Chapter-I

Morphological and biochemical assessment of the ability of ellagic acid to prevent selenite-induced cataractogenesis in an in vivo system

1. INTRODUCTION

Human studies, as well as in vitro and in vivo animal experiments, strongly suggest that there is an association between oxidative stress and the development of cataract (Varma et al., 1995; Spector et al., 1998; Gupta et al., 2003; 2005; Truscott, 2005; Vinson, 2006). Oxidative (free-radical) damage is considered as an initiating or a very early event in the overall sequence leading to cataract (Spector, 1995). Due to the transparency of the cornea, aqueous humor, lens, vitreous and retina and consequent intraocular flooding of light during the long periods of photopic vision, the eye provides a unique situation for an in situ photochemical generation of reactive oxygen species (ROS) (Varma et al., 1979). The lens in vitro is highly susceptible to damage by ROS, as evidenced by loss of transparency and decreased active transport of cations, reduced glutathione (GSH) and adenosine triphosphate (ATP), as well as protein insolubilization and generation of lipid peroxides (Varma et al., 1979, 1995; Varma and Hegde, 2004).

Like other organs, the lens has a well-designed system of defense against oxidation (Augusteyn, 1981). It uses primary defenses to neutralize oxidative species and to repair, recover or degrade molecules that do get damaged. Primary antioxidants include non-enzymatic (e.g., GSH, vitamin C, vitamin E and carotenoids) and enzymatic (e.g., superoxide dismutase, glutathione peroxidase and catalase) systems. With advancing age, antioxidant mechanisms appear to suffer a decline and; hence; need to be supplemented. This has led to the evaluation of different compounds that are
known to have antioxidant properties, for their efficacy in retarding or preventing cataractogenesis. In experimental cataractogenesis induced by exposure to selenite, nutrients such as vitamin C (Devamanoharan et al., 1991), pantethine (Matsushima et al., 1997), aqueous extract of green tea (Thiagarajan et al., 2001; Gupta et al., 2002), aqueous extract of black tea (Thiagarajan et al., 2001), an extract of *Gingko biloba* (Thiagarajan et al., 2002), a procyanidin-rich extract of grape seeds (Yamakoshi et al., 2002), an extract of the oyster mushroom *Pleurotus ostreatus* (Isai et al., 2009), the physiological compound acetyl-L-carnitine (ALCAR), (Geraldine et al., 2006), *Vitex negundo* (Rooban et al., 2009), rutin (Isai et al., 2009) and an extract of *Cineraria maritima* (Anitha et al., 2010) have all been found to prevent the progression of cataract formation. However, other compounds need to be evaluated as dietary supplements to prevent or retard cataractogenesis. Ellagic acid is considered to be one such compound with potent antioxidant properties.

Ellagic acid is a polyphenolic compound found in nature in a wide variety of fruits and nuts, such as raspberries, pomegranate, walnuts, grapes and blackcurrants. Ellagic acid has been found to possess antioxidant (Priyadarsini et al., 2002), antimutagenic (Kaur et al., 1997), and anti-inflammatory (Ihantola-Vormisto et al., 1997) properties, to scavenge both oxygen and hydroxyl radicals, and to inhibit lipid peroxidation and 8-OhdG formation in vitro and in vivo (Cozzi et al., 1995; Takagi et al., 1995; Laranjinha et al., 1996; Iino et al., 2001). In view of the antioxidant properties of ellagic acid, and since oxidative stress has been implicated in cataractogenesis, it is reasoned that ellagic acid might exhibit anticataractogenic potential. To evaluate this hypothesis, the ocular lenses in an in vivo experimental animal model were studied. The morphological assessment of the lens in normal, selenite-challenged, untreated and selenite-challenged, ellagic acid-treated rat pups was made and correlated with lens antioxidant enzyme levels.
2. MATERIALS AND METHODS

The rat pups, grouping of animals and treatment regimen used in this phase of the study were as described in the General Materials and Methods section.

2.1 Morphological assessment of cataract

When the rat pups first opened their eyes (approximately 16 days after birth), a slit-lamp biomicroscopic examination was performed on each eye of the rat to provide a morphological assessment of any lenticular opacification. The occurrence of cataractogenesis in the rat eyes was assessed by slit-lamp biomicroscopy once a week for 3 consecutive weeks after selenite injection. At the final examination (postnatal day 30) any cataracts that had developed were photographed and graded (Hiraoka and Clark, 1995) as follows.

Stage   0 - normal transparent lens.

1 - lens with initial signs of a posterior subcapsular or nuclear opacity involving tiny scatters.

2 - lens with a minimal nuclear opacity with swollen fibers or posterior subcapsular scatterings.

3 - lens with a diffuse nuclear opacity.

4 - lens with a partial nuclear opacity.

5 - lens with a nuclear opacity not involving the lens cortex.

6 - lens with a mature dense opacity involving the entire lens.

Following the final morphological examination, the animals were sacrificed by cervical dislocation; the lenses were at once dissected out for various biochemical studies. A sample of blood was also drawn from each rat just before sacrifice, in order to determine the level of malondialdehyde in the erythrocytes; each blood sample was collected in a heparinized polypropylene tube.
Paired lenses from each individual rat were pooled together and considered as one individual unit when estimating the various values.

2.2 Biochemical analysis of antioxidant enzymes

Lenses from each group of rats were homogenized in 10 times their mass of 50 mM phosphate buffer (pH 7.2), and centrifuged at 14,000xg for 15 min. The supernatant thus obtained (lens homogenate) was used for the analysis of enzyme activities. To calculate the specific enzyme activity, protein in each sample was estimated by the method of Bradford (1976).

2.2.1 Catalase (CAT)

CAT activity was determined by the method of Sinha (1972). In this method, the dichromatic acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H₂O₂), with the formation of perchloric acid as an unstable intermediate. First, an assay mixture containing 0.5 ml of 0.2 M H₂O₂, 1.0 ml of sodium phosphate buffer (0.01 M, pH 7.0) and 0.4 ml distilled water was prepared. Following this, 20 µl of the lens homogenate were added to initiate the reaction. Then, 2.0 ml of dichromate-acetic acid reagent (containing potassium dichromate 1 part and glacial acetic acid 3 parts) were added after 15, 30, 45 and 60 seconds to arrest the reaction. The tubes were then heated for 10 min and allowed to cool; the green color developed was read at 590 nm against blank (containing all the components except the lens homogenate) in a spectrophotometer. The activity of catalase was expressed as units/mg protein (one unit was the amount of enzyme that utilized 1 µmole of H₂O₂/min).

2.2.2 Glutathione peroxidase (Gpx)

The activity of Gpx was determined essentially as described by Rotruck et al. (1973). Briefly, the rate of glutathione oxidation by H₂O₂, as catalysed by the Gpx
present in the supernatant, was determined. An assay mixture containing 0.5 ml sodium phosphate buffer, 0.1 ml 10 mM sodium azide, 0.2 ml of 4 mM GSH and 0.1 ml 2.5 mM H₂O₂ was prepared. To this, 20 µl of the lens homogenate were added, and the total volume was made up to 2.0 ml with distilled water. This was incubated at 37°C for 3 min and the reaction was finally terminated by adding 0.5 ml of 10% trichloroacetic acid (TCA). To determine the residual glutathione content, centrifugation was performed, and the supernatant was obtained; to this, 4.0 ml of disodium hydrogen phosphate (0.3 M) solution and 1.0 ml of dithio-bisnitrobenzoic acid (DTNB) were added. The colour that developed was read against a reagent blank (containing only the phosphate solution and DTNB reagent) at 412 nm on a spectrophotometer. Suitable aliquots of a standard were also treated similarly. The enzyme activity was expressed as units/mg protein (one unit was the amount of enzyme that converted 1 µmole of GSH to the oxidized form of glutathione [GSSH] in the presence of H₂O₂ / min).

2.2.3 Superoxide dismutase (SOD)

SOD activity was determined by the method of Marklund & Marklund (1974). Here, the degree of inhibition of pyrogallol auto-oxidation by supernatant was measured. 0.5 ml of lens homogenate was added to an assay mixture containing 0.25 ml absolute ethanol and 0.15 ml chloroform. After 15 min of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant was used for the assay. The reaction mixture for auto-oxidation consisted of 2 ml Tris-HCl buffer (pH 8.2), 0.5 ml 2 mM pyrogallol and 2 ml distilled water. Initially, the rate of auto-oxidation of pyrogallol was noted every minute for 3 min. This was considered as 100% auto-oxidation. The assay mixture for the enzyme contained 2 ml of Tris-HCl buffer pH 8.2, 1.5 ml of distilled water, 0.5 ml of the supernatant of the lens homogenate and 0.5 ml of 2 mM pyrogallol. This was immediately read at 470 nm against blank (containing all components except the lens homogenate and pyrogallol) every minute for 3 minutes on a spectrophotometer. The enzyme activity was expressed as units/mg protein.
2.3 Estimation of malondialdehyde (MDA) content in erythrocytes and lenses

Each blood sample collected was centrifuged (3,000xg, 4°C, 5 min) to allow separation of the erythrocytes from the other blood cells. These erythrocytes were washed twice with equal volumes of 0.9% sodium chloride solution and then hemolyzed with twofold volumes of cold distilled water. A supernatant was obtained after centrifugation at 5,000xg for 30 minutes; the levels of MDA in the supernatant were then measured.

The extent of lipid peroxidation was determined by the method of Ohkawa et al. (1979). Briefly, to 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 tissue sample (hemolysate or lens homogenate) was added. The mixture was 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. The mixture was then centrifuged at 5,000xg for 15 minutes. The upper organic layer was separated, and the intensity of the resulting pink colour was read at 532 nm. Tetramethoxypropane was used as an external standard. The level of lipid peroxide was expressed as nmoles of MDA formed/g wet weight for lenses and nmoles of MDA formed/g Hb for erythrocytes.

2.4 Statistical analysis

The mean value of each parameter in each individual group of rats was calculated from at least five individual values and was expressed as mean±SD. Statistical analysis was done by using the Student’s t-test and chi-square test. P values < 0.05 were considered significant.
3. RESULTS

3.1. Morphological assessment of changes in lenses

At the final examination on postpartum day 30, 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 cataractous lenses (Fig. 1.1). In Group II, all (100%) rats, which had received a single subcutaneous (s.c.) injection of sodium selenite (19 µmol/kg body weight) on postpartum day 10, developed cataracts that were graded as being between Stage 4 and Stage 6 (Figs. 1.2-1.4). However, in Group III, only 7 of 15 (47%) rats, which had received a single s.c. injection of sodium selenite on postpartum day 10 and intraperitoneal injections of ellagic acid (200 mg/kg body weight) on postpartum days 9-14, developed cataracts that were graded as being between stage 1 and stage 3 (Figs. 1.5-1.7) (Table 1.1); This difference was statistically significant ($\chi^2 \ [df = 1] = 10.9; P < 0.01$). The remaining 8 of 15 (53%) rats of Group III had clear, normal lenses.

3.2. Effect of ellagic acid on antioxidant enzymes

3.2.1 Catalase

The mean activity of CAT in lenses of Group II (selenite-challenged, untreated) rats (Table 1.2) was significantly ($P < 0.001$) lower than that in lenses of Group I (normal) rats and was also significantly ($P < 0.001$) lower than that in Group III rat (selenite-challenged, ellagic acid-treated) lenses. However, the mean catalase activity in the lenses of Group III rats was also significantly ($P < 0.05$) lower than that in Group I rat lenses (Table 1.2).

3.2.2 Glutathione peroxidase

The mean activity of Gpx enzyme was significantly less ($P < 0.001$) in Group II (selenite-challenged, untreated) rat lenses than that in Group I (normal) and Group III
Table 1.1. Morphological assessment of cataract formation in Wistar rat pups

<table>
<thead>
<tr>
<th>Groups</th>
<th>S-6</th>
<th>S-5</th>
<th>S-4</th>
<th>S-3</th>
<th>S-2</th>
<th>S-1</th>
<th>S-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=15)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>---</td>
<td>15</td>
</tr>
<tr>
<td>Group II (n=15)</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Group III (n=15)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

Group I : normal rat pups  
Group II : selenite-challenged, untreated group (rat pups exposed to selenite only)  
Group III : selenite-challenged, ellagic acid-treated group (rat pups exposed to selenite and treated with ellagic acid)  
S - stage of cataract  
n = pairs of eyes
**Fig. 1.1.** Stage 0 cataract: Normal clear lens observed on postpartum day 30. Seen in all Group I rats that had received a single intraperitoneal injection of saline; also seen in 53% of Group III pups that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of ellagic acid on postpartum days 9 to 14.

**Fig. 1.2.** Stage 4 cataract: Lens with a partial nuclear opacity observed on postpartum day 30. Seen in 33% of Group II rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10.
Fig. 1.3. Stage 5 cataract: Nuclear opacity observed on postpartum day 30. Seen in 40% of Group II rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10.

Fig. 1.4. Stage 6 cataract: Opacity in entire lens observed on postpartum day 30. Seen in 27% of Group II rats that had received a single subcutaneous injection of sodium selenite on postpartum day 10.

Fig. 1.5. Stage 1 cataract: Initial sign of subcapsular or nuclear opacity involving tiny scatters observed on postpartum day 30. Seen in 20% of Group III rat pups that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of ellagic acid on postpartum days 9 to 14.
**Fig. 1.6.** Stage 2 cataract: Slight nuclear opacity observed on postpartum day 30. Seen in 20% of Group III pups that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of ellagic acid on postpartum days 9 to 14.

**Fig. 1.7.** Stage 3 cataract: Lens with a diffuse nuclear opacity observed on postpartum day 30. Seen in 7% of Group III rat pups that had received a single subcutaneous injection of sodium selenite on postpartum day 10 and intraperitoneal injections of ellagic acid on postpartum days 9 to 14.
(selenite-challenged, ellagic acid-treated) rat lenses (Table 1.2). The mean activity of Gpx in lenses of Group III rats was significantly less ($P < 0.001$) than that in lenses of Group I rats (Table 1.2).

### 3.2.3 Superoxide dismutase

The mean activity of SOD in Group II (selenite-challenged, untreated) rat lenses was significantly lower than that in lenses of Group I (normal) rats ($P < 0.01$) and Group III (selenite-challenged, ellagic acid-treated) rats ($P < 0.05$) (Table 1.2). However, no significant differences were observed between the mean SOD activities in Group III and Group I rat lenses (Table 1.2).

### 3.3. Effect on MDA levels in erythrocytes and lenses

The mean concentrations of MDA in erythrocytes and lenses of Group II (selenite-challenged, untreated) rats were significantly ($P < 0.001$) higher than the concentrations in Group I and Group III rats (Table 1.3). However, there was also a significant ($P < 0.001$) difference in mean concentrations of MDA in erythrocytes and lenses between Group III and Group I rats (Table 1.3).

### 4. DISCUSSION

Although cataract is the most prevalent condition leading to visual impairment, there is currently no routine pharmacological treatment for this condition. Ideally, a medicament for cataract should prevent or delay cataractogenesis and should also be able to cause regression of established cataractous changes. A key impediment to the formulation of an ideal anti-cataract medication is, perhaps, the continued imperfect understanding of the mechanisms leading to cataractogenesis.

Various experimental models have been developed to delineate the mechanism of cataractogenesis and to identify crucial targets in the process. Selenite-induced
Table 1.2. Activities of antioxidant enzymes in lenses removed from Wistar rat pups

<table>
<thead>
<tr>
<th>Enzymes studied</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (mmol H$_2$O$_2$ consumed/min/mg protein)</td>
<td>7.40 ± 0.22</td>
<td>3.86 ± 0.17$^{a}$</td>
<td>6.28 ± 0.56$^{b}$</td>
</tr>
<tr>
<td>Glutathione peroxidase (µmol glutathione oxidized/min/mg protein)</td>
<td>34.17 ± 2.01</td>
<td>27.89 ± 1.16$^{a}$</td>
<td>29.79 ± 0.79$^{c}$</td>
</tr>
<tr>
<td>Superoxide dismutase (units/min/mg protein)</td>
<td>2.29 ± 0.26</td>
<td>1.0 ± 0.11$^{d,e}$</td>
<td>1.71 ± 0.11$^{NS}$</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SD of five determinations

$^{a}$ Statistically significant difference ($P < 0.001$) when compared with group I and group III values

$^{b}$ Statistically significant difference ($P < 0.05$) when compared with group I value

$^{c}$ Statistically significant difference ($P < 0.001$) when compared with group I value

$^{d}$ Statistically significant difference ($P < 0.01$) when compared with group I value

$^{e}$ Statistically significant difference ($P < 0.05$) when compared with group III value

$^{NS}$ Value not significantly different from group I value

Group I: normal rat pups
Group II: selenite-challenged, untreated group (rat pups exposed to selenite only)
Group III: selenite-challenged, ellagic acid-treated group (rat pups exposed to selenite and treated with ellagic acid)
Table 1.3. Mean levels of malondialdehyde in erythrocytes and lenses of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA in erythrocytes* (nmoles/g Hb)</th>
<th>MDA in lenses* (nmoles/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>285.4 ± 71</td>
<td>60.13 ± 3</td>
</tr>
<tr>
<td>Group II</td>
<td>384.2 ± 46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.84 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>334.4 ± 75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.24 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SD of five determinations

<sup>a</sup> Statistically significant difference ($P < 0.001$) when compared with both group I and group III values

<sup>b</sup> Statistically significant difference ($P < 0.001$) when compared with group I value

MDA - Malondialdehyde

Group I: normal rat pups
Group II: selenite-challenged, untreated group (rat pups exposed to selenite only)
Group III: selenite-challenged, ellagic acid-treated group (rat pups exposed to selenite and treated with ellagic acid)
Cataract is reported to be the most reliable and reproducible animal model of cataractogenesis, compared with radiation, galactose, streptozotocin, and Royal College of Surgeons (RCS) models, especially for advanced cataract evaluation (Clark et al., 1996). Selenite-induced cataractogenesis in young rats has been shown to mimic human senile cataract with respect to several morphological and biochemical changes in the lens (Shearer et al., 1997). Although various biochemical changes associated with selenite-induced cataract have been reported, the mode of action of selenite is not completely understood. It is hypothesized that early changes in the lenticular epithelium may result from oxidative damage caused by selenite, possibly to critical sulfhydryl groups on molecules such as Ca$^{2+}$-ATPase or ion channels, leading to calcium accumulation. In rodent lenses, the influx of calcium then causes 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. This is accompanied by a decrease in the activity of antioxidant enzymes, such as SOD, Gpx, CAT, glutathione-s-transferase (GST), and glutathione reductase (GR) (Shearer et al., 1997). Therefore, there is an increase of free radical species in the aqueous humor and a significant reduction in nicotinamide adenine dinucleotide phosphate (NADPH; reduced form) and GSH content in lens (Bhuyan et al., 1981). Various synthetic compounds and natural resources that are rich in antioxidants have been reported to prevent cataractogenesis in animal models (Tamada et al., 2001; Gupta et al., 2003, 2005; Doganay et al., 2006; Geraldine et al., 2006).

Ellagic acid, a naturally-occurring polyphenolic compound, is endowed with antimutagenic, anti-inflammatory, antiviral, anticarcinogenic, and antioxidant activities. Previous studies have indicated that ellagic acid possesses scavenging action against both oxygen and hydroxyl radicals, and inhibits lipid peroxidation and 8-OhdG formation in vitro and in vivo (Cozzi et al., 1995; Takagi et al., 1995; Laranjinha et al., 1996; Iino et al., 2001). Although ellagic acid exhibits minimum solubility in water, it
is highly soluble in organic solvents, such as methanol and dimethyl sulphoxide (DMSO). This lipophilic property, along with its ability to scavenge peroxyl radicals, suggests that ellagic acid is a lipophilic, chain-breaking antioxidant.

In the present study, the mean activities of CAT, SOD and Gpx were found to be significantly lower in lenses of Group II (selenite-challenged, untreated) rats than in those of Group I (normal) rats (Table 1.2). Other workers have made similar observations in cataractous lenses (Bhuyan and Bhuyan, 1977; Varma et al., 1982; 1984; Fecondo and Augusteyn, 1983; Dwivedi and Pratap, 1987; Cekic et al., 1999; Gupta et al., 2002). However, it was observed that the mean activities of CAT and Gpx in Group III lenses (treated with ellagic acid in selenite-challenged pups) were significantly higher than the values in Group II lenses (although still lower than the values in Group I lenses) (Table 1.2). Majid et al. (1991) observed an increase in the level of Gpx and GR in liver and lungs of ellagic acid-treated mice; similarly, Gpx was also found to be increased in regions of the brain (Hassoun et al., 2004). The present results suggest that ellagic acid can partially prevent the reduction in lenticular antioxidant enzyme activities wrought by exposure of rat pups to selenite; this protective effect was manifested morphologically as a decreased frequency and intensity of lenticular opacification (Table 1.1, Figs. 1.5-1.7). The higher mean activities of the antioxidant enzymes seen in Group III rat lenses (selenite-challenged, ellagic acid-treated) compared to Group II (selenite-challenged, untreated) rat lenses highlight the putative anti-radical and antioxidant effects of ellagic acid (Priyadarsini et al., 2002).

Selenite is known to cause significant damage to membranes, which is indicated by increased levels of MDA (Gupta et al., 2002). In the present study, ellagic acid was found to prevent cataract formation in the eyes of 53% of Group III (selenite-challenged, ellagic acid-treated) rats; in the eyes (of the 47% of rats) in which
cataractous changes occurred, the degree of opacification was less intense than that seen in Group II rats (Table 1.1). Lenses in Group III rats had lower mean MDA concentrations than did Group II rats. These data suggest that the putative anticitractogenic effect of ellagic acid, as manifested in the gross morphological study, may also have occurred due to decrease in the MDA concentration.

From a study of structure-function relationships, it has been suggested that both phenolic hydroxy groups and lactones are necessary for the activity of ellagic acid under different conditions (Barch et al., 1996); these mechanisms are likely to have been operative in retarding the initial selenite-induced oxidative damage to lenticular membranes in the present study. Phospholipids in the lenticular membrane are known to be affected by oxidative stress, with a consequent increase in lipid peroxidation, which is manifested by a rise in the level of MDA (Varma et al., 1979; 1995; Varma and Hegde, 2004). The efficacy of ellagic acid in inhibiting lipid peroxidation has been well-documented in rat liver microsomes (Priyadarsini et al., 2002), N-nitrosodietylamine (NDEA)-induced lung tumorigenesis (Khanduja et al., 1999) and in hyperlipidemic rabbit serum (Yu et al., 2005). This property of inhibiting lipid peroxidation possibly accounted for the observed decline in MDA levels in the present study in selenite-challenged rats that received ellagic acid.

In the present study, ellagic acid may have prevented or retarded oxidative damage to sulfhydryl groups in the lenticular epithelium (the initial event in selenite cataractogenesis) by direct radical scavenging activity (Priyadarsini et al., 2002). The scavenging action of ellagic acid on both oxygen and hydroxyl radicals, and inhibition of lipid peroxidation and 8-OhdG formation in vitro and in vivo, has already been referred to (Cozzi et al., 1995; Takagi et al., 1995; Laranjinha et al., 1996; Iino et al., 2001). The protective effects of ellagic acid can thus be attributed to several factors.
including binding of DNA, inhibition of the production of ROS, scavenging of ROS, and protection of DNA from alkylating injury.

5. CONCLUSION

The results of the present in vivo study suggest that ellagic acid provides significant protection against lenticular nuclear opacification; this protective effect was manifested morphologically by decreased frequency and intensity of lenticular opacification and biochemically by maintaining the antioxidant defense system at near normal activity and by inhibition of lipid peroxidation in rat lenses. These results highlight the antioxidant effect of ellagic acid and suggest its anticataractogenic potential in an in vivo model of selenite-induced oxidative insult.