Chapter 5

Summary and Conclusion

Cataract is the major cause of blindness and ocular morbidity throughout the world, and any means of delaying or preventing its onset would have enormous social and economic benefits. At present some 1.2 million cataract extractions are performed every year in India. In spite of this huge efforts, evidences from recent prevalence data of WHO suggests that cataract blindness is increasing. The cataract blindness problem in India is too massive to be solved by the surgical programme alone, particularly in view of the ageing population trend, which is expected substantially to increase the new cases of blindness from cataract. Efforts in controlling cataract blindness should be directed at developing strategies to reduce the incidence of blinding cataract.

The mechanism which leads to senile cataractogenesis is of complex nature since it is known as a multifactorial disease. Any step to cure or prevent cataract needs a basic knowledge of the mechanism and chemistry of cataract development in man. Because of the difficulty in obtaining enough human lens samples to do extensive morphological and biochemical studies, various experimental cataract models are being used to elucidate the underlying mechanism of cataract formation. Selenite cataract model in the Wistar rat is an appropriate specimen for the study of cataractogenesis, incorporating many of the characteristics observed in the morphology of human senile cataract.
The present investigation was designed to work out the cataractogenic potency of sodium selenite in Wistar rats, histomorphology of lens after selenite administration, and of different stages after cataract development, as well as the biochemistry of selenite cataractogenesis after various low (chronic), and high (acute) dosages. The anti-cataractogenic potency of vitamin-E was also studied.

Sodium selenite is capable of producing cataract both with high and low dosages. The cataractogenic potency of sodium selenite was found both age- and dosage-dependent. The minimum age for maximum cataract development was found as eighth day post partum for low dosages and 10th day post partum for high dosages. This reveals that the delivery of selenite to the lens is depended on critical developmental events. With multiple low (chronic) dosages at eighth day post partum a cent per cent cataract was not observed. But the high (acute) dosages produced a cent per cent cataract with a single dose for most dosages. The minimum amount required to produce a cent per cent cataract was calculated as above 32 μg. dosage. The appearance of dosage-dependent selenite cataract is the result of increased distribution, retention and metabolism of selenite in the body of the rat.

With high dosages (acute) as the number of doses increases, a high mortality rate was observed. A high selenium level demands an efficient detoxification mechanism involving the reduction and methylation system, which is liable to fail due to decreased antioxidants in the lens during these stages.

A decreased body weight was observed with most experimental groups because of decreased food consumption, and altered body metabolism as a result of selenite interaction.
A 2.5 mg. vitamin-E dosage depending upon the number of dosages produced a 15-91% anticataractogenic effect when studied on 12 days *post partum* rat pup after a 32 μg. selenite administration. This is attributed to the antioxidant property of vitamin-E. Vitamin-E protects the lens membranes from oxidation; and increases the fluidity of the cell membrane. This results in keeping the membrane integrity intact, and preserving the membrane permeability and ion homeostasis constant, which were found altered during selenite cataractogenesis.

The light microscopic studies revealed that the inducement of opacity was first observed at the adult nuclear area, and slowly progressed to a pinhead opacity enclosing the whole nuclear area. The important morphological changes observed were enlargement of nuclear fibres due to swelling, faulty denucleation of the fibres cells at the bow region and vacuolisation of lens fibres. The cortical involvement of selenite cataract constitutes the extensive vacuolisation and degradation of cortical fibres. Extensive epithelial damage and hyperplasia were also observed.

The SEM studies confirmed the light microscopic observations. Nuclear and cortical damages were represented by loss of surface modifications in the lens fibres and extensive fragmentation of fibres. Capsule shows shrinkage and pore formation during advanced cataract stages.

The present microscopic studies confirmed that formation of selenite cataract follows a typical pattern from nuclear region to cortical area. This occurred by a series of events based on the development of the lens fibres. Thus the damage occurred at the epithelial layer is concentrated to the centre of the lens as the fibre matures, due to inward migration of older fibres. The cortical damage and vacuolisation are due to extensive hydration because of
osmotic imbalance created by the damaged peripheral layer of lens. This explains the cortical involvement of mature and hyper mature selenite cataractogenesis. The loss of surface structures and cortical fibre degeneration are due to the denaturation of the membrane and the structural proteins because of selenite action.

Biochemical studies were done to understand the involvement of various lens constituents in cataract formation. In general with all the experimental groups, the total protein showed a decline during advanced cataract stages. The soluble protein starts declining from the early stages with an increase in the insoluble fraction. All the changes observed were found dosage-dependent. The aqueous humour and blood samples showed a slight decline in total protein mainly during advanced stages of cataract formation. One of the main causes of selenite cataractogenesis is the oxidation of lenticular proteins. The oxidation of protein SH groups by selenite causes its denaturation. This results in conformational change and insolubiliation of soluble proteins. The loss of total protein is due to the inhibition of protein synthesis and increased degradation of lens proteins.

The lens glutathione concentration declined from the very beginning after selenite administration. The decrease in GSH concentration was found dosage-dependent. The high affinity of selenite to SH groups results in the drastic loss of GSH. The oxidation of GSH accelerates the oxidation of protein and the non-protein SH groups. This weakens the defensive mechanism of the lenticular system.

The lens TSH, PSH and NPSH declined progressively from the very beginning which was found dosage-dependent. The high affinity of selenite to
SH groups, and the loss of GSH accounts for the loss of these sulfhydryl groups, during selenite cataractogenesis.

Ascorbic acid level was found to decline progressively during selenite cataractogenesis in lens. A highly significant decrease was observed during hyper mature stage in the lens. Selenite caused alteration in the oxidation reduction cycle of GSH, and the decline in GSH level, account for the loss of ascorbic acid, during cataractogenesis.

A progressive increase in MDA content was noted after selenite administration. This is due to the external entry of lipid peroxides through damaged lens membrane. However by the maturation of cataract an increase in MDA content was observed. It is because of lipid peroxides generated by ROS in the lens due to break-down of the defensive system.

A dosage-dependent decrease in the RNA and DNA content was observed with the maturation of selenite cataract. This denoted a decline in the protein synthesis. The decrease in the nucleic acid content observed is due to the abnormal denucleation of lens fibre cells and extensive cellular damage in the cortical and nuclear fibres.

A slight progressive dosage-dependent increase in glucose and fructose was observed after selenite administration. The increase is due to diffusion from aqueous humour and blood through the blood-aqueous-lens barrier because of the altered membrane function due to selenite interaction with membrane proteins.

A dosage-dependent increase in sodium and calcium and a decrease in magnesium and potassium, was observed after selenite administration with all experimental groups studied. This denotes an impairment of cation pump. Inactivation of Na-K-ATPase and Ca-ATPase alters the membrane
permeability and this explain the alterations in ion homeostasis observed. Inactivation is brought by the oxidation of critical SH group of ATPases. An elevated calcium level leads to activation of proteolytic enzyme calpain and results in the proteolysis of crystallins especially β-crystallin polypeptides and cytoskeletal proteins. A decreased synthesis of ATPase enzymes due to the lack of viable cells in the epithelial layer also causes the impairment of cation pump during selenite cataractogenesis.

An increased hydration was found to accompany selenite cataract as it matures with a decrease in solid water. This is mainly due to syneresis. Additionally, an altered membrane permeability of cataract lenses causes the entry of some free water from outside the lens due to degradation of membranes and inactivation of sodium pump.

A progressive dosage-dependent decline in glutathione reductase and γ-glutamyl cysteine synthetase, was observed after selenite administration. However a sharp decline was observed during advanced cataract stages. The observations revealed that selenite does not attack the enzyme system initially. A decrease in these two enzymes results in the decreased regeneration or synthesis of GSH. This weakens the defensive system of the lens.

A notable decrease in ATPases activity was found with the maturation of selenite cataract. This is due to a decrease in active enzyme sites because of a reduction in the number of viable cells as a result of hyperplasia formation. The ATPase enzyme got highly reactive SH groups. The oxidation of SH groups by selenite also causes the inactivation of ATPase enzymes.

A dosage-dependent increase in the acid phosphatase activity was observed after selenite administration during the initial period. By the hyper mature stage ACP activity declines. Hydrolytic enzymes are postulated to play
an essential role in the break-down of extracellular materials under pathological conditions and tissue repair. Thus the initial increase in ACP activity is associated with tissue hydrolysis and repair after selenite interactions on lens tissue. But the loss of ACP activity during hyper mature stage is due to the extensive tissue damage occurred at the epithelial and cortical layers as the cataract advances.

The glucose-6-phosphatase dehydrogenase activity was found prominently decreased by advanced cataract stages. This results in the decreased production of NADPH, which is the main reducing equivalent needed to regenerate GSH. This confers less protection to the lens SH groups, whose oxidation is the main cause of selenite cataractogenesis. The decline in the G-6-PDH activity is due to the action of selenite up on the SH groups of the enzyme.

Succinic dehydrogenase showed a gradual dosage-dependent decline after selenite administration. A prominent decrease was observed as the cataract matures. This points to the fact that considerable alterations in the citric acid cycle had taken place in the lens after selenite administration. This denoted a considerable loss of energy metabolism. The decline in activity is the result of extensive deterioration of the epithelial and cortical layers of the lens.

The electrophoretic studies revealed in general, a decrease in soluble protein and an increase in SDS soluble (insoluble) protein during selenite cataractogenesis. The formation of insoluble protein aggregates and opacification of the lens after selenite administration, are associated with a decrease in several lens cytoskeletal proteins besides the crystallins as observed by the present study. The study confirmed that the loss of
cytoskeletal proteins and soluble crystallins due to oxidation, caused selenite cataract in Wistar rats.

The administration of vitamin-E prevented the loss of cytoskeletal proteins, degradation of crystallins and insolubilization of proteins as revealed by the electrophoretic studies. This is because of the antioxidative property of vitamin-E. Histomorphological and biochemical studies also revealed that vitamin-E protects the lens from selenite action. The biochemical mechanism behind the protective action of vitamin-E on lens proteins, the various other lens constituents, and the prevention of opacification is due to its antioxidative property.

There are reports from previous studies that vitamin-E or C prevented many forms of experimental cataracts. But it may not be a part of medical dogma yet, that swallowing pills of vitamin-E or C everyday keeps cataract away. However, there seems enough favourable evidence to evoke optimism. In the present study a high dosage of vitamin-E is needed to prevent the selenite cataractogenesis. It must be remembered that when talking of nutrition and the eye that the lens is avascular. Nutrients must go in to it through diffusion. The protective effect of vitamin-E observed in vivo on the lens is strongly limited by its low diffusion in the aqueous, at least in normal conditions of the blood-aqueous barrier.