and aggregation of lens proteins and leads to lens opacity[30] in selenite model which was seen as higher levels of MDA in selenite-induced group. Lower levels of MDA in AcTp and FFTp treated groups are an indication to the prevention of lipid peroxidation. *Tephrosia purpurea* has been shown to possess potent free radical scavenging activity *in vitro* by various researchers[33,34] which support our observation *in vivo*. Many studies have shown that flavonoids can prevent oxidative damage and experimental cataract progression.[35,36] Further, rutin, a major flavonoid present in *T. purpurea*, has been also reported to retard the progression of cataract in selenite-induced cataract.[37]

One of the pathological events leading to protein precipitation in selenite model is mediated through disulfide cross-linking of protein via sulfhydryl oxidation, leading to high-molecular-weight aggregate formation, protein precipitation and lens opacification.[38,39] At 4 weeks post-selenite injection, selenite-induced group had significantly lower levels of protein sulfhydryl content over control animals. In the treated groups, protein sulfhydryl content was found to be increased, again confirming its protective effect against oxidative damage. This may be due to its restoration of the glutathione reductase activity. Oxidative damage of the critical sulfhydryl groups of proteins could lead to the inactivation of membrane proteins like Ca^{2+}-ATPase as well as inhibition of selective calcium permeability. Selenite cataract shows significant decrease in Ca^{2+}-ATPase activity and increase in lenticular calcium levels. Increased lens calcium could be due to inhibition of outwardly directed Ca^{2+}-ATPase pumps or inhibition of Na/Ca exchange. Moreover, in rodent lenses, the calcium influx was observed to cause activation of calcium dependent proteases, which partially degrade the crystallins and thereby resulting in the protein insolubilization.[16] In the treated groups, lower levels of calcium and higher levels of Ca^{2+}-ATPase activity were observed attributing to their protective effect on cataract. Disulfiram and Verapamil hydrochloride have also reported similar effects.[40,41]

Babizhayev et al.[42] reported that lipid peroxidation causes the oxidative inhibition of Ca^{2+}-ATPase in several tissues including the lens, and induces increase in membrane permeability of Ca^{2+}. Since the plant shows potential reduction in the lipid peroxidation and increases the protein sulfhydryl content, it may be responsible for the restoration of the Ca^{2+}-ATPase activity as well as the Ca^{2+} levels.

Nitric oxide (NO) reacts readily with the superoxide anion, to form a strong oxidant, peroxynitrite.[43,44] This reaction is highly favorable under reduced concentrations of SOD which is found in the selenite-induced group. Peroxynitrite, which is known to oxidize sulfhydryls induces membrane lipid peroxidation.[45] Moreover, NO can affect the levels of both free protein thiols and total glutathione levels in cells[46] causing opacification. Nitrite levels, an indirect measurement of NO was found to be significantly higher in the selenite-induced group. These levels of nitrite were found to be decreased with treatment which may be responsible for increasing Ca^{2+}-ATPase activity and hence decreasing the Ca^{2+} accumulation. Thus, suggesting the protective effect of the extracts in preventing the cataract formation.

CONCLUSIONS

The results of the present study indicate that treatment with *T. purpurea* is effective in preventing the progression of cataract by maintaining the antioxidant status and preventing protein oxidation and lipid peroxidation in lens. This protective effect was also manifested morphologically as a decreased frequency and intensity of lenticular opacification. However, further studies are required to justify its use in humans for prophylaxis or treatment of cataract.

DECLARATION OF INTEREST

There is no conflict of interest in the study.

REFERENCES

1. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol 2012;96:614–618.
2. Chang MA, Congdon NG, Baker SK, Bloem MW, Savage H, Sommer A. The surgical management of cataract: barriers, best practices and outcomes. Int Ophthalmol 2008;28:247–260.
3. Brian G, Taylor H. Cataract blindness-challenges for the 21st century. Bull World Health Organ 2001;79:249–256.
4. Klein BEK, Howard KP, Lee KE, Iyengar SK, Sivakumaran TA, Klein R. The relationship of cataract and cataract extraction to age-related macular degeneration: the Beaver Dam Eye Study. Opththalmology 2012;119:1628–1633.
5. Hegde KR, Varma SD. Prevention of cataract by pyruvate in experimentally diabetic mice. Mol Cell Biochem 2005; 269:115–120.
6. Jacques PF, Hartz SC, Chylack Jr LT, McGandy RB, Sadowski JA. Nutritional status in persons with and without senile cataracts: blood vitamin and mineral levels. Am J Clin Nutr 1988;48:152–158.
7. Taylor A, Jacques PF, Chylack Jr LY, Hankinson SE, Khu PM, Rogers G, et al. Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. Am J Clin Nutr 2002; 75:540–549.
8. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J. 1995;9:1173–1182.
9. Varma SD, Kovtun S, Hegde KR. Role of ultraviolet irradiation and oxidative stress in cataract formation-medical prevention by nutritional antioxidants and metabolic agonists. Eye Contact Lens 2011;37:233–245.
10. Javadi M, Zarei-Ghanavati S. Cataracts in diabetic patients: a review article. J Ophthalmic Vis Res 2008;3:52–65.
11. Ho M, Peng Y, Chen S, Chiou S. Senile cataracts and oxidative stress. J Clin Gerontol Geriatr 2010;1:17–21.
12. Reddy VN, Giblin FJ, Lin LR, Chakrapani B. The effect of aqueous humor ascorbate on ultraviolet-B-induced DNA damage in lens epithelium. Investig Ophthal Vis Sci 1988; 39:344–350.
13. Young RW, editor. Age-related cataract. New York: Oxford University Press; 1991.
14. Altomare E, Grattagliano I, Vendemaile G, Micelli-Ferrari T, Signorile A, Cardia L. Oxidative protein damage in human diabetic eye: evidence of a retinal participation. Eur J Clin Invest 1997;27:141–147.
15. Shearer TR, David LL, Anderson RS. Selenite cataract: a review. Curr Eye Res 1987;6:289–300.
16. Shearer TR, Ma H, Fukiage C, Azuma M. Selenite nuclear cataract: review of the model. Mol Vis 1997;3:8–15.
17. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal plants: a compendium of 500 species. Vol. 5. Chennai, India: Orient Longman Private Ltd; 2004.
18. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002.
19. Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144–158.
20. Baharam T, Gressier B, Trotin F, Brunet C, Dine T, Pinkash M. Oxygen species scavenging activity of phenolics extracts from Hawthorn fresh plant organs and pharmaceuticals preparations. Drug Res 1996;46: 1086–1089.
21. Lowry OH, Rosenbrouch NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193:265–275.
22. Grattagliano I, Vendemiale G, Sabba C, Buonamico P, Altomare E. Oxidation of circulating proteins in alcoholics: role of acetaldehyde and xanthine oxidase. J Hepatol 1996; 25:28–36.
23. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882–888.
24. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170–3175.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.
26. Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization. Methods Enzymol 2008;440:361–380.

27. Hjerten S, Pan H. Purification and characterization of two forms of low affinity Ca^{2+}-ATPase from erythrocyte membrane. Biochim Biophys Acta 1983;728:281–288.
28. Srivastava OP, Srivastava K. BetaB2-crystallin undergoes extensive truncation during aging in human lenses. Biochem Biophys Res Commun 2003;301:44–49.
29. Liem-The KN, Hoenders HJ. HM-crystallin as an intermediate in the conversion of water-soluble into water-insoluble rabbit lens proteins. Exp Eye Res 1974;19: 549–557.
30. Manikandan R, Thiagarajan R, Beulaja S, Sudhandiran G, Arumugam M. Effect of curcumin on selenite induced cataractogenesis in Wistar rat pups. Curr Eye Res 2010;35: 122–129.
31. Harding JJ. Can drugs or micronutrients prevent cataract? Drugs Aging 2001;18:473–486.
32. Anitha TS, Muralidharan AR, Annadurai T, Jesudasan CAN, Thomas PA, Geraldine P. Putative free radical scavenging activity of an extract of *Cineraria maritima* in preventing selenite induced cataractogenesis in Wistar rat pups. Mol Vis 2013;19:2551–2560.
33. Patel A, Patel A, Patel A, Patel NM. Estimation of flavonoid, polyphenolic content and in-vitro antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). Int J Pharma Sci Res 2010;1:66–77.
34. Nile SH, Khobragade CN. Phytochemical analysis, antioxidant and xanthine oxidase inhibitory activity of *Tephrosia purpurea* Linn. root extract. Ind J Nat Prod Resour 2011;2:52–58.
35. Kilic F, Bhardwaj R, Trevithick JR. Modelling cortical cataractogenesis, XVIII: *In vitro* diabetic cataract reduction by venoruton, a β-flavonoid which prevents lens opacification. Acta Ophthalmol Scand 1996;74:372–378.
36. Durukan AH, Evereklioglu C, Hurmeric V, Kerimoglu H, Erdurman C, Bayraktar MZ, et al. Ingestion of IH636 grape seed proanthocyanidin extract to prevent selenite-induced oxidative stress in experimental cataract. J Cataract Refract Surg 2006;32:1041–1045.
37. Isai M, Sakthivel M, Ramesh E, Thomas PA, Geraldine P. Prevention of selenite-induced cataractogenesis by rutin in Wistar rats. Mol Vis 2009;15:2570–2577.
38. Dong D, Lu A, Liu Y, Jia W, Hou W. The early biochemical changes of cataractous lenses of rats cultured *in vitro*. Zhonghua Yan Ke Za Zhi 2000;36:344–347.
39. Guo SB, Manabe S, Wada O. The decomposition and aggregation of rat lens protein induced by selenite in vitro and in vivo. Nihon Eiseigaku Zasshi 1989;44: 615–621.
40. Nabekura T, Koizumi Y, Nakao M, Tomohiro M, Inomata M, Ito Y. Delay of cataract development in hereditary cataract UPL rats by disulfiram and aminoguanidine. Exp Eye Res 2003;76:169–174.
41. Ettl A, Daxer A, Gottinger W, Schmid E. Inhibition of experimental diabetic cataract by topical administration of RSverapamil hydrochloride. Br J Ophthalmol 2004;88: 44–77.
42. Babizhayev MA, Deyev AI, Yermakova VN, Brikman IV, Bours J. Lipid peroxidation and cataracts: N-acetylcarnosine as a therapeutic tool to manage age-related cataracts in human and in canine eyes. Drugs R D 2004;5: 125–139.
43. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620–1624.
44. Beckman JS, Crow JP. Pathological implications of nitric oxide, superoxide and peroxynitrite formation. Biochem Soc Trans 1993;21:330–334.
45. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481–487.
46. Onoda M, Inano H. Effect of curcumin on the production of nitric oxide by cultured rat mammary gland. Nitric Oxide 2000;4:505–515.