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Anticataractogenic Effect of an Extract of the Oyster Mushroom, *Pleurotus ostreatus*, in an Experimental Animal Model

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ABSTRACT

Purpose: To evaluate the efficacy of *Pleurotus ostreatus* extract in preventing selenite-induced cataractogenesis. *Methods*: *In vitro*, enucleated rat lenses, divided into one control and three experimental groups (selenite only, simultaneous selenite and extract, initial extract and subsequent selenite), underwent morphological and biochemical evaluation. The anti-cataractogenic effect was also evaluated *in vivo*. *Results*: *In vitro*, simultaneous incubation of extract with selenite-challenged lenses caused a decrease in lens opacification by maintaining antioxidant components at near normal levels. *In vivo*, *P.ostreatus* (300 mg/kg body weight) prevented cataract in 75% of rats. *Conclusion*: Extract of *P. ostreatus* prevents experimental selenite-induced cataractogenesis.

Keywords: antioxidant enzymes; cataractogenesis; oxidative stress; *Pleurotus ostreatus*; selenite cataract

INTRODUCTION

Cataract, an opacification of the crystalline lens in the eye, is a major ocular problem, and the current estimate of 20 million people blinded by cataract is expected to double by the year 2020.[1] Cataract can be remedied surgically by extirpation of the cataractous lens.[2] However, the cost of cataract surgery may place it beyond the reach of less affluent individuals; moreover, post-surgical infection may impair recovery of effective vision. Thus, the development of a pharmacological solution to prevent or delay the onset of cataract will be of great benefit.

Cataract is the end result of the interaction of multiple factors that include oxidative stress, glycation, transamidation, phosphorylation,[3] and proteolysis of cytoskeletal protein due to calpain elevation.[4] Oxidative stress, which occurs due to the formation of free radicals in the cell as a consequence of both enzymatic and non-enzymatic reactions, has been established as a major initiating factor involved in the development of most types of cataract.[5] Further, the incidence of cataract has been reported to increase with age and with a concomitant decrease in the activity of antioxidant enzymes.[6] The antioxidant defense system is composed of antioxidant enzymes and biological antioxidants; the former include the major enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and the latter include reduced glutathione (GSH), vitamin C, and vitamin E.[7] The occurrence of decreased antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and subsequent development of cataract.[8]

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Several important experimental studies have highlighted the potential role of antioxidants in the prevention of cataract; the antioxidants evaluated include butylated hydroxytoluene (BHT),[9] vitamin C,[10] diethyldithiocarbamate,[11] pantethine,[12] lycopene,[13] α-ketoglutarate,[14] resveratrol,[15] acetyl-L-carnitine (ALCAR),[16,17] melatonin,[18] and ellagic acid.[19] In addition, naturally occurring antioxidant compounds, such as an aqueous extract of black tea,[20,21] an extract of *Gingko biloba*,[22] *Ocimum sanctum*,[2] procyanidin-rich extract of grape seeds,[23] flavonoids from *Emilia sonchifolia*,[24] and an aqueous extract of soya bean[25] have been found to prevent the progression of cataract formation in experimental animal models.

Mushrooms have long constituted an integral part of the normal human diet. Mushrooms are also reported to be highly potent, safe, and bioavailable free radical scavengers that possess a broad spectrum of pharmacological and medicinal properties against oxidative stress and offer excellent protection against free radicals.[26–28] An extract of the oyster mushroom *Pleurotus ostreatus* has been found to possess potent antioxidant activity by scavenging free radicals and by inhibiting lipid peroxidation in vital organs of aged rats.[29] In addition, an extract of *P. ostreatus* has also been found to act as a hepatoprotective agent in CCl_4-induced oxidative stress in Wistar rats.[30] Encouraged by these results, the present study was undertaken to evaluate whether a *P. ostreatus* extract, a potential source of antioxidants, could prevent selenite-induced cataractogenesis in an experimental rat model under both *in vitro* and *in vivo* conditions. In addition, the effects of this extract on the status of antioxidant enzymes and on malondialdehyde (MDA), a marker of lipid peroxidation, were observed.

MATERIALS AND METHODS

Preparation of an Ethanolic Extract of the Mushroom

The mushroom *P. ostreatus* was cultivated adopting the "layer spawning" method. Freshly harvested whole mushrooms were dried in the shade and then finely powdered. Five grams of the powder were extracted with 100 ml of 95% ethanol using a Soxhlet apparatus. The residue was filtered and concentrated to a dry mass by vacuum distillation; the filtrate thus obtained was used as the mushroom extract (5.86%).

Animal Experiment

In Vitro Phase of the Study

The animals in the current study were treated in accordance with institutional guidelines and with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Research. Wistar rats (75–90 g in weight) were anesthetized with diethylether and then killed by cervical dislocation. Their lenses were removed at once, immersed in Dulbecco's Modified Eagle Medium (DMEM, HiMedia, Mumbai, India), which contained sodium bicarbonate (0.2% w/v) but did not contain calcium; the medium also contained streptomycin (60 μg/ml) and penicillin (60 μg/ml) to prevent microbial contamination. After 2 hr, the lenses that developed artifactual opacities were discarded and only transparent undamaged lenses were taken[2]; these were divided into four groups:

Group I ($n = 8$) comprising normal control lenses incubated in DMEM alone;
Group II ($n = 8$) comprising lenses incubated in DMEM containing 100 μM sodium selenite (Sigma Chemical Co., St. Louis, MO, USA);
Group III ($n = 8$) comprising lenses incubated in DMEM containing 100 μM sodium selenite and 250 μg/ml of the *P. ostreatus* extract (co-treated lenses);
Group IV ($n = 8$) comprising lenses first incubated in DMEM containing 250 μg/ml of the *P. ostreatus* extract for 2 hr and then in fresh DMEM containing 100 μM sodium selenite (pre-treated lenses).

The lenses were incubated for 24 hr at 37°C in cell culture test plates maintained in an incubator with an atmosphere of 95% air and 5% CO_2. All lenses were subjected to gross morphological examination at 24 hr after the start of the experiment. In addition, after the 24-hr incubation period, quantitative analyses of enzyme activities and determination of lipid peroxidation were done for the lenses of all groups.

In order to exclude the possibility that the simultaneous addition of the extract and selenite in the DMEM medium affected the concentration of selenite, a time course of selenite concentration in the presence of extract without lenses was performed at different time intervals (0 hr, 6 hr, 12 hr, 24 hr) using Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES) (PERKIN ELMER OPTIMA 2000DV).

Morphological Examination of Lenses

This was performed by gross examination of lenses under the dissecting microscope against a background of black grid lines. The degree of opacification was graded as follows:

0: when there was absence of opacification (grid lines clearly visible);
+: when there was a slight degree of opacification (minimal clouding of grid lines, with grid lines still visible);

++: when there was presence of diffuse opacification involving almost the entire lens (moderate clouding of grid lines, with grid lines faintly visible);
+++: when there was presence of extensive thick opacification involving almost the entire lens (total clouding of grid lines, with grid lines not seen at all).

Preparation of Lens Supernatant

Prior to biochemical analysis, the lenses from each group were washed with normal physiological saline, weighed, and processed. Each lens was homogenized in 10 times its mass of 50 mM phosphate buffer (pH 7.2) and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant obtained was used for analysis of enzyme activity (see below). To calculate the specific enzyme activity, protein in each sample was estimated by the method of Bradford,[31] using bovine serum albumin as a standard.

Quantitative Analysis of Enzyme Activities

Catalase (CAT). CAT activity was determined by the method of Sinha.[32] In this method, the dichromic acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate. In the test, the green color developed was read at 590 nm against a suitable blank on a spectrophotometer. The activity of catalase was expressed as units/mg protein (one unit was the amount of enzyme that utilized 1 mmole of H_2O_2/min).

Superoxide Dismutase (SOD). SOD activity was determined according to the method of Marklund and Marklund.[33] In this test, the degree of inhibition of pyrogallol auto-oxidation by supernatant of the lens homogenate was measured. The change in absorbance was read at 470 nm against blank every minute for 3 min on a spectrophotometer. The enzyme activity was expressed as units/mg protein.

Glutathione Peroxidase (GPx). The activity of GPx was determined essentially as described by Rotruck et al.[34] The principle of this method is that the rate of glutathione oxidation by H_2O_2, as catalyzed by the GPx present in the supernatant, is determined; the color that develops is read against a reagent blank at 412 nm on a spectrophotometer. In the test, the enzyme activity was expressed as units/mg protein (one unit was the amount of enzyme that converted 1 μmole of reduced glutathione to the oxidized form of glutathione (GSSH) in the presence of H_2O_2/min).

Glutathione Reductase (GR). The activity of GR was determined by the method of Stall et al.[35] GR utilizes nicotinamide adenine dinucleotide phosphate (NADPH) to convert oxidized glutathione to the reduced form; the change in absorbance was read at 340 nm for 2 min, at intervals of 30 sec, in a UV spectrophotometer. The activity of glutathione reductase was expressed as nmoles of NADPH oxidized/min/mg protein.

Determination of Reduced Glutathione (GSH)

The GSH content was estimated by the method of Moron et al.[36] Each lens was homogenized in 1 ml of 0.1 M phosphate buffer and was centrifuged at 5000 rpm for 15 min at 4°C. To the supernatant of the lens homogenate, 0.5 ml of 10% trichloroacetic acid was added and re-centrifuged. The protein-free supernatant thus obtained was reacted with 4 ml of 0.3 M Na_2HPO_4 (pH 8.0) and 0.5 ml of 0.04% (w/v) 5,5-dithiobis-2-nitrobenzoic acid. The intensity of the resulting yellow color was read spectrophotometrically at 412 nm. A parallel standard was also maintained. The results were expressed in μmoles/g wet weight.

Determination of Lipid Peroxidation

The extent of lipid peroxidation was determined by the method of Ohkawa et al.,[37] the principle of this method being that MDA, an end product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a pink chromogen. For this assay, lens material was homogenized in 1.0 ml of 0.15 M potassium chloride. In a reaction tube, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.81% thiobarbituric acid aqueous solution were added in succession. To this reaction mixture, 0.2 ml of the lens homogenate was added and then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol:pyridine (15:1 v/v) solution were added and then centrifuged at 5000 rpm for 15 min. The upper organic layer was aspirated, and the intensity of the resulting pink color was read at 532 nm using tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nmoles of MDA formed/mg protein.

Preliminary *In Vivo* Phase of the Study

Nine-day-old Wistar rat pups were used in this phase of the study. Pups of the same litter were housed with parents in large spacious cages, and the parents were given food and water *ad libitum*. These pups were randomly assigned to one control group and four experimental groups, each comprising eight pups. In group I (normal), saline was injected intraperitoneally and,

in group II (cataract-untreated), a single subcutaneous injection of sodium selenite (19 μmol/kg body weight) was given on postpartum day 10. Group III pups (cataract-treated, with the extract of *P. ostreatus*) were further subdivided into three groups, IIIa, IIIb, and IIIc, which were injected intraperitoneally with the *P. ostreatus* extract in concentrations of 100, 200, and 300 mg/kg body weight, respectively. The first dose of the extract was administered one day prior to the selenite challenge and was repeated once daily for six consecutive days thereafter. The development of cataract was assessed by slit lamp biomicroscopy on the 30th day after birth. Mydriasis was achieved by using a topical ophthalmic solution containing tropicamide with phenylephrine (Maxdil Plus; Hi-Care Pharma, Chennai, India). One drop of the solution was instilled every 30 min for 2 hr into each eye of the rats, which were kept in a dark room. The eyes were viewed under a slit lamp biomicroscope using a magnification of 12×; the observer did not know the identity of the animals before scoring the cataracts.

Statistical Analysis

The values are expressed as the mean ± SD for 8 lenses in each group. Differences between groups were assessed by a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software package for Windows (version 11.5). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test, and the chi-square test was applied wherever relevant. Values corresponding to $p < 0.001$, 0.01, and 0.05 have been denoted by distinct symbols in the tables.

RESULTS

In Vitro Phase of the Study

Effect on Morphology of Lenses

None of the 8 lenses incubated in DMEM alone (group I) exhibited opacification (Grade 0 opacification) even after 24 hr incubation. All (100%) lenses in group II (incubated in DMEM and selenite) exhibited total (Grade +++) opacification after 24 hr incubation. In contrast, only 2 of 8 lenses (25%) in group III (incubated in DMEM + selenite + mushroom extract added simultaneously) exhibited Grade + opacification after 24 hr incubation, while the remaining (75%) lenses did not show any opacification (Grade 0) (Table 1, Fig. 1). However, in group IV lenses, a moderate degree of opacification (Grade ++) was noted in 4 of 8 lenses (50%), while no opacification (Grade 0) was noted in the remaining (50%) lenses. The difference was statistically significant when group III was compared with group II ($\chi 2\ [df = 1] = 9.6$; $p < 0.01$) and group IV was compared with group II ($\chi 2\ [df = 1] = 5.2$; $p < 0.05$).

Table 1. *In vitro* study: Morphological examination of cultured rat lenses

Groups	No. of lenses	Degree of opacification	Changes observed in lenses
Group I	8	0	All 8
Group II	8	+++	All 8
Group III	8	+	2 of 8
Group IV	8	++	4 of 8

Group I: Normal; Group II: Selenite; Group III: Co-treated; Group IV: Pre-treated.

The concentration of selenite in DMEM was found to be 15.8 mg/l. The concentration of selenite in the presence of extract in DMEM was found to be 15.79 mg/l at 0 hr, 15.77 mg/l at 6 hr, 15.74 mg/l at 12 hr, and

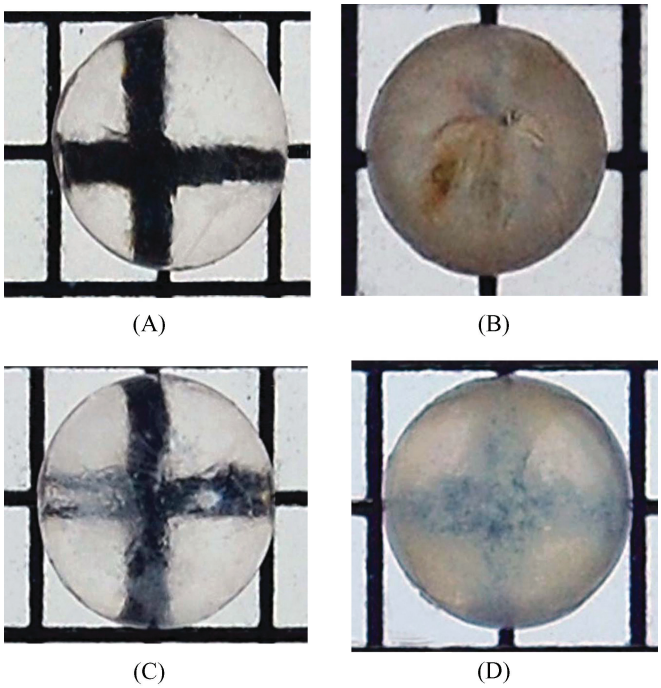

Figure 1. (A) Normal; (B) Lens exposed to selenite only; (C) Lenses simultaneously exposed to selenite and to the *P. ostreatus* extract; (D) Lenses first exposed to the *P. ostreatus* extract only (2 hr) and then to selenite only.

15.71 mg/l at 24 hr, indicating that the concentration of the selenite did not undergo any alteration in the incubation medium.

Quantitative Analysis of Enzyme Activities

CAT

The mean activity of CAT in group II lenses was significantly lower ($p < 0.001$) than that in group I and group III lenses and was also significantly lower ($p < 0.01$) than that in group IV lenses. The mean CAT activity in group IV lenses was significantly lower ($p < 0.001$) than that in group III lenses. However, a significantly lower mean activity of CAT was noted in group III ($p < 0.01$) and group IV ($p < 0.001$) lenses than in group I lenses (Table 2).

SOD

A significantly lower ($p < 0.001$) mean activity of SOD was observed in group II lenses when compared to group I and group III lenses and was also significantly lower ($p < 0.05$) than that of group IV lenses, while the mean SOD activity in group IV lenses was significantly ($p < 0.01$) lower than that in group III lenses. However, a significantly lower ($p < 0.01$ and $p < 0.001$, respectively) mean activity of SOD was observed in group III and group IV lenses compared to that in group I lenses (Table 2).

GPx

The mean activity of GPx enzyme in group II lenses was significantly lower ($p < 0.001$) than that in lenses of group I and the values in group III and group IV lenses. A significantly lower ($p < 0.01$) mean activity of GPx was observed in group IV than that in group III lenses. The mean GPx activity in group III and in group IV lenses was significantly lower ($p < 0.001$) than that in group I lenses (Table 2).

GR

The mean activity of GR was significantly lower ($p < 0.001$) in group II lenses than in groups I, III, and IV. However, the mean activity of GR was significantly lower ($p < 0.05$) in group IV than in group III lenses. The mean activity of GR in group III and in group IV lenses was significantly lower ($p < 0.001$) than that in group I lenses (Table 3).

GSH

A significantly lower ($p < 0.001$) mean level of GSH was observed in group II lenses than in group I lenses; similarly, the mean GSH level was significantly lower ($p < 0.01$) in group II than in group III and group IV lenses. However, no significant differences were observed between the mean GSH levels in group IV and group III lenses, while significantly lower ($p < 0.01$) mean levels of GSH were observed in group III and group IV lenses when compared with group I lenses (Table 3).

MDA

The mean concentration of MDA in group II lenses was significantly greater ($p < 0.001$) than that in group

Table 2. Activity of antioxidant enzymes in cultured lenses

Enzymes (unit of activity)	Group I (normal)	Group II (selenite)	Group III (co-treated)	Group IV (pre-treated)
Catalase (μmol H_2O_2 consumed/min/mg protein)	8.21 ± 0.35	$4.41 \pm 0.20^{a,b}$	7.06 ± 0.49^{c}	$5.69 \pm 1.08^{d,e}$
Superoxide dismutase (unit/min/mg protein)	2.44 ± 0.17	$1.21 \pm 0.26^{a,f}$	1.97 ± 0.18^{c}	$1.51 \pm 0.27^{g,e}$
Glutathione peroxidase (μmol glutathione oxidized/min/mg protein)	33.96 ± 1.14	$23.65 \pm 0.87^{a,h}$	28.94 ± 0.53^{e}	$27.36 \pm 0.82^{g,e}$

All values are expressed as mean ± SD of six determinations.
Group I: Normal; Group II: Lenses exposed to selenite only; Group III: Lenses simultaneously exposed to selenite treated and *P. ostreatus* extract; Group IV: Lenses first exposed to *P. ostreatus* extract only (2 hr) and then to selenite only.
[a]Statistically significant difference ($p < 0.001$) when compared with Group I and Group III values.
[b]Statistically significant difference ($p < 0.01$) when compared with Group IV value.
[c]Statistically significant difference ($p < 0.01$) when compared with Group I value.
[d]Statistically significant difference ($p < 0.001$) when compared with Group III value.
[e]Statistically significant difference ($p < 0.001$) when compared with Group I value.
[f]Statistically significant difference ($p < 0.05$) when compared with Group IV value.
[g]Statistically significant difference ($p < 0.01$) when compared with Group III value.
[h]Statistically significant difference ($p < 0.001$) when compared with Group IV value.

Table 3. Components of redox system in cultured lenses

Component analyzed (unit of activity)	Group I (normal)	Group II (cataract-untreated)	Group III (co-treated)	Group IV (pre-treated)
Glutathione reductase (nmoles of NADPH oxidized/min/mg protein)	0.29 ± 0.01	0.21 ± 0.01^{a}	0.27 ± 0.003^{b}	$0.25 \pm 0.01^{b,c}$
Reduced glutathione (μmol/gm tissue)	8.23 ± 0.64	$6.11 \pm 0.64^{b,d}$	7.64 ± 0.30^{e}	$7.07 \pm 1.21^{e,NS}$

All values are expressed as mean ± SD of six determinations.
Group I: Normal; Group II: Lenses exposed to selenite only; Group III: Lenses simultaneously exposed to selenite treated and *P. ostreatus* extract; Group IV: Lenses first exposed to *P. ostreatus* extract only (2 hr) and then to selenite only.
[a]Statistically significant difference ($p < 0.001$) when compared with Groups I, III, and IV values.
[b]Statistically significant difference ($p < 0.001$) when compared with Group I value.
[c]Statistically significant difference ($p < 0.05$) when compared with Group III value.
[d]Statistically significant difference ($p < 0.01$) when compared with Groups III and IV values.
[e]Statistically significant difference ($p < 0.01$) when compared with Group I value.
[NS]Value not significantly different from Group III value.

I lenses and was also significantly greater ($p < 0.001$) than that in group III and group IV lenses. However, the mean MDA concentration in group IV lenses was significantly higher ($p < 0.01$) than in group III lenses and also significantly higher ($p < 0.001$) than in group I lenses (Table 4).

In Vivo Phase of the Study

Subcutaneous injection of sodium selenite led to the development of dense opacification of the lens (Grade +++ opacity) in all (100%) 8 animals in group II (received only selenite) (Table 5, Fig. 2B). In contrast, only 2 of 8 (25%) rat pups in group IIIc (received selenite + 300 mg/kg body weight of the mushroom extract) (Table 5, Fig. 2E) and 4 of 8 (50%) pups in group IIIb (received selenite + 200 mg/kg body weight of the mushroom extract) exhibited slight to moderate lenticular opacification (Grades + or ++) (Table 5, Fig. 2D). However, 6 of 8 (75%) group IIIa rat pups (received selenite + 100 mg/kg body weight of the mushroom extract) revealed moderate to thick lenticular opacification (Grades ++ or +++) (Table 5, Fig. 2C). This difference was statistically significant when group IIIa was compared with group II ($\chi 2\ [df = 1] = 4$; $p < 0.01$), group IIIb was compared with group II ($\chi 2\ [df = 1] = 5.2$; $p < 0.05$) and group IIIc was compared with group II ($\chi 2\ [df = 1] = 9.6$; $p < 0.01$).

DISCUSSION

Although cataract is the most prevalent disorder leading to visual impairment, the prospect of pharmacological intervention to inhibit or to delay the onset of cataract is still at the experimental stage. Several factors are involved in cataractogenesis, but the exact mechanism of cataract formation is still not very clear. Various experimental models have been developed to delineate the mechanism of cataractogenesis and to focus on possible therapeutic targets. Selenite-induced

Table 4. Quantitative analysis of malondialdehyde in cultured lens

Lipid peroxidation (unit of activity)	Group I (normal)	Group II (selenite)	Group III (co-treated)	Group IV (pre-treated)
Malondialdehyde (nmol/gm wet weight)	62.42 ± 1.61	81.41 ± 2.37^{a}	68.27 ± 2.04^{b}	72.74 ± 2.81^{c}

All values are expressed as mean ± SD of six determinations.
Group I: Normal; Group II: Lenses exposed to selenite only; Group III: Lenses simultaneously exposed to selenite treated and *P. ostreatus* extract; Group IV: Lenses first exposed to *P. ostreatus* extract only (2 hr) and then to selenite only.
[a]Statistically significant difference ($p < 0.001$) when compared with Groups I, III, and IV values.
[b]Statistically significant difference ($p < 0.001$) when compared with Group I value.
[c]Statistically significant difference ($p < 0.01$) when compared with Group III value.

Table 5. Morphological examination of lenses of rat pups

Experimental groups	Number of pups	Number of pups with different degree of opacification			
		0	+	++	+++
Group I (normal)	8	8	–	–	–
Group II (selenite)	8	–	–	–	8
Group IIIa (100 mg/kg b.w.)	8	2	–	4	2
Group IIIb (200 mg/kg b.w.)	8	4	2	2	–
Group IIIc (300 mg/kg b.w.)	8	6	2	–	–

Group I rat pups received only saline; Group II rat pups received only selenite; Group IIIa rat pups received selenite and an extract of *P. ostreatus* (100 mg/kg b.w.); Group IIIb rat pups received selenite and an extract of *P. ostreatus* (200 mg/kg b.w.); Group IIIc rat pups received selenite and an extract of *P. ostreatus* (300 mg/kg b.w.).
The degree of opacification was graded as follows: 0—normal transparent lens; +—initial sign of nuclear opacity involving tiny scatters; ++—partial nuclear opacity; +++—mature nuclear opacity.

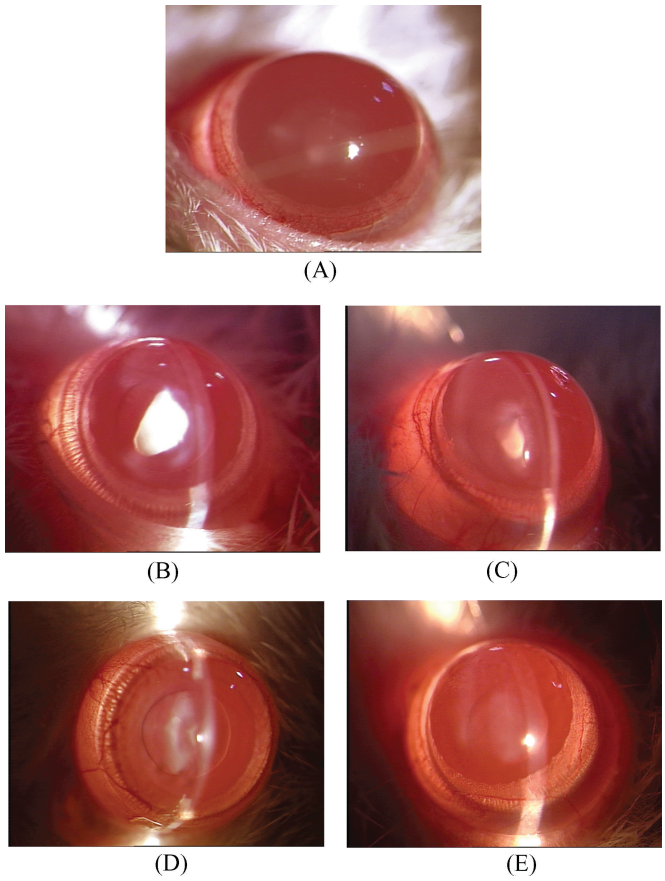

Figure 2. (A) Normal clear lens observed on postpartum day 30, seen in all Group I rats that had received a single intraperitoneal injection of saline. (B) Nuclear opacity observed on postpartum day 30, Seen in 100% of Group II rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10. (C) Dense nuclear opacity in entire lens observed on postpartum day 30, seen in 100% of Group IIIa rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of 100 mg/kg body weight of the *P. ostreatus* extract on postpartum days 9–15. (D) Slight nuclear opacity observed on postpartum day 30, seen in 50% of Group IIIb rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of 200 mg/kg body weight of the *P. ostreatus* extract on postpartum days 9–15. (E) A slight degree of opacity observed on postpartum day 30, seen in 25% of Group IIIc rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of 300 mg/kg body weight of the *P. ostreatus* extract on postpartum days 9–15.

cataractogenesis in young rats has been shown to be an extremely rapid and convenient model for evaluation since it mimics some biochemical and morphological changes in senile cataract.[38]

Various biochemical changes associated with selenite-induced cataract have been reported. Hightower and McCready[39] reported that incubation of rabbit lens epithelial homogenate with 0.1 mM selenite caused a 56% loss of protein sulfhydryl groups. The oxidative damage to sulfhydryl groups is likely to be accompanied by lipid peroxides[13]and by breakage of DNA bonds.[40] Further studies showed that the transport of cations and activity of Na^+ K^+-ATPase progressively declined in the lens membrane following exposure to selenite.[41]Furthermore, the inactivation of calcium pumping in selenite cataractogenesis may lead to influx of calcium.[3] In rodent lenses, the influx of calcium is followed by activation of the calcium-dependent proteases calpain II and Lp82, which partially degrade α and β crystallins, ultimately causing insolubilization of protein and scattering of light.[3]In addition, elevation of lenticular calcium levels and parallel calpain proteolysis of fodrin, the cytoskeletal protein in the lens, have been shown to contribute to the development of cortical opacities.[4]

It has also been suggested that the changes in lenticular calcium level are enough to independently induce aggregation of proteins.[38] This is accompanied by a decrease in the activities of lenticular antioxidant enzymes such as CAT, SOD, GPx, and GR and in the level of GSH in the lens.[3]Various pharmacological agents possessing antioxidant properties have been shown to protect against selenite-induced cataractogenesis in experimental animal models.[16,17,19,42] The oyster mushroom *P. ostreatus* contains many biologically active flavonoids. A recent report indicated a comparatively

high content of chrysin (40 mg/100 g) and rutin (31.2 mg/100 g) in an extract of *P. ostreatus* while the other constituents detected included β-carotene (3.1 mg/100 g), the antioxidant vitamins ascorbic acid (25 mg/100 g) and α-tocopherol (30.3 mg/100 g), and total phenol (54.9 mg/g).[43] *P. ostreatus* has been reported to possess higher concentrations of cysteine, methionine, and aspartic acid than those in other edible mushrooms.[44] In addition, an extract of *P. ostreatus* has been shown to have hepatoprotective activity in CCl_4-induced oxidative stress in rats[30] and to improve the antioxidant status of various organs during aging.[29]

In the present *in vitro* evaluation of the anticataractogenic potential of the extract of *P. ostreatus*, the results of the gross morphological examination of the lenses appear to suggest that the mushroom extract is able to significantly retard selenite-induced cataractogenesis. The efficacy of *P. ostreatus* was best seen when lenses were incubated in DMEM and simultaneously exposed to selenite and the extract of *P. ostreatus*. However, when lenses were pretreated with the extract of *P. ostreatus* for 2 hr and then subsequently exposed to selenite for 24 hr, only diffuse opacification of the lenses was observed. This result suggests that the action of the *P. ostreatus* extract is not merely obtained by blocking the uptake of selenite from the test medium.

CAT, SOD, and GPx are important components of the innate enzymatic defenses of the lens. CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2.[45] SOD catalyzes the removal of superoxide radicals ($O_2^{\cdot-}$),which would otherwise damage the membrane and biological structures.[46] The enzyme GPx, first demonstrated in the lens by Pirie,[47] has been reported to maintain the integrity of the phospholipid bilayer of membranes by inhibiting lipid peroxidation. Thus, CAT and GPx catalyze the transformation of H_2O_2 within the cell to harmless by-products, thereby curtailing the quantity of cellular destruction inflicted by products of lipid peroxidation. A reduction in the activities of these enzymes in tissues has been associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes.[45,48,49] In the present investigation, the mean activities of CAT, SOD, and GPx were found to be significantly lower in selenite-treated (Group II) lenses than the mean activities in normal (Group I) lenses; such a reduction in the activities of these enzymes in selenite-induced cataractogenesis has been well documented in *in vitro* [2,15,20,24] and in *in vivo* [16,18,23] experimental models. However, in lenses treated with the *P. ostreatus* extract, such a decline in activities of antioxidant enzymes was prevented. This observation that treatment of lenses exposed to selenite with an antioxidant helps to maintain antioxidant enzymes at near normal activities is consistent with findings reported in the literature.[2,15,16,18,20,23,24]

Glutathione, a major non-protein thiol, coordinates the innate antioxidant defense mechanisms. It is involved in the maintenance of the normal structure and functions of cells, probably by its redox and detoxification reactions.[50] The intracellular level of GSH is maintained by GR by preserving the integrity of cell membranes and by stabilizing the sulfhydryl groups of proteins.[51] Reduced GSH has been implicated to play a significant role in the maintenance of the reduced state in the lens. Ganea and Harding[52] reported that the effect of oxidative stress on the lens is characterized by a diminishing level of GSH and by reduced activities of antioxidant enzymes. It has also been shown that GSH is decreased in early diabetic cataract.[53]In addition, GSH has been found to decrease concomitantly with selenite-induced cataractogenesis.[17,19]

The present investigation has yielded similar results. Moreover, when the lenses were incubated with the mushroom extract in addition to selenite (Group III and Group IV lenses), the mean GSH level was found to be significantly higher than that in the selenite-exposed (Group II) lenses. A high concentration of GSH has been found to protect the lens from oxidative damage and toxic chemicals.[39]

Lipid peroxidation, a highly destructive process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids. The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation,[54] which causes changes in biochemical processes and structural integrity and leads to cellular damage.[55] MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage.[37] In the present investigation, such a disruption of membrane lipids possibly accounted for the observed increase in MDA levels in the lenses exposed to selenite alone (Group II), when compared to normal lenses (Group I). The observed reduction in the MDA level in the *P. ostreatus* extract-treated groups (Groups III and IV) suggests that the extract possibly preserved the structural integrity of the lens, thereby preventing the opacification of the lens. These data corroborate the findings of earlier investigations.[16,17,19,56] Having established by *in vitro* studies that the *P. ostreatus* extract could retard selenite-induced cataractogenesis, we sought to determine whether a similar effect could be obtained in an *in vivo* condition. Gross morphological examination of the lenses in 30-day-old Wistar rat pups appeared to suggest that *P. ostreatus* is able to significantly retard selenite-induced cataractogenesis in these animals. The

degree of lens opacification appeared to decrease with increasing concentrations of *P. ostreatus* extract administered. We observed that the *P. ostreatus* extract, in a dose of 300 mg/kg b.w., was able to prevent selenite-induced cataractogenesis in 75% of rats. In previous studies that have demonstrated the ameliorating effect of *Pleurotus* extracts in various pathological states, the doses employed have ranged between 200 mg and 250 mg/kg b.w.[29,30,57] Similarly, relatively high doses (single or multiple) of antioxidant compounds have been required to demonstrate efficacy in the selenite cataract model.[16,17,19,22,58–61] Further studies are required to determine whether, and in what concentration, this mushroom *Pleurotus ostreatus* would exert similar beneficial effects in humans, and such studies would clarify the relevance of our present results.

In conclusion, the present study appears to demonstrate that an ethanolic extract of the oyster mushroom *P. ostreatus* prevents selenite-induced cataractogenesis by reducing the extent of lipid peroxidation, and by enhancing the activities of antioxidant enzymes and functions of the redox system. The ethanolic extract of this mushroom was found to contain a perceptible amount of total phenols in addition to other constituents such as ascorbic acid, α-tocopherol, β-carotene and flavonoid compounds (rutin and chrysin), all of which probably contributed to the observed antioxidant activity. This preliminary study is encouraging, but further studies are required to determine whether a similar anti-cataractogenic effect can be demonstrated in humans.

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