

ANTICATARACT ACTIVITY OF ERDOSTEINE AGAINST EXPERIMENTAL CATARACTOGENESIS: AN *IN VITRO* & *IN VIVO* STUDY

CHITRA V., MANIMARAN U., SHRINIVAS SHARMA*, DEEPTHI T.

*Department of Pharmacology, Sri Ramaswamy Memorial College of
Pharmacy, Sri Ramaswamy Memorial University, Kattankulathur -
603203, Tamil Nadu, India*

**corresponding author: shrinivas.sharma29@gmail.com*

Abstract

Several studies have suggested that antioxidants retard the process of cataractogenesis by scavenging free oxygen radicals. The present study assessed the efficacy of erdosteine in preventing experimental cataractogenesis in an experimental setting. In the *in vitro* phase of the study, goat lenses were used and lenses were subjected for photographic evaluation and estimation of the biochemical parameters. In the *in vivo* phase of the study, nine days old Wistar rat pups were used; on the 16th *postpartum* day cataract assessment was performed by slit lamp biomicroscope. In the *in vitro* phase of the study erdosteine significantly restored the lens reduced glutathione (GSH), Ca^{2+} , catalase (CAT), superoxide dismutase (SOD), total protein and water soluble protein levels. Conversely, erdosteine showed a significant decrease in the malondialdehyde (MDA) content. In the *in vivo* study, erdosteine treatment delayed the development of selenite-induced cataract in rat pups. Erdosteine protects against experimental cataract development due to its antioxidant properties.

Rezumat

Lucrarea prezintă un studiu de farmacologie experimentală privind evaluarea eficacității erdosteinei în prevenirea și terapia cataractogenezei. Au fost evaluate proprietățile antioxidante ale erdosteinei prin determinarea activității catalazei, a superoxid dismutazei și a concentrației de malondialdehidă la nivelul cristalinului din ochiul de capră.

Keywords: erdosteine, catalase, cataract, antioxidant, reduced glutathione, lenses

Introduction

Cataract is a disease causing clouding of the eye lens that reduces the amount of incoming light and resulting in deteriorating vision. Cataract is often described as being similar to looking through a waterfall or waxed paper. Daily functions such as reading or driving a car may become difficult or impossible. Cataract is one of the most ancient diseases known being an age-related disorder. It is for this reason that there is no medical solution or cure for this affliction. Many of the approaches that have been taken to

mitigate the problem have thus been revolving around preventive measures, and towards delaying its onset or progression. Considerable research has been done on the complex aspects of this disease. Important leads have come from studies on the epidemiological, nutritional, photochemical and genetic aspects of cataract. In addition, the role of behavioral habits such as smoking, alcohol intake, or drug addiction has also been investigated. During the past fifteen years, a number of cross sectional surveys have provided data on the prevalence of cataract, which indicate that cataract is by far the most common cause of blindness and visual disability worldwide. Cataract can include cases of family/genetic origin, or secondary to trauma, systemic diseases, drugs and age-related factors. Senile or age-related cataract is responsible for more than 80% of the total number of cataract cases. The proportion of cataract patients with diabetes has been found to range from 8.7 to 21%, which shows a high risk of cataract associated with diabetes [18,20].

Erdosteine was introduced in the market as a mucolytic agent for chronic pulmonary diseases more than 10 years ago. The drug contains two blocked sulfhydryl groups one of which, after hepatic metabolization and opening of the thiolactone ring, becomes available both for the mucolytic and free radical scavenging and also for its antioxidant activity (figure 1). There are several experimental evidences which support the protective effect of erdosteine in acute injuries induced by a variety of pharmacological or noxious agents, mediated by products of oxidative stress [5,10].

In the present study, we aimed to investigate the cellular and molecular mechanisms behind the protective effects of erdosteine against eye lens damage induced by glucose and sodium selenite as potent inducers of cataract. We investigated the morphological and biochemical aspects of erdosteine in experimental induced cataractogenesis in animal models.

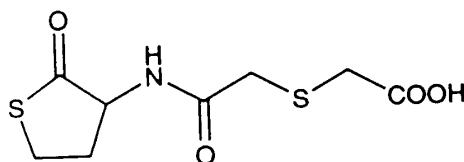


Figure 1

Erdosteine chemical structure

Materials and methods

Erdosteine was kindly provided by Glenmark Pharmaceuticals Ltd. (Nasik, Maharashtra, India.); the approximate purity is 99.5%. Bovine Serum Albumin, antibiotic-antimycotic solution and sodium selenite were

purchased from Sigma Chemical Company, St. Louis, MO. 24 wells Falcon plastic culture plate was acquired from Genei, Bangalore India. All other analytical grade chemicals and solvents were from SRL laboratories, Mumbai, India.

***In vitro* phase of the study**

Lens culture: Thirty fresh goat eyeballs were obtained from slaughterhouse immediately after slaughter and transported to the laboratory at 4°C and used within 24 hours. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humour (NaCl 140 mM, KCl 5 mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH(PO₄)₂ 0.5mM, CaCl₂ 0.4 mM and glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55 mM was used to induce cataract [7].

Grouping of lens: A total of 30 lenses were divided into the following categories, each group consisted of 10 lenses.

Group I: Normal group (Glucose 5.5 mM)

Group II: Control group (Glucose 55 mM)

Group III: Test group (Erdosteine 20 µg /mL) + (Glucose 55 mM)

Photographic evaluation: Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of lines clearly visible through the lens) was observed through the lens as a measure of lens opacity [7].

Biochemical analysis: After 72 h of incubation, the homogenate of half of the lenses in each group was prepared for the estimation of the forward described parameters.

The protein content of the samples was determined by the method of Lowry *et al.* [8], using bovine serum albumin as the standard. For total protein estimation the lens homogenate was prepared in 5% trichloroacetic acid. The precipitated protein was dissolved in sodium hydroxide and used as aliquots for the estimation of total proteins. A soluble fraction of the protein was estimated by preparing the homogenate in double distilled water. The water-soluble supernatant was used for estimation of soluble proteins.

The extent of lipid peroxidation was determined by the method of Ohkawa *et al.* [13]. Tetramethoxypropane was used as an external standard. The level of lipid peroxide was expressed as nmoles of malondialdehyde (MDA) formed/g wet weight for lenses.

The reduced glutathione (GSH) content was estimated by the method of Moron *et al* [11]. Half of the lenses from each group were weighed and

homogenized in 1 mL of 5% trichloroacetic acid (TCA), and a clear supernatant was obtained by centrifugation at 5000 rpm for 15 min. Reduced GSH was used as a standard. The dry weight of the lens was measured after heating at 100°C for 20 h. The lenses were then digested with 0.2 mL concentrated HCl at room temperature overnight and adjusted to 1 mL with deionised water. The mixtures were centrifuged at $10,000 \times g$ for 10 min to remove the insoluble material, if any [14]. Ca^{2+} concentration was estimated by a Ca^{2+} measurement kit (Agappe Diagnostics Ltd.).

Catalase (CAT) activity was determined by the method of Sinha [17]. The activity of CAT was expressed as units/mg protein (one unit is the amount of enzyme that used 1 mmol of H_2O_2 /min).

Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund [9]. The enzyme activity was expressed as units/mg protein.

In vivo study

Nine days old rat pups (Wistar strain) were used in this phase of the study. Rats used for the study were obtained from the animal house stock of the Department of Pharmacology, SRM College of Pharmacy, Kattankulathur, India and handle in accordance with the guidelines as per the “Institutional Animal Ethical Committee” and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules. The pups were housed with parents in large spacious cages, and the parents were given food and water *ad libitum*. The animal room was well ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period [14]. The rat pups were divided into three groups each containing 10, Group I (normal) rats received an intraperitoneal (i.p.) injection of normal saline on *postpartum* day 10; group II (cataract-untreated) rats received a single subcutaneous (s.c.) injection of sodium selenite (19 μ mol/kg body weight) on *postpartum* day 10; group III (cataract-treated) pups received a single s.c. injection of sodium selenite on *postpartum* day 10 and intraperitoneal injections of erdosteine (10 mg/kg body weight) on *postpartum* day 9, and repeated once daily upto 16th day. On the 16th day, when they opened their eyes, cataract assessment was performed by slit lamp biomicroscope.

Prior to each examination, mydriasis was achieved in each eye, by instilling one drop of a topical ophthalmic solution containing tropicamide with phenylephrine, for every 30 min while keeping the rats in a dark room for 2 h. The eyes were observed under a slit-lamp biomicroscope at 10 $\times$ magnification; the observer did not know the identity of the animals before the examination. At the final examination, only presence or absence of cataract was documented.

Results and discussion

In vitro study

Effect on morphology

After 72 h of incubation in glucose 55 mM, lens become completely opaque (Group II) as against lenses incubated in glucose 5.5 mM (Group I). Incubation of lenses in the presence of erdosteine 20 $\mu\text{g/mL}$ with glucose 55 mM (Group III), seemed to retard the progression of lens opacification, compared with group II (glucose 55 mM). This is because the gridlines were clearly more visible in group III (Glucose 55 mM + Erdosteine 20 $\mu\text{g/mL}$) than in group II (glucose 55 mM), as shown in figure 2.

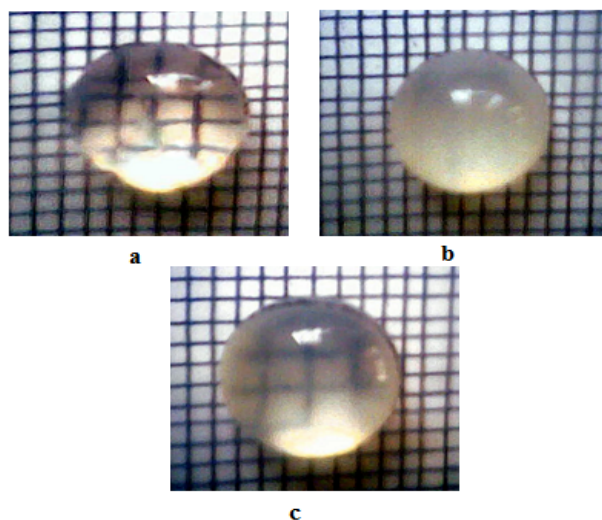


Figure 2

Effect of the treatment on lens morphology

a : Normal group (Glucose 5.5 mM); **b** : Control group (Glucose 55 mM);
c : Test group (Glucose 55 mM) + (Erdosteine 20 $\mu\text{g/mL}$)

Biochemical parameters changes

The mean GSH value in the normal lenses (Group I) was 4.92 ± 0.29 $\mu\text{mol/g}$ of wet lens tissue. A significant decrease in GSH level was observed in the presence of glucose 55mM in the control lenses (Group II) as opposed to the normal group ($P < 0.01$). In the presence of erdosteine, there was a significant restoration of GSH level in the treated lenses (Group III) ($P < 0.01$) as opposed to the control lenses. The mean GSH values in the control and test groups were 2.71 ± 0.21 and 3.69 ± 0.48 $\mu\text{mol/g}$ of wet lens tissue, respectively [Table I]. A significant increase in MDA level was

found in the control (Group II) opposed to the normal lenses (Group I) 1.39 ± 0.27 nmoles/g of wet lens tissue ($P < 0.01$). Erdosteine supplementation significantly protected ($P < 0.01$) the test group lenses (Group III) from lipid peroxidation; the MDA content was 12.5 ± 1.27 nmoles/g of wet lens tissue [Table I]. Control group lenses (Group II) showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate ($P < 0.01$) compared with the normal group lenses (Group I) as shown in Table I. Test group lenses (Group III) had significantly restored the lower concentrations of lens proteins (total and water soluble proteins) ($P < 0.01$), compared with the control lenses in Group II. The calcium content in the lenses of the three groups of lenses is depicted in Table I.

The calcium concentration 1.1 ± 0.05 meq/g was significantly ($P < 0.01$) higher in the control group lenses (Group II) that had received glucose 55mM than in the erdosteine treated lenses (Group III) (glucose 55mM + erdosteine 20 μ g/mL) 0.71 ± 0.1 meq/g and normal group (Group I) (glucose 5.5 mM) lenses 0.52 ± 0.03 meq/g. The mean activity of catalase (CAT) expressed as mmol H₂O₂ consumed/min/mg protein in lenses from the control group (Group II) 0.87 ± 0.30 was significantly ($P < 0.01$) lower than that in normal lenses (Group I) 2.05 ± 0.25 and was also significantly ($P < 0.01$) lower than that in test lenses (Group III) 2.55 ± 0.41 (Table I). The mean activity of SOD expressed as units/min/mg protein in control lenses (Group II) 0.27 ± 0.03 was significantly lower than that in lenses of normal (Group I) 1.39 ± 0.2 lenses ($P < 0.01$) and test lenses (Group III) 1.09 ± 0.31 lenses ($P < 0.01$) (Table I).

Table I
Levels of GSH, MDA, Ca²⁺, total protein and water-soluble protein in the three studied lenses group

Parameter	Group I	Group II	Group III
GSH (μ mol/g)	$4.92 \pm 0.29^*$	2.71 ± 0.21	$3.69 \pm 0.48^*$
MDA (nmol/g)	$1.39 \pm 0.27^*$	32.8 ± 2.91	$12.5 \pm 1.27^*$
Total protein (mg/g)	$189.12 \pm 3.34^*$	112.0 ± 2.71	$168.8 \pm 2.74^*$
Water soluble protein (mg/g)	$96.4 \pm 2.89^*$	59.5 ± 1.94	$78.4 \pm 1.84^*$
Ca ²⁺ content (meq/g)	$0.52 \pm 0.03^*$	1.1 ± 0.05	$0.71 \pm 0.1^*$
CAT (U/min/mg protein)	$2.05 \pm 0.25^*$	0.87 ± 0.30	$2.55 \pm 0.41^*$
SOD (U/min/mg protein)	$1.39 \pm 0.2^*$	0.27 ± 0.03	$1.09 \pm 0.31^*$

All values are expressed as mean $\pm$ standard deviation (SD) of five determinations. One-way ANOVA followed by Dunnett's test was performed. Group I: lenses exposed to glucose 5.5 mM. Group II: lenses exposed to glucose 55 mM. Group III: lenses exposed to glucose 55 mM and erdostein 20 μ g/mL. Statistically significant difference (* $P < 0.01$) when compared with group II values.

In the present study, for the *in vitro* phase of the study to initiate cataractogenic changes, isolated goat lenses were maintained in artificial aqueous humour supplemented with high contents of glucose. Oxidative stress may also be implicated in the cataract induced by glucose, due to the formation of superoxide ($O_2^{\bullet-}$) radicals and H_2O_2 . High glucose (55 mM) concentration has been shown to reduce the activity of antioxidant enzymes, suggesting the presence of oxidative stress in the cells [3]. In this study, GSH levels were significantly lower and MDA levels were significantly higher in the high glucose (55 mM) group, compared with the normal glucose (5.5 mM) group. The GSH levels were significantly high and MDA levels were significantly low in the erdosteine treated (glucose 55 mM + erdosteine 20 μ g/mL) group. The enzymatic activities of SOD and CAT were significantly hampered with glucose (55mM) and positively modulated in the presence of erdosteine. Interestingly, the enzymatic activities of SOD and CAT were higher than normal in the presence of erdosteine. The data clearly demonstrated that erdosteine significantly improves the antioxidant defense mechanisms of the normal lens. Routine consumption of erdosteine may offer a prophylactic measure against the onset and progression of cataract. Significantly raised SOD activity in the presence of erdosteine in the present study is in accordance with previous findings and further confirms its higher oxygen quenching activity as demonstrated by Di Mascio and colleagues [3]. Significantly enhanced CAT activity in the presence of erdosteine offers additional protection not only by the efficient removal of hydrogen peroxide formed *in situ* in the presence of glucose but also by the action of activated SOD.

The *in vivo* study

At the final examination on *postpartum* day 16, the pups were evaluated for cataract development and photographed. None of the group I (control) rats, which had received an intraperitoneal (i.p.) injection of normal saline on *postpartum* day 10, developed cataract lenses. In group II, all (100%) rats, which had received a single subcutaneous (s.c.) injection of sodium selenite (19 mmol/kg body weight) on *postpartum* day 10, developed cataract. However in group III, only 3 of 10 (30%) rats, which had received a single s.c. injection of sodium selenite on *postpartum* day 10

and intraperitoneal injections of erdosteine (10 mg/kg body weight) on *postpartum* days 9 to 16, developed slight peripheral opacification. This difference was statistically significant, when evaluated by Chi-square test, ($\chi^2=10.76$; $df=1$; $P<0.01$).

The selenite cataract is an extremely rapidly induced and convenient model of cataractogenesis. It is a useful *in vivo* rodent model for initial drug testing, which explains why a number of potential anti-cataract agents have been evaluated in the selenite cataract model [15].

Initial osmotic stress followed by membrane damage and a weakened antioxidant defence mechanism may be responsible for some of the early changes in diabetes [12]. In a previous study, it was reported that erdosteine prevents the accumulation of free radicals when their production is accelerated and increases antioxidant cellular protective mechanisms in the brain. Erdosteine effectively protects alpha 1-antitrypsin against the smoke injury and, after alkaline hydrolysis; it appears to be as effective as glutathione. From the current study, it is obvious that erdosteine also protects the lens against oxidative stress [4]. Our results on selenite- and glucose-induced cataracts *in vivo* and *in vitro* not only demonstrate the protective effect of erdosteine, but also indicate that erdosteine prevents cataractogenesis due to its antioxidant properties. Erdosteine, therefore, may be useful for the prophylaxis or therapy of cataract.

Conclusion

In conclusion, we have demonstrated, for the first time, the prevention of selenite-induced and glucose-induced cataractogenesis by erdosteine. This effect is associated with increased GSH, decreased MDA levels, maintaining of protein level, mineral homeostasis and increased CAT and SOD enzyme activities. These biochemical changes reiterate the important role of oxidative stress in selenite and glucose induced cataractogenesis, with erdosteine playing the role of antioxidant. Our preliminary results of the *in vitro* and *in vivo* studies are encouraging for the use of erdosteine as an anticataract agent.

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