Chapter 2

REVIEW OF LITERATURE
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Cataract is the leading cause of blindness all over the world. Prevention of cataract or delaying its progress would constitute a major achievement in human welfare. Many investigators studied the chemistry of cataract formation and forwarded many hypothesis. Animals viz, primates, rabbits, guineapigs and rats (Mackic et al., 1994) form the models of the research. The selenium and galactose induced cataract model in Wistar strain of rat is an appropriate specimen for the study of cataractogenesis, incorporating many of the characteristics observed in the morphology of human senile cataract.

Selenium is present in nearly all materials of earth's crust, fundamentally in magnetic (<0.05 ppm) and sedimentary rocks (sandstones and lime stone). Selenium is usually concentrated in sulphur and sulphide minerals (200 ppm) (Allel, 1996). Selenium which ranks about 15th in the order of abundance, was discovered by Berzelius and Gahn in the sediments of a sulphuric acid plant at Gripsholm, Sweden in 1817. It occurs in soils, in different oxidative states, selenium organic complexes, selenides, selenites and selenates. The order of availability is selenates, selenites, se-organic complexes. It has a profound influence on oxidative processes, because it is an effective sulfhydryl binder, particularly of enzymatic and membrane proteins as well as carboxyl group of amines.
Selenium, a component of glutathione peroxidase, is known today as an essential trace element in the mammalian diet (Schingoethe et al., 1982) and their dietary requirement is well established (Hilton et al., 1980, Imai et al., 1997). Equally important are its toxic effects both in experimental animals and in man (Hilton et al., 1980 and Julius et al., 1983).

Selenium toxicity in livestock that consumed selenium accumulator plants can be traced back to Marcopolo (Spallholz, 1994). Experimental chronic selenium toxicity in animals affect the major organs including the liver, spleen, heart and kidney (Turan et al., 1996). More particularly selenium compounds have been shown to cause cellular dysfunction or cytotoxicity in a number of tissues including the blood cells (Kumar et al., 1989 and Zia, 1993) lungs (Bell et al., 1997), hepatocytes (Aunudi et al., 1982) skeletal musculature (Lin-Shiau et al., 1989) skin (Mani, 1994) and ocular tissue (Rosenthal and Adler, 1962).

The cataractogenic potential of selenium was first reported by Algina et al., (1957). Since then cataract was reported in experimental animals associated with selenium deficiency (Sprinkler et al., 1971, Lawrence, 1974 and Langle et al., 1997) and selenium excessive intake as in young suckling rat pups given a single subcutaneous dose of 20 \( \mu \)mol sodium selenite per kg body weight on the 10th day postpartum development (Ostadalova, 1978). Selenium induced cataract was also reported in rabbit...
and guinea pig besides rats, by Bhuyan et al., (1980). Advanced stages of cataract have been produced within three to six weeks by injecting twice a week with selenite. Later the cataractogenic potency was confirmed by many workers, including Bunce et al., (1980), Bunce and Hess (1981), Shearer et al., (1987), Huang et al., (1992) and Hess et al., (1996). Ganther and Corcoran (1969) suggested that selenium in cataractogenesis may be due to the oxidation of lens GSH and formation of proteinaceous disulfide cross links, causing protein aggregation and light scatter.

The current updated working hypothesis for selenium nuclear cataract (Shearer et al., 1992) formation, is that selenium oxidises critical sulfhydryl groups in the lens epithelium. This oxidation leads to impaired calcium homeostasis, elevation of calcium in the nucleus, and activation of a Ca-dependent proteolytic enzyme-calpain. Limited proteolysis of crystalline especially β-crystalline polypeptides leads to abnormal interaction of crystallins, insolubilization of proteins and light scatter. In addition abnormal fibrogenesis also seems to contribute to selenite cortical cataract. Ganther (1975) suggest that selenium is a strong sulfhydryl oxidant and thus the selenium induced cataract may serve as a useful model for studies on the role of oxidant stress in cataractogenesis. Selenium cataract provides a useful model for the study of the invivo effect of elevated lenticular calcium. During
selenium cataractogenesis, a fivefold increase in whole calcium was localized in the lens nucleus (Shearer et al., 1983).

Galactose cataract can consistently be produced in young rats by feeding a diet containing 25-70% galactose (Keiding and Mellemgraad 1972). At higher doses of galactose, the potential of cataract development hastens and inclusion of lower doses in diet requires longer time for opacification of lens (Meydani et al., 1994). He also observed that 20% -30% galactose in the diet induces cataract in rat lens. Galactose feeding can rapidly produce cataract, retinopathy and nephropathy (Cheng et al., 1990). Biochemical studies of diabetic lenses have revealed a variety of metabolic abnormalities including changes in the level of electrolytes, glutathione, nucleotides and sugars. Similarly biochemical changes have also been observed in cataract associated with galactosaemia which suggests that the sugar cataract has a common biochemical aetiology (Kador and Kinoshita 1984). In addition to galactose, diet rich in xylulose with increase in blood sugar can also cause cataract. Xylulose is a more effective cataractogenic agent than galactose (Obazava et al., 1974). Balasubramanian et al., (1993) reviewed the epidemiology, classification, genetics, photochemical etiology and the role of nutrition in cataract. In human infants, galactose cataract is the result of an "in born error of metabolism", the infant being unable to metabolize galactose.
Galactosaemia is caused by the deficiency of anyone of the three possible enzymes involved in the metabolism of galactose viz, galactokinase, transferase and epimerase. Stambolian (1988) suggested that deficiency of any enzyme can cause cataract through the accumulation of galacitol in the lens. Studies by Lelior (1951) and Maxwell (1956) have elucidated the manner whereby galactose is converted to glucose. The steps involved in Lelior pathway are given in figure 2.1.

Figure 2.1. Lelior pathway

Lelior (1951) and Kalcker (1957) suggested that lack of enzyme galactose-1-phosphate uridyl transferase causes the marked increase of
glucose-1-phosphate in erythrocytes. In experimental animals fed with high galactose diet, a similar ten fold accumulation of galactose-1-phosphate was observed in the lens (Schwarz and Goldberg 1955). It achieves significance in the transferase and galactokinase deficient patients where accumulation of galactitol has been demonstrated by Quan-ma et al., (1966).

The second pathway is the polyol pathway which involves the reduction of galactose to galactitol by aldose reductase, an NADPH requiring enzyme possessing broad specificity for aldehyde though it is not a major route in normal individual. Unakar et al., (1979) observed high levels of polyols in cataractous lens of new born and postulated that the accumulation of sugar alcohol, galactitol, induces cataract in the lens through an osmotic mechanisms. Arola et al., (1992) ruled out this possibility in case of senile cataract and demonstrated the presence of lower levels of galactitol in senile cataractous lens.

![Fig: 2.2 The Polyol Pathway](image)
The third pathway (fig 2.3) involves oxidation of galactose to xylulose by NAD⁺ requiring enzyme. The galactonic acid is then oxidized to 3-ketogalactonic acid which is converted to xylulose and further metabolised (Cuatrecases and Segal 1966. Bergren et al., 1972). Conversion of galactose-1-phosphate to galactose-6-phosphate (fig 2.4) by phosphoglucomutase has been demonstrated in red cell of patients with transeferase deficiency. Galactose -6-phosphate can be oxidised by glucose-6-phosphate dehydrogenase and NADP to 6 phosphogalactonic acid as under (Cohn and Segal 1973).

![Fig: 2.3 Oxidation of galactose](image)
The enzyme aldose reductase (AR), an important constituent of polyol (sorbitol) pathway is of particular interest in ophthalmology (Varma and Kinoshita 1974). The pathway was first discovered in sperm (Hers, 1956) later found to be in many tissues including lens (Van Heyningen, 1959). The polyol pathway consist of two enzymes aldose reductase and sorbitol dehydrogenase. Affinity of AR for glucose (Jedziniak et al., 1981) and galactose are low, higher concentration of these sugars are needed for the production of sugar alcohol viz. sorbitol and galactitol. The enzyme possesses greater affinity for galactose which is not further metabolised by sorbitol dehydrogenase which is responsible for the elevation of sugar alcohol (Kinoshita et al., 1962). A series of experimental cataract has been characterized by abnormalities in fiber
permeability which results in vacuoles or clefts in the lens cortex (Harding and Crabbe, 1984). The swelling of lens led Kinoshita (1965) to postulate that high levels of sugar alcohol draws water, rupturing lens fibers and causing vacuolation in lens cortex. The formation of sugar alcohol from either glucose or galactose by the enzyme aldose reductase is the common factor for initiation of both types of cataract i.e galactose and diabetic cataract. Increased level of sugar alcohol have a hyper osmotic effect which leads to swelling of lens fibers, formation of vacuoles and subsequent opacification. Kinoshita et al., (1962) suggested that as the dulcitol (sugar alcohol) accumulates, water is drawn into the lens fibres to maintain the osmotic equilibrium. The resulting increase in volume of water may be sufficient to cause the lens fibers to swell and perhaps rupture their membrane.

Lee et al., (1995), demonstrated the accumulation of polyol in cataract using transgenic mice expressing aldose reductase gene in lens. Shi et al., (1992) and Shi and Bekhor (1994) proved that the transient elevation of aldose reductase mRNA in lenses of rats developing galactose cataract. Peterson et al., (1979) and Ohta et al., (1999) were also confirmed the polyol as the major aetiologic factor in the formation of sugar cataract.

Oxidative damage to lens constituents has been recognised as an integral part of the pathological events in the genesis of many forms of cataract. Glutathione is a ubiquitous thiol containing tripeptide, which
plays a central role in cell biology. A number of workers studied the
importance of glutathione on cataract formation (Srivastava and Beutler,
1968, Dwivedi and Prathap, 1987 and Kamei 1993). The importance of
reduced glutathione (GSH) stem from the findings that the tripeptide is
decreased in most forms of cataracts (Rawal et al., 1978 and Harding et
al., 1996). The lens epithelium is known to have the highest
concentration of GSH (Reddy 1971). The depletion of glutathione in all
types of cataract were due to its decreased biosynthesis and increased
permeability of lens membranes (Srivastava and Beutler, 1968, Ohrloff
et al., 1984 and Ohta et al., 1999). Augsteyn (1979) reported that the
decrease in GSH will result in protein sulphydryl oxidation and alterations
in protein linkages, solubility and transparency.

Bunce and Hess (1981) reported that GSH level was decreased in
lens in selenium cataract. Augsteyn (1979) opined that the GSH involved in
the maintenance of protein sulphydryl groups in lens. Giblin, McCready and
Reddy (1982) studied the role of glutathione metabolism in the detoxification
of H$_2$O$_2$ in rabbit lens. Reim et al. (1974) studied glutathione peroxidase
(GSH-pX) in the lens and observed that glutathione reductase (GR) is an
important enzyme for the maintenance of cellular pool of GSH, which serve
as a reductant and prevent the formation of protein disulphide leading to
molecular aggregation and cataract formation (Balasubrahmanian et al.,
1993). Dwivedi and Prathap (1987) studied glutathione metabolism during cataract formation and observed a significant decrease in the activation of GR, GSH-pX and glucose-6-phosphate dehydrogenase (G-6-PD). Srivastava et al., (1980) opinioned that glutathione provides the lens with a major detoxifying mechanism via the enzyme GSH-pX. Selenium uptake causes cataract but there is no significant difference between the levels of GSH-pX (Ursini and Bindole 1987) in the plasma of normal and cataractous patients (Babicky et al., 1985 and Akesson et al., 1987). Huang et al., (1992) again observed that GsT and GR were significantly reduced but GSH-pX appeared to be unchanged. Selenium cataractous lenses also exhibit a rapid rise in lens insoluble protein and a decrease in reduced Glutathione (Bunce et al., 1981 and David and Shearer 1984). Cherian and Rawal (1991) observed that cataractous lens showed a significant decrease in glutathione and related enzymes in busulphan treated rat lens. Bhat and Gopalan (1974) observed a decreased GR activity in erythrocytes of cataractous patients.

The HMP shunt is active in lens and generates NADPH that maintains glutathione in reduced state (Rao et al., 1983 and Harding and Crabbe 1984). Fuji et al., (1984) observed that GSH dependent protection against lipid peroxidation is mediated by one or more proteins other than GSH-pX and glutathione transferase (GsT). Saneto et al., (1981) reported
that two electrophilic forms of GsT exist in bovine ocular lens. Rao et al., (1983) reported a decreased activity of GR, GSH-pX and GsT in human senile cataractous cases. An increase in GsT activity was found in liver, kidney, brain and blood of Streptozotocin induced diabetic rats (Mukherjee et al., 1994). Bhuyan and Bhuyan (1978) studied the role of superoxide dismutase (SOD) in lens. He suggested that catalase present in the eye protects the lens from inhibitory effects of hydrogen peroxide (H$_2$O$_2$). SOD in turn protects the lens from $\dot{O}_2^-$ (superoxide radical) and it is possible that both SOD and catalase might prevent the formation of O$\dot{H}$ (hydroxyl radical). Catalase content of eye tissue regulates the endogenous H$_2$O$_2$ in eye humors to the physiologic level and speculated that H$_2$O$_2$ might be the triggering factor in cataract (Bhuyan and Bhuyan 1977). SOD and the two metabolizing enzymes catalase and peroxidase may constitute an important defense mechanism against oxygen toxicity in cells (Bhuyan and Bhuyan, 1978).

The loss of GSH would make GSH-pX in operative and potential loss of catalase would allow free and abnormally high levels of H$_2$O$_2$ to react nonspecifically with lenticular proteins that leads to the disintegration of lens cell membranes (Spector and Garner 1981). The lens is highly susceptible to superoxide anions or its derivatives (Bhuyan and Bhuyan, 1978) and humans exposed to hyperbasic oxygen develop cataract (Palmquist et al.,
1984). Kakkar et al., (1995) studied the activity of SOD, catalase GSH-px and found that they protect the cells and tissue against oxidative injury. Ozmen et al., (2002) observed that the level of SOD and catalase are decreased in diabetic cataract.

Many workers provided evidence for the involvement of lipid peroxidation in cataract (Bhuyan et al., 1986; Babizhayev et al., 1988; Ozmen et al., 1997). Lipid as structural components of lens fiber membranes, are intimately associated with insoluble lens proteins (Cotlier 1989). The peroxide damage of the lens fiber membrane may be the initial cause of cataract development (Eabizhayev et al., 1988). Stocks and Dormandy (1971) suggested that autoxidation or non enzymatic oxidation of polyunsaturated fats leading to the formation of malodialdehyde(MDA). Simonelli et al., (1989) suggest that two fold increase in the levels of MDA in diabetic cataract as compared with non-diabetic senile cataractous lenses. Riley and Harding (1993) also noticed that MDA level was increased in human nuclear and cortical cataract. Babizhayev et al., (1994) noticed lipid peroxidation is one of the mechanisms of cataractogenesis which induced by the production of oxygen free radicals in the eye fluids and tissues and impaired enzymatic and non-enzymatic defence of the lens. Mibu et al., (1994) showed that lipid peroxides like linoleic acid peroxides can cause lenticular damage leading to cataractogenesis. Many workers elucidated
involvement of MDA and lipid peroxidation in cataracts (Bhuyan et al., 1986; Costagliola et al., 1988 and Ohta et al., 1999).

Glycoproteins are usually defined as complexes between carbohydrates and proteins in which two components are linked by covalent bond (Dische 1965) and that glycoprotein contains glucose, mannose, fucose, sialic acid and hexosamine.

**Schematic representation of structure of glycoprotein**

![Schematic diagram of glycoprotein structure](image)

Spiro et al., (1968) suggested that the synthesis of these components (sialic acid, fucose, hexosamine, glucose and mannose) involves a series of glycosyl transferase which transfer activated sugars from nucleotides to specific protein acceptors on the membranes of endoplasmic reticulum. In crystalline lens, glycoproteins are found in the capsule (Spiro and Fukushi 1969) of the membrane, insoluble proteins of fibers, epithelial cells and in the inter fibrillar cement substance (Dische 1965). Among the glycoproteins of the isolated lens capsule, Spiro and
Fukushi (1969) found that two carbohydrate-peptide units, one a glucose and galactose disaccharide linked by an O-glycosidic bond to hydroxy-xyline and other a hetero polysaccharide compound of mannose, hexosamine, fucose and sialic acid joined to either serine or threonine.

Sialic acid constitutes an important part of the structure of glycoprotein (Winzler 1958 and Whitehouse and Zillken 1961) which increases in serum of certain disease conditions (Popenoe and Drew 1957 and Winzler 1958). Hadded (1961) demonstrated that an increase of sialic acid concentration occurs in the lens in the course of senile cataract. Morone et al., (1965) demonstrated an increase in the amount of sialic acid in serum from patients affected with diabetes in comparison to normal. Fiore and Daniele (1967) suggested that sialic acid content increases in the lens during senile cataract formation but at the same time there is no change in blood sialic acid between normal and patient with cataract. The concentration of sialic acid have been related to ageing of the eye, particularly that of lens (Hadded 1962). Auricchio and Testa (1959) suggested that total amount of hexosamine increased in the lens of cataractous patients. But Testa and Delogu (1961) was of the opinion that hexosamine content in the serum of normal and cataractous subjects is not influenced by the presence of the senile cataract.
All glycoprotein profiles are elevated in diabetic patients Srinivasan et al., (1970) and Donald et al., (1972) observed that elevated level of serum protein-bound fucose in diabetic patients. Fucose, glucosamine and sialic acid is elevated in fluids from diabetic subjects (Walker and Patrick 1967 and Clamp et al., 1979). The characteristics of lens glycosidase involved in the breakdown of the carbohydrate portion of glycoproteins and glycolipids are reported by Carlin and Cotlier (1971). Fushimi and Tarui (1976) reported that glycosidase activities are increased in serum of diabetic patients. Increased activities of enzymes associated with glycoprotein synthesis in both tissues and blood were reported by Spiro (1973) and Price et al., (1978). Fushimi and Tarui (1976) again reported that deposition of Pas-positive materials and thickening of basement membrane in vascular lesions are characteristic findings in diabetes mellitus and seems to indicate that the metabolism of glycoprotein is altered.

Ooman (1958) found cataract among children suffering from kwashiorkor disease. He postulated a probable biological damage to the lens in early age which later gets affected by protein, malnutrition and UV-light resulting in clinical cataract formation. Singh et al., (1979) opinioned that cataract is the net result of all nutritional insult, acute or chronic, mild or severe, suffered by the patients over months and years. Gupta et al., (1982) suggested that malnutrition played a significant role in cataract formation.
because of decrease in body weight, protein, calorie intake, blood hemoglobin and serum albumin.

In human, current evidences points to the role of sugars (high) proteins (low) tryptophan (oxidative photo product), calcium (hyper and hypo calcemia) and lower intake of antioxidants (riboflavin, vitaminC, vitaminE and beta carotene) in the production of senile cataract (Balasubramanian et al., 1990). It is identified among many other factors that a decrease in the antioxidant level contribute to cataract formation. Vitamins, minerals and micronutrients which will encounter these are important as part of daily diet. The oxidation of lens proteins by free radicals is believed to play an important role in the multifactorial process leading to the lens opacification. The process is mediated by micronutrients, such as α-tocopherol, β-carotene and selenium (Jacques ard Taylor 1991). It has been found that the serum concentration of the antioxidant vitamins namely α- tocopherol and β-carotene are the major risk factors for end stage of senile cataract (Knekt et al., 1992). Varma et al., (1979) showed that the lens lipid peroxidation in vitro induced by ultraviolet light was attenuated by vitamin E (Varma et al., 1995). Increase in vitaminE intake was found to retard the cataract formation in diabetes (Ross et al., 1982 a) or association with genetic factors (Varma et al., 1984). VitaminE is able to prevent glucose induced cataract in cultured lenses (Creighton and Trevithick 1979).
Vitamin E is known to inhibit a number of experimental cataracts in vitro (Creighton and Threwhick 1979 and Ross et al., 1983) and in vivo (Bhuyan et al., 1981 a, Ross et al., 1982 a and Varma et al., 1984). Vitamin E mainly in the form of α tocopherol, is one of the most important and effective biological antioxidants in the body. It is known for its free-radical scavenging ability (McKay and King 1980) and it participates in redox reactions, by protecting glutathione and P-SH groups from oxidations. Moreover, vitamin E maintains the intracellular cation gradients by stabilizing membrane structures, through its ability to increase the fluidity of cell membrane. (Ross et al., 1983). The presence of adequate concentration of vitamin E in the lens, an endogenous antioxidant suggests a protective function (Varma et al., 1979; Libondi et al., 1985 and Schemehl and Lohmann 1989). The possible correlation between oxidative stress and cataract formation, has been discussed by several authors (Dwivedi and Pratap 1987 and Sohal et al., 1990). It is observed that cataract occur as a result of the oxidative damage of the lens epithelial cell (Spector et al., 1985 and Spector 1995) or of damaged lens fibers (Kikugawa et al., 1991).

Bensch et al., (1985) and Tissie et al., (1988) noticed that vitamin C and vitamin E plays a fundamental role in antioxidant protection in the lens. Vitamin C and vitamin E are known to be effective in scavenging free radicals and eliminating pro oxidants (Halliwell 1996). Hankinson et al., (1992)
suggested that the long term use of Vitamin C supplements was linked with decrease in the development of cataract.

Vitamin C significantly prevents light induced damage to ocular lens cation pumps in vitro (Varma et al., 1979). Ascorbate is shown to be an effective scavenger of superoxide, OH radical and H₂O₂, especially at the levels present in the primates aqueous. Recent epidemiological evidences indicate that the higher ascorbate status has diminished the risk of various forms of cataract (Taylor et al., 1991). L-ascorbic acid or vitamin C is a well known metabolite and antioxidant that has been tested because of its action as a free radical scavenger in the prevention of cataract (Devamanoharan et al., 1991). L-ascorbic acid was studied for its protective effect in cornea (Saika et al., 1993). Vitamin C was found to be effective in preventing galactose cataract in guinea pigs (Kosegarten and Maher 1978). Ascorbic acid is capable of both slowing down the progress as well as speeding up the regression of galactose induced cataract in rats (Vinson et al., 1986). Yokoyama et al., (1994) suggested that ascorbate can inhibit development of the early stages of sugar cataract. It is by a pro-oxidant rather than an antioxidant mechanism which involves a preferential consumption of NADPH associated with reduction of DHA and H₂O₂. This finding may help to explain the reported efficacy of ascorbic acid in slowing the formation of cataract in diabetic and galactose fed rats. (Linkcater et al., 1990).
Endogenous oxidative damage to proteins, lipids and DNA is thought to be an important etiologic factor in ageing and the development of chronic diseases such as cancer, atherosclerosis and cataract formation. Vitamin C, vitamin E and β-carotene have been suggested to limit oxidative damage in human thereby lowering the risk of the chronic disease (Mark et al., 1999). Reddey et al., (2001) also mentioned about the protective role of antioxidant micronutrients such as vitamin E and vitamin C.

Animal models have demonstrated that supplements of vitamin C or vitamin E can limit lens damage after oxidative insult (Varma 1991) and experimental studies have provided some evidences that high intake of this vitamins may protect the tissues of human (Rouhiainen 1996 and Lyle et al., 1999). Shang et al., (2003) pointed out that GSH depletion increases the susceptibility of cells to stress induced cell death. In that situation supplemented with vitamin C or vitamin E enhances the resistance of GSH-depleted lens epithelial cells to peroxide induced death.