CHAPTER
2

REVIEW OF LITERATURE
Cataract is the subject of intense research for years. Many investigators studied the chemistry of cataract formation and forwarded many hypothesis. Animals viz., rats, rabbits, primates and guinea pig (Mackic, Ross-Cisneros, McComb, Bekhor, Weiss, Kannan, and Zlokovic, 1994) form the models of research. Galactose cataract can consistently be produced in young rats by feeding a diet containing 25-70% galactose (Lerman, 1962; Keiding & Mellemgrad, 1972). Meydani, Martin, Sastre, Smith, Dallal, Taylor and Blumberg (1994) observed that 20% - 30% galactose in the diet induces cataract in rat lens in a dose and time dependent fashion and also opined that rat is a useful model for studying the sequel of cataractogenesis. At higher dose of galactose, the potential of cataract development hastens and the inclusion of lower dose in the diet requires longer time for opacification of lens (Meydani et al, 1994). The most studied cataract is that which is caused by high concentrations of various sugars (Kinoshita, 1974). Older rats are more resistant to this type of cataract and different strains of rats vary in their susceptibility (Pirie & van Heyningen, 1956; Lerman & Ishida, 1961; Lerman, 1962; Ikebe, Terubayashi, Tsutsumi, Mori, Akagi, Kawasaki and Tanimoto, 1991). There are many causes of cataract viz., physical, mechanical or chemical insult (Crabbe & Harding, 1982). The galactose induced cataract has been extensively employed as a model for understanding the mechanism which mediates the lens opacities (Williams, Chaplain and Meakem, 1985). Experimental diabetic and galactosemic animal models are widely used to study diabetes induced complications. Galactose feeding can rapidly produce cataract, retinopathy and
nephropathy, it is therefore favoured over the diabetic model (Cheng, Xiong, Xiong and Tsubota, 1990). Biochemical studies of diabetic lenses have revealed a variety of metabolic abnormalities including changes in the level of electrolytes, glutathione, nucleotides and sugars. Similar biochemical changes have also been observed in cataract associated with galactosaemia, suggesting that the sugar cataract has a common biochemical aetiology (Kador & Kinoshita, 1984).

Unakar, Smart, Reddan and Devlin (1979) observed that sugar cataract formed during the period of prenatal development appears to be completely reversible. In addition to galactose, diet rich in xylose with increase in blood sugar can also cause cataract. Xylose is a more effective cataractogenic agent than galactose (Obazava, Merola, and Kinoshita, 1974). The development of cataract is slower in diabetic rats in comparison with other two sugars and usually the lens does not become completely opaque (Kinoshita, 1963). Cheng (1989) reviewed the various causative factors of cataract and observed that cataract is induced not only by ageing and sugars but also by sunlight and UV radiation which can act as causative factors.

Balasubramanian, Bansal, Basti, Bhatt, Murthy and Rao (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 “inborn error of metabolism”, the infant being unable to metabolize galactose. Beutler, Matsumoto, Kuhl, Krill, Levy, Sparkes and Degnan (1973) studied the galactokinase and galactose-1-phosphate uridyl transferase from blood samples of persons who developed cataract before the age of 40. They pointed out the necessity of screening for galactokinase deficiency at early stage. Galactosaemia is a disorder caused by the deficiency of any one of the three possible enzymes involved in the metabolism of galactose viz., galactokinase, transferase and
epimerase. Any single deficient enzyme can result in cataract through the accumulation of galactitol in the lens (Stambolian, 1988). Studies by Leloir (1951) and Maxwell (1956) have elucidated the manner whereby galactose is converted to glucose. The steps involved in Leloir pathway are given in figure 2.1.

![Figure 2.1: The Leloir Pathway.](image)

The lack of enzyme galactose-1-phosphate uridyl transferase causes the marked increase of glucose-1-phosphate in erythrocytes, with subsequent developments cause the symptoms characteristic of the disease (Leloir, 1951; Kalckar, 1957). In experimental animals fed with high galactose diet, a similar tenfold accumulation of galactose-1-PO₄ was observed in the lens (Schwarz and Goldberg, 1955).

A second alternate pathway (Fig. 2.2) involves the reduction of galactose to its polyol galactitol by aldose reductase, an NADPH requiring enzyme possessing
broad specificity for aldehydes though it is not a major route in normal individuals. It achieves significance in the transferase and galactokinase deficient patients where accumulation of galactitol has been demonstrated (Quan-Ma, Wells, Wells, Sherman and Egan, 1966). The Km value of this enzyme in the purified lens underscores the fact that this enzyme will not be quantitatively significant until galactose accumulation occurs (Hayman & Kinoshita, 1965). Unakar et al, (1979) observed high levels of polyol in cataractous lens of new born and postulated that the accumulation of sugar alcohol, galactitol, induces cataract in the lens through an osmotic mechanism. Arola, Sillanaukee, Aine, Koivula and Isokoski (1992) ruled out this possibility in case of senile cataract and demonstrated the presence of lower levels of galactitol in senile cataractous lens.

The third route involves oxidation of galactose (Fig. 2.3) to galactonate by NAD⁺ requiring enzyme whose Km for galactose is 26 mM and has been described in rat liver. The galactonoic acid is then oxidised to 3-ketogalactonic acid which is converted to xylulose and further metabolized (Cuatrecases and Segal, 1966; Bergren, Ng and Donnel, 1972). Conversion of galactose-1-phosphate to galactose-6-phosphate (Fig. 2.4) by phosphoglucomutase has been demonstrated in red cells of patients with transferase deficiency, galactose-6-phosphate has been found in erythrocytes of such patients. The galactose-6-phosphate can be oxidised in vitro by glucose-6-phosphate dehydrogenase (G6PD).
and NADP to 6-phosphogalactonic acid (Inouye, Schneider and Hsia, 1964; Cohn & Segal, 1973).

Figure 2.3: Oxidation of galactose.

Galactose → NAD⁺ → Galactonolactone

Galactonic acid

Xylulose → NAD⁺ → 3Ketogalactonic acid

Figure 2.4: Galactose metabolism in RBC of transferase deficient persons.

Galactose-1-phosphate → Galactose-6-phosphate → NADP⁺

NADPH → NADP⁺ → NADPH

Xylulose-5-phosphate → 6-Phosphogalactonate

The enzyme aldose reductase, an important constituent of polyol (Sorbitol) pathway, is of particular interest in ophthalmology (Varma and Kinoshita, 1974). The pathway was first observed in sperm (Hers, 1956), later found to be active in many tissues including lens (Van Heyningen, 1959). The polyol pathway consists of two enzymes aldose reductase and sorbitol dehydrogenase. Aldose reductase has broad substrate specificity and can reduce a variety of aromatic and aliphatic aldehydes including aldoses. As the affinity of aldose reductase for glucose (Jedziniak, Chylack, Cheng, Gillis, Kalustian and Tung, 1981) and
galactose are low, higher concentration of these sugars are needed for the production of sugar alcohol viz., sorbitol or galactitol. The enzyme possesses greater affinity for galactose which is not further metabolized by sorbitol dehydrogenase which is responsible for the elevation of sugar alcohol (Kinoshita, Merola and Dikmak, 1962). A series of experimental cataract has been characterised by abnormalities in fiber permeability which results in vacuoles or clefts in the lens cortex (Harding & Crabbe, 1984). The swelling of lens led Kinoshita (1965) to postulate that high levels of sugar alcohol draws water, thus rupturing lens fibres and causing vacuolation in lens cortex. In contrast with other sugar alcohol dulcitol is not a suitable substrate for polyol dehydrogenase (Kinoshita et al, 1962). In diabetic cataract and galactose cataract the common factor found to initiate both types of sugar cataract is the formation of sugar alcohol from either glucose or galactose by the enzyme aldose reductase. Increased intracellular levels of these polar alcohol have a hyperosmotic effect which leads to swelling of lens fibres, formation of vacuoles and subsequent opacification. Patterson and Bunting (1965) analysed the sugar alcohol, fructose, glucose, ATP, dry weight hydration and extracellular spaces before and after the sugar cataract and observed the appearance of mature cataract with the disruption of fibres. Kinoshita, Merola and Dikmak, (1962) studied the osmotic changes in galactose cataract. They observed that as the dulcitol 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 fibres to swell and perhaps to rupture their membranes. Lee, Chung and Chung (1995) demonstrated the accumulation of polyol in cataract using transgenic mice expressing aldose reductase gene in lens. Birlouez-Aragon, Ravelontscheno, Villate-Cathelineau, Cathelineau and Abitbol (1993) conducted a study to evaluate the relationship between milk and yogurt consumption and galactose metabolism and cataract. They concluded that
the cataractogenic action of lactose depends on the disturbance of galactose metabolism in elderly subjects and the yogurt is not cataractogenic, although the mechanism of the protective effect of yogurt remains unknown. Shi, Unakar, Wen, Tsui and Bekhor (1992) & Shi and Bekhor (1994) studied and proved the transient elevation of aldose reductase mRNA in lenses of rats developing galactose cataracts. Thus polyol as the major aetiologic factor in the formation sugar cataract was confirmed by the researchers of last century (Peterson, Sarges Aldinger and MacDonald, 1979; Ohta, Yamasaki, Niwa, Goto, Majima and Ishiguro, 1999).

After the discovery of the aldose reductase enzyme a lot of inhibitors like sorbinil, tolrestat, statil, AL 1576, M79 and ONO2235 were established by many workers (Dvronik, Simrad-Duquesne, Krami, Sestanj, Gabbay, Kinoshita, Varma and Merola, 1973; Kinoshita, Fukushi, Kador and Merola, 1979; Datiles, Fukui, Kuwabara and Kinoshita, 1982; Hu, Datiles and Kinoshita, 1983; Gonzalez, Sochor, Hothersall and Mclean, 1986; Unakar, Tsui and Johnson, 1989; Kato, Nakayama, Ohata, Murakami, Murakami, Mizota, Miwa and Okuda, 1990; Toshima, Taura and Okamura, 1991; Sabasinski and Andrzejewska-Buczko, 1997). Plant product like flavonoids were forwarded by many workers as the inhibitors and thus forwarded as a preventive measure for galactose and diabetic cataract (Varma, Mikuni and Kinoshita, 1975; Varma & Kinoshita, 1976; Varma, Mizuno and Kinoshita, 1977; Parmar & Ghosh, 1979).

A number of workers studied the effect of glutathione on cataract formation (Srivastava and Beutler, 1968; Harding, 1970; Diwvedi and Prathap, 1987; Xic, Kanai, Nakajima, Kitahara, Ohisu and Fujii, 1991; Kasuya, Itoi, Kobayashi, Sunaga and Suzuki, 1992; Kamei, 1993). Rawal, Patel and Desai (1978) studied the amount of glutathione in human cataractous lenses and found decreased. Reddy (1971) studied the mechanism by which high levels of GSH are
maintained and the possible functions of the tripeptide are discussed. Lens proteins contain reduced sulphydryl groups and oxidised disulphide groups, maintaining high levels of GSH (Rathbun, 1976). Kamei (1993) suggested that the presence of GSH may play an important role in preventing the oxidation by various oxidants. The depletion of glutathione in all types of cataract are due to its decreased biosynthesis and increased permeability of lens membranes (Srivastava & Beutler, 1968; Ohrloff, Hockwin, Olson and Dickman, 1984; Kasuya, 1992; Xie et al, 1991; Kannan, Fernandez-Checa, Garcia-Ruiz, Mackic and Zlokovic, 1997; Ohta et al, 1999). The decrease in GSH will result in protein sulphydryl oxidation and alterations in protein linkages, their solubility and transparency (Spector & Roy, 1978; Augusteyn, 1979). Davidson and Tanaka (1972) studied the factors affecting pentose phosphate pathway activity in human red cells and observed that the pentose phosphate pathway activity vary according to pH. Szczypka, Gajewski, Laskowska-Klita and Zbieg-Sendecka, (1990) studied glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-s-transferase (GST), G6PD, catalase, glutathione and lipid peroxides in red blood cells of galactosemic children. Augusteyn (1979) opined that the GSH is 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 \( \text{H}_2\text{O}_2 \) in rabbit lens. The results indicate that GSH metabolism and HMP shunt pathway contribute significantly to the detoxification of \( \text{H}_2\text{O}_2 \) in lens. Kasuya et al, (1992) studied the changes in glutathione and taurine concentration in lens of rats induced by galactose cataract. They observed a decrease of sulphur containing compounds in cataractous lens. Reim, Heuvels and Cattepoel (1974) studied GSH-Px in lens and observed that GR is an important enzyme for the maintenance of cellular pool of GSH, which serves as a reductant and prevents the formation of protein disulphide leading to molecular aggregation and cataract
formation (Balasubrahmanian et al., 1993). Dwivedi & Prathap (1987) studied the glutathione metabolism during cataract formation and observed a significant decrease in the activities of GR, GSH-Px and G6PD. Srivastava and Beulter (1973) studied the cleavage of lens protein, GSH mixed disulphide by GR. They suggested that this mechanism keeps lens protein in reduced form thereby preventing the precipitation or inactivation. Glutathione provides the lens with a major detoxifying mechanism via., the enzyme GSH-Px (Srivastava, Lal and Ansari, 1980; Fecondo & Augusteyn, 1983).

Cherian and Rawal (1991) studied the glutathione and glutathione related enzymes in busulphan treated rat lens. They observed that the cataractous lens showed a significant decrease in glutathione and related enzymes. Fujii, Dale and Beutler (1984) studied the glutathione dependent protection against oxidative damage of human red cell membrane and the observations indicate that GSH dependent protection against lipid peroxidation is mediated by one or more proteins other than GSH-Px and GsT. Bhat and Gopalan (1974) studied the erythrocyte GR activity in normal and cataractous patients and observed a decreased GR activity in erythrocytes of cataractous patients.

Kinoshita and Masuray (1957) studied various factors influencing the state of bovine lens glutathione and suggested possible interaction of dehydrogenase of the shunt and glutathione. The HMP shunt is active in lens and generates NADPH that maintains glutathione in reduced state (Harding & Crabbe, 1984; Rao, Sadasivudu and Cotlier, 1983). Balaji, Sasikala, Sundararajulu, Ravindran and Latheef sathar (1995) analysed the G6PD in cataractous lens and observed an increase of G6PD in middle aged cataractous patients while the same was lower in aged patients.

Auriccho and Libondi (1983) reviewed the physiologic and pharmacologic
factors protecting the lens transparency and thereby preventing the cataract. Bhuyan and Bhuyan (1978) studied the role of super oxide dismutase (SOD) in lens. He opined that catalase present in the eye protects the lens from H$_2$O$_2$ and it also protects superoxide dismutase of the lens from inhibitory effects of H$_2$O$_2$. SOD in turn protects the lens from O$_2^-$ and it is possible that both SOD and catalase might prevent the formation of OH'. Bhuyan and Bhuyan (1977) studied the effect of 3-aminotriazole (a catalase inhibitor) on the catalase and GSH-Px of rabbit eye. They found that 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. The lens is highly susceptible to superoxide anion or its derivatives (Bhuyan & Bhuyan, 1978) and humans exposed to hyperbasic oxygen develop cataract (Palmquist, Philipson and Barr, 1984). Peroxide is catalysed by catalase and peroxidase are synthesized through SOD.

Lipid peroxidation has been associated with a number of specific manifestations related to cataract (Fernandes, Pereira, Ramalho, Mota and Oliveria, 1996). The cataract formation through the polyol pathway is associated with free radical production and accelerates the damage of lens fibres (Kubo, Miyoshi, Fukuda and Akagi, 1999). Lipids as structural components of lens fiber membranes, are intimately associated with insoluble lens proteins (Cotlier, 1989). The increased insolubility of proteins with cataract formation may be due to derangements in the stereochemical arrangement between lipids and proteins in the membrane and the soluble proteins inside the fibers (Rosenfeld & Spector, 1982; Spector, 1984). Bhuyan, Bhuyan and Podos (1981) provided experimental evidence to demonstrate the involvement of lipid peroxidation in pathogenicity of cataract. They observed an increased malondialdehyde content in cataractous lens. Altomare, Vendemiale, Grattagliano, Angelini, Micelli-Ferrari and Cardia,

Sreekumar, Jyothi, Betzy & Shashidhar (1997) studied the effect of galactose diet on protein and taurine content in albino rat lens. They observed a gradual and significant decrease in total protein and taurine content with the maturity of cataract and these parameters were found related to lens opacification and weight. Priya and Shashidhar (1996) observed an increase in the glycoprotein in diabetic cataractous lens when compared to non diabetic cataractous lens. Kamei (1990) studied the lens protein aggregation and observed that the protein aggregation in cataractous lens is due to the disulfide bond. Kador, Zingler and Kinoshita (1979) studied the alterations of lens protein synthesis in galactosemic rats and observed a depression in synthesis of the lens crystalline along with cataract development, where as noncrystalline proteins found unaffected. The removal of galactose from the diet results in gradual recovery of crystalline synthesis. Williams et al (1985) studied the events of synthesis and degradation of water soluble and insoluble rat lens proteins for 21 days in galactose induced cataractogenesis. They observed that epithelial cells continued to synthesise both
fractions of proteins, while both water soluble and insoluble proteins synthesis found inhibited in cortical cells. Remya, Sreekumar and Shashidhar (2000) studied the effect of vitamin A deficiency on the integrity of crystalline lens and they observed a decrease in lens weight and protein content in vitamin A deficient rats. Gona (1984) studied the morphological alterations to lens in galactose fed rats and hypothesized that the loss of meridional row integrity is a phenomenon common to development of most cortical cataracts. Srivastava (1988) studied age-related increase in concentration and aggregation of degraded polypeptides in human lens. The results suggested an apparent age-related polymerization of degraded polypeptides into heavy molecular weight proteins leading to their insolubilization. Kinoshita, Barber, Merola and Tung, (1969) studied the changes in the levels of free amino acids and myo-inositol in the galactose exposed lens. The results suggest that depressed levels of these soluble components in the galactose exposed lens may be due to osmotic effects caused by the retention of dulcitol.

Fournier and Patterson (1971) observed that galactose feeding resulted in a marked lowering of Mg ATPase activity in the epithelium after six days and a marked decrease in the activity of Na ATPase in fibers after fifteen days. Harding, Unaker, Bobrowski, Dang, Tsui and Harding, (1987) was of the opinion that after six days of galactose cataract, cortex undergoes loss of potassium and gain in Na, Cl and Ca. But after 20 days of galactose cataract, this region except central nuclear region retains normal elemental composition. Libondi, Menzione, Iuliano, Corte, Latte and Auricchio (1985) studied the change of some biochemical parameters of the lens in galactose treated weaned rats with and without Vitamin E therapy. It becomes evident that the Vitamin E does not prevent biochemical changes caused by galactosemia. Creighton, Ross, Stewart-DeHann, Sanwal and
Trevithick (1985) studied the possibility of Vitamin E and other antioxidants which might prevent cataract by incubating rat lenses in vitro in galactose enriched medium or by treating rat fed with a diet containing 50% galactose. They observed that Vitamin E has only very minor role in preventing cataractogenesis. However, Ohta et al, (1999) showed that vitamin E containing liposome can prevent cataractogenesis.