Summary and Conclusion

The ocular lens needs to be maintained in a state of complete transparency so that light rays from the exterior can be transmitted and focused onto the retina. For this to occur, the lens must be maintained in a reduced state. In order to maintain the lenticular tissues in a reduced state, a healthy lens is endowed with various antioxidants and oxidation defense systems. However, in the aging lens, protection and repair mechanisms against oxidative stress slowly deteriorate or become ineffective so that the lens is no longer able to counteract the effects of H$_2$O$_2$ or other oxidative agents. If such conditions persist over a long period, cataract formation occurs.

Age-related cataract is the leading cause of preventable visual loss worldwide, with developing nations shouldering most of this burden. Currently, surgical removal is generally accepted as the only treatment for human cataract. However, the cost of surgery, post-operative complications and use of expensive operating facilities and microsurgical equipment may render cataract surgery inaccessible to less affluent individuals. Hence, there has been considerable interest in finding an alternative source, that is, a non-surgical approach, for the management of cataract.

Various natural and synthetic compounds of differing chemical structures have been reported to prevent selenite-induced cataract in vitro as well as in vivo. However, there is a need to evaluate additional compounds for their anticataractogenic effects. Ellagic acid is a polyphenolic compound found in nature in a wide variety of fruits and nuts. Ellagic acid has been found to possess antioxidant, antimutagenic and anti-inflammatory properties, to scavenge both oxygen and hydroxyl radicals, and to inhibit lipid peroxidation and 8-OhdG formation in vitro and in vivo. Since oxidative stress is considered to be the stimulus leading to cataract formation, and since ellagic acid is a compound with proven antioxidant properties, it is hypothesized that ellagic acid could
possibly prevent the process of cataractogenesis. Selenite-induced cataract is reported to be the most reliable and reproducible animal model of cataractogenesis, especially for advanced cataract evaluation. Hence this model was chosen for this study. To test the validity of this hypothesis, a multipronged approach was adopted in the present thesis.

Nine day-old rat pups (Wistar strain) were used for the various experiments in this study. For each set of experiments, the rat pups were divided into three groups, each group consisting of 15 pups. The groups were designated as I, II and III:

1) Group I, received only saline (normal rats);
2) Group II, received selenite alone (selenite-challenged, untreated rats);
3) Group III, received selenite and ellagic acid (selenite-challenged, ellagic acid-treated rats).

In the first phase of the study, the putative preventive effect of ellagic acid on selenite-induced cataractogenesis was assessed by morphological and biochemical investigations. In the morphological investigations, none of the Group I (normal) rat pups developed cataractous lenses, all (100%) Group II (selenite-challenged, untreated) rat pups developed dense cataracts (graded as stages 4-6) while only seven of 15 Group III (selenite-challenged, ellagic acid-treated) rat pups, developed mild cataracts (graded as stages 1-3). In biochemical analysis, the mean activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx) were found to be significantly lower in samples from lenses of Group II (selenite-challenged, untreated) rats than in the lenticular samples from Group I (normal) rats. However, the mean activities of CAT and Gpx in sample from lenses of Group III rats were significantly higher than the mean values in lenticular samples from Group II rats (although still lower than the values in lenticular samples from Group I rats). In addition, the mean level of malondialdehyde (MDA) was found to be significantly higher in the lenticular
samples from Group II rats than that in Group I rat lenses. However, in samples from lenses of ellagic acid-treated (Group III) rats, the mean level of MDA was significantly lower than that in lenticular samples from Group II rats, presumably due to limitation of lipid peroxidation. These observations suggest that ellagic acid can significantly prevent the reduction in lenticular antioxidant enzyme activities that follows exposure of rat pups to selenite; this protective effect is manifested morphologically as a decreased frequency and intensity of lenticular opacification. These results highlight the antioxidant effect of ellagic acid and suggest its anticataractogenic potential in an \textit{in vivo} model of selenite-induced oxidative insult.

In the second phase of the present investigation, an attempt was made to study the putative regulatory effect of ellagic acid on the various components of the redox system. In selenite-challenged, untreated (Group II) rat lenticular samples, the mean concentration of reduced glutathione (GSH) and the mean activities of glutathione S-transferase (GST) and glutathione reductase (GR) were significantly lower when compared to the mean concentrations in samples from lenses of normal (Group I) rats. However, there were no significant differences between the mean concentrations of GSH and the mean activities of GST and GR in samples from lenses of normal (Group I) rats and samples from lenses of selenite challenged, ellagic acid-treated (Group III) rats. It was also observed that the mean activities of \(\gamma\)-glutamyl cysteine synthetase (\(\gamma\)-GCS) and glutaredoxin (Grx) were lower in the samples of lenses from selenite-challenged, untreated rats, when compared to the mean activities in lenticular samples from normal rats. However, in the samples from lenses of selenite-challenged, ellagic acid-treated rats, the mean activities were found to be comparable to the mean activities observed in lenticular samples from normal rats. The mRNA transcript levels of the \(\gamma\)-GCS and Grx1 genes were also found to parallel the activities of the \(\gamma\)-GCS and Grx enzymes. Thus, ellagic acid appears to maintain the level of the principal redox
component, namely GSH, by maintaining GR at normal levels which, in turn, is involved in replenishment of GSH and γ-GCS, involved in the biosynthesis of GSH.

In the third phase of the study, an attempt was made to determine whether ellagic acid could maintain calcium homeostasis and calpain activation in the lenses of Wistar rats. In Group II (selenite-challenged, untreated) rat lenticular samples, the actual mean concentration of calcium was significantly higher than that in the samples from lenses of Group III (selenite-challenged, ellagic acid-treated) rats and that in lenticular samples from Group I (normal) rats. With reference to calpain activity, the mean activity in Group II rat lenticular samples was significantly lower than that in the lenticular samples from Group III and Group I rats probably due to autolysis after its activation. In the lens, the intracellular calcium level is regulated by the activities of plasma membrane calcium ATPase1 (PMCA1) and elevated levels of intracellular calcium have been found to modify the relative expression of cytochrome c oxidase-I (COX-I), the terminal enzyme of the electron transport chain that plays a major role in the process of oxidative dephosphorylation, as well as that of the early growth response protein-1 (EGR-1), an immediate early gene. In samples from lenses of selenite-challenged untreated (Group II) rats, increased levels of mRNA transcripts of the PMCA1 gene and the EGR-1 gene, and lowered levels of mRNA transcripts of the COX-I gene and the m-calpain gene, were noted, when compared to levels in lenticular samples from normal (Group I) rats. Additional immunoblot analysis confirmed the lowered concentration of COX-I protein and rodent lens-specific calpain, Lp82 protein, in samples from selenite-challenged, untreated (Group II) rats. Spearman correlation analysis revealed a significant negative correlation between the intensity of the band corresponding to Lp82 and the lenticular calcium concentration. However, in samples from lenses of selenite-challenged, ellagic acid-treated (Group III) rats, the mean calcium concentration, calpain activity, mRNA transcript levels of PMCA1, EGR-1, COX-I and m-calpain genes, and concentrations of Lp82 and COX-I proteins were very
similar to the values obtained in normal rat lenticular samples. These results suggest that ellagic acid can prevent selenite cataractogenesis in Wistar rats by maintaining lenticular calcium concentrations, and associated lenticular calpain activity, expression of key genes and concentrations of certain proteins involved in the maintenance of calcium homeostasis, at near normal states.

In the fourth phase of the study, an attempt was made to determine the modulatory effect of ellagic acid on selenite-induced apoptosis of lenticular epithelial cells of Wistar rats. The mean levels of caspase-3 gene mRNA transcripts were found to be significantly higher in Group II (selenite-challenged, untreated) rat lenticular samples than those in samples from lenses of Group I (normal) and Group III (selenite-challenged, ellagic acid-treated) rats. Western blot analysis suggested the activation of caspase-3 in lenticular samples from selenite-challenged, untreated rats since cleavage products were found to occur. However, in samples from lenses of selenite-challenged, ellagic acid-treated rats, such a cleavage appear to be prevented, suggesting that there was no activation of caspase-3. The caspase substrates, poly ADP-ribose phosphorylase (PARP) and cytoskeletal proteins such as vimentin and spectrin, were found to be cleaved in lenticular samples from selenite-challenged, untreated (Group II) rats due to the activation of caspase-3. However, administration of ellagic acid to selenite-challenged (Group III) rats prevented such an enzymatic activation of caspase-3, which was evident by the absence of cleaved products in lenticular samples from Group III rats. The transmission electron microscopic (TEM) study also appear to confirm the apoptotic nature of the epithelial cell region in ultrathin sections of lenses from selenite-challenged, untreated (Group II) rats whereas normal architecture appeared to be maintained in section of lenses from selenite-challenged, ellagic acid-treated (Group III) rats. Thus, the results of the present investigation appeared to suggest that ellagic acid prevents selenite-induced apoptosis of lenticular epithelial cells in Wistar rats.
In the final phase of the study, an attempt was made to evaluate whether ellagic acid is able to prevent alterations in the lenticular protein profile in an experimental model of selenite cataract. Two dimensional electrophoresis (2DE) and image analysis of samples from lenses of selenite-challenged, untreated (Group II) rats revealed approximately 45 and 60 prominent spots in soluble and insoluble protein fractions, respectively. Analysis of the pI and molecular weight of protein spots revealed differences in the expression patterns of crystallin proteins in soluble and insoluble fractions. Western blot analysis confirmed changes in the expression of αA- and βB1-crystallins in both soluble and insoluble protein fractions, while mass spectrometry confirmed the degradation of αA-crystallin, in lenticular samples from selenite-challenged, untreated (Group II) rats. Western blot analysis confirmed the occurrence of altered expression of the cytoskeletal proteins studied, namely actin and tubulin, in insoluble fractions. However, the lenticular protein profile in samples from selenite-challenged, ellagic acid-treated (Group III) rats was essentially similar to that noted in samples from lenses of normal (Group I) rats. Ultrastructural studies appeared to confirm the putative role of ellagic acid in preventing structural alterations to the lens during selenite cataractogenesis. The present study confirms the importance of structural and cytoskeletal proteins in the maintenance of lenticular transparency; the results also suggest that ellagic acid prevents the alterations to lenticular proteins that are induced by selenite in an experimental setting.

In conclusion, administration of ellagic acid appeared to prevent selenite-induced cataractogenesis in an experimental animal model. The results of the present investigation suggest that ellagic acid can prevent the reduction in lenticular antioxidant enzyme activities and redox system components wrought by exposure of rat pups to selenite. The present study also demonstrated that ellagic acid modulates calcium homeostasis and the calpain cascade in the lenses of Wistar rats exposed to selenite. In addition, ellagic acid appeared to prevent selenite-induced apoptosis of lenticular
epithelial cells by modulating the activities of caspases. The results of the present investigation also suggested that ellagic acid could prevent selenite-induced alterations to the lenticular protein profile. The ultrastructural studies appeared to confirm that ellagic acid prevented structural alterations, therein maintaining the normal transparency of the lens. This protective effect was manifested morphologically as a decreased frequency and intensity of lenticular opacification. These results provide considerable promise for the development of ellagic acid as a potential anti-cataract drug. Although these experimental studies are of relevance, further study is required to extrapolate the use of ellagic acid to humans for the prevention of age-related cataractogenesis.