Review of Literature

I Cataract and its genesis

1. The lens

The crystallin lens of the eye is positioned behind the iris with its posterior aspect embedded in the vitreous body (Fig. 1). It is a transparent tissue, comprising highly elongated fiber cells, which are derived from the differentiation of a single layer of polarised epithelial cells that underlie the anterior surface of the lens (Fig.2). The lens plays a passive role in the process of accommodation by which light rays, which have passed through the cornea and aqueous humor, are focused upon the retina. It is the remarkable property of the lens that allows it to play an important role in normal vision.

Fig. 1. The structure of the human eye (longitudinal section) (adapted from http://www.colorado.edu/intphys/Class/IPHY3430-200/image/eye.jpg)

Fig. 2. The structure of the human lens (adapted from Roberts et al., 1995)
2. Transparency

Only a transparent lens can efficiently transmit light of wavelengths from 390 nm to 1200 nm, extending well above the limit of visual perception (about 720 nm). Lenticular transparency results from the appropriate architecture of lenticular cells and tight packing of their proteins, resulting in a constant refractive index over distances approximating the wavelength of light (Benedek, 1971; Delaye and Tardieu, 1983). The cellular architecture and arrangement of the fiber cells, and particularly, their sutures, are critical for light transmission and lenticular transparency (Kuszak et al., 2004). In addition, the stability and close ordering of lenticular crystallins, which make up 80-90% of the soluble proteins in the lens, are also critical for lenticular transparency. The high protein content of the lenticular nucleus, approximately 60% of its wet weight, is particularly important for refraction and focusing of light.

3. Histoarchitecture of the lens

The human lens consists of three metabolically different zones: the epithelium, the cortex and the lens nucleus or core (Harding, 1991). Epithelial cells are found just beneath the predominantly collagenous capsule that surrounds the lens. These are the most metabolically-active cells. Some of these cells divide, migrate to the lenticular equator and turn the corner 180° (apex to apex) as they elongate up to 1000 times to form lenticulate fiber cells. In these cells, the major gene products of the lens, the crystallins, are produced. The outer layer of these fiber cells, which are laid down at the rate of about one cell layer per day (like tree rings), make up the cortex. These cells also undergo changes as they denucleate and age, and they become compressed as new cells are being formed externally. Buried under the cortical cells are the oldest cells of the lens, called nuclear or core cells. Therefore, a gradient exists in the lens, with the most recent proteins occurring in the epithelium and the oldest proteins, arising during embryonic states, in the nuclear cells. Posterior subcapsular opacities are primarily due to aberrations in the outermost layers of the lens. Cortical opacities occur in the inner
and outer cortical tissue. Nuclear opacities are found in the oldest, and most central zone which is metabolically quiet (Vinson, 2006). The hypothesis is that opacification in each of these three zones arises due to different etiologies, and thus most epidemiological studies treat them separately.

4. **Cataract**

Cataract, which can be defined as lenticular opacities, arises due to multiple causes, but is often associated with breakdown of the lenticular microarchitecture (Vrensen et al., 1990; Kuszak et al., 2004); this may include vacuole formation and disarray of lenticular cells, which can cause large fluctuations in density, resulting in light scattering. In addition, light scattering and opacification occur due to the formation of significant amounts of protein aggregates of high molecular weight (approximately 1000\textdegree{}A or more) (Benedek et al., 1987; Bettelheim et al., 1995). For transparency, crystallins must exist in a homogeneous phase with significant short-range spatial ordering. This condition is abrogated in the presence of aggregates of partially denatured or even native proteins. In fact, disruptions of lenticular microarchitecture and protein denaturation are not mutually exclusive events, and both may play a part in the development of some cataracts (Delaye and Tardieu, 1983).

5. **Classification of cataract**

There are three main types of cataracts based on their clinical appearance: nuclear, cortical, and posterior subcapsular. They can present alone or in combination. Typically the changes are bilateral, but they are commonly asymmetrical (Vinson, 2006).

**Nuclear:** As the lens ages, new layers of fibres are added and the lens nucleus is compressed and becomes harder (nuclear sclerosis cataract), with associated yellowing of the lens. Nuclear sclerosis progresses slowly, over years. In some cases it does not
significantly affect vision or causes only a change in refraction (myopic shift),
sometimes called second sight, since glasses might no longer be needed for reading.
With further progression, there can be loss of color discrimination and also loss of
vision, typically greater for distant than for near vision.

**Cortical:** The cortex of the lens is made of the newest lens fibres. No fibres are lost
with ageing, and new fibres are added to the outer part of the lens, under the outer
coating or capsule of the lens. With ageing, discrete opacities (cortical spokes) develop
within the cortex of the lens. These typically cause no visual symptoms unless they
involve the visual axis or the entire cortex, in which case the lens becomes white and is
said to be mature.

**Posterior subcapsular:** These cataracts are granular opacities occurring mainly in the
central posterior cortex just under the posterior capsule. They may occur in younger
patients, and are commonly associated with a complaint of glare, such as when driving
at night, and tend to reduce near vision more than distant visual acuity.

6. **Oxidative stress and cataract**

Oxidative stress arises due to an imbalance between the rate of production and
the rate of degradation of oxidants (Sies, 1991). The complete reduction of the oxygen
molecule occurs within the mitochondria, the end-product being water. However, a
partial reduction produces superoxide and various reactive oxidative intermediates (free
radicals and reactive oxygen species [ROS], including hydroxyl radicals, singlet
oxygen radicals and hydrogen peroxide). In addition to these endogenous oxidants,
there are exogenous sources of oxidants, including food, air pollutants, tobacco smoke,
exercise, ionizing radiation, infrared (IR) and sunlight. Although cells are also equipped
with a well-organized antioxidant defence system (ADS), comprising many substances
such as enzymes and vitamins, an imbalance between generation of free radicals and
their disposal by antioxidants occurs with advancing age, resulting in degenerative changes and senescence (Reiter, 1995). Increased production of superoxide ($O^-$) and hydrogen peroxide ($H_2O_2$) with increasing age has been documented (Sohal et al., 1994).

Oxidative damage appears to be a major factor in the initiation and progression of numerous age-related conditions, including cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and cataract (Finkel and Holebrook, 2000). Oxidative stress plays a significant role in the degradation, oxidation, cross-linking, and aggregation of lenticular proteins that contribute to the loss of lenticular transparency (Lou, 2003). This loss of transparency leads to the formation of cataract, which is the most frequent cause of preventable blindness worldwide (WHO, 1997). The major enzyme systems involved in the degradation and detoxification of ROS in the lens include superoxide dismutase (SOD) (Behndig et al., 1998) and catalase (CAT) (Yang et al., 1998), which constitute a primary line of defense against the superoxide anion ($O_2^-$) and $H_2O_2$, respectively. Another class of antioxidant enzymes is represented by ‘classic’ glutathione peroxidases (Gpx) that reduce small organic peroxides through a catalytic mechanism involving selenocysteine for the active site (Flohe, 1999). However, there is restricted substrate utilization by these enzymes so that their inhibitory roles in cataractogenesis caused by a broad spectrum of hydroperoxides are not fully understood. Although cataract is a multifactorial disease associated with several risk factors (Harding, 1991), free radical-induced oxidative stress is postulated to be the major factor leading to senile cataract formation (Gerster, 1989). This hypothesis has been tested by attempts to supplement the diet with micronutrients possessing antioxidant capabilities in a bid to prevent/retard cataractogenesis (Devamanoharan et al., 1991; Thiagarajan et al., 2001; 2002; Gupta et al., 2002).
7. Senile cataract

The ageing human lens has been the subject of intense research over the past two decades, for a number of quite disparate reasons. The fact that the prevalence of cataract doubles with each decade of life after 40 years, means that most people in their 80s and 90s will be affected (Chong and Wong, 2008). Increased exposure to oxygen has been identified as a risk factor for nuclear cataract, the most common type of age-related cataract. Age-related changes in the lens are likely to make it more susceptible to oxygen-induced nuclear cataracts. Nuclear cataracts are caused by excessive oxidation of proteins in the center of the lens, namely the nucleus (Truscott and Augusteyn, 1977; Lohmann et al., 1986; Spector, 1995; Truscott, 2005). The cells of the lenticular nucleus have minimal metabolic capabilities. The proteins in these cells are protected from oxidative damage by antioxidant compounds that are produced in metabolically active cells near the surface of the lens. These molecules, especially reduced glutathione (GSH), diffuse through gap junctions that connect cells on the surface of the lens to the cells of the lenticular nucleus (Sweeney and Truscott, 1998). If antioxidant compounds in the nucleus become oxidized, they must diffuse back to the metabolically active cells at the lenticular surface to be reduced or re-synthesized. With increasing age, the rate of diffusion in the lenticular cytoplasm is significantly reduced (Sweeney and Truscott, 1998; Moffat and Pope, 2002; Truscott, 2005; McGinty and Truscott, 2006) and oxidized glutathione levels in the lenticular nucleus increase (Reddy, 1990; Giblin, 2000; Truscott, 2005). Small increases in exposure to oxygen, which might be tolerated by a younger lens, may lead to protein oxidation and nuclear cataract in the lenses of older individuals. This view of nuclear cataract formation suggests interventions that could be successful in preventing or delaying this disease.

8. Risk factors

A growing body of research has addressed risk factors that might contribute to the multifactorial nature of cataract development and preventive factors that might
retard their growth (West and Valmadrid, 1995; VanNewkirk et al., 2002). Personal factors, such as increasing age, have been repeatedly reported to be associated with nuclear and cortical opacities (Asbell et al., 2005). Ethnic variation has been reported to be associated with different cataract types and varying prevalence rates (Asbell et al., 2005). Genetic factors may account for up to 50% of the severity of nuclear cataract (Hammond et al., 2000) and may also be important in the development of cortical cataracts (Hammond et al., 2001). Several studies have suggested that women are at slightly greater risk than men of cataract development, and there is conflicting evidence of a possible beneficial effect from hormone replacement therapy in postmenopausal women (Congdon, 2001; Weintraub et al., 2002). Cataractogenesis is associated with cigarette smoking, exposure to sunlight, alcohol use, and lower education. Many of these factors are associated with other health problems, so are of general public-health interest. Published epidemiological research on ultraviolet exposure and lenticular opacities strongly suggests that exposure to ultraviolet B causes changes leading to cortical cataract (Hollows and Moran, 1981; West and Valmadrid, 1995; Hightower, 1995; McCarty and Taylor, 2002). Common medical problems have also been investigated, such as obesity, diabetes, hypertension, diarrhoea, dehydration, steroid use, and use of systemic medications. There is clear evidence that diabetes mellitus contributes to the development of cortical and posterior subcapsular cataracts, related to the duration of disease and degree of control, and diabetes also seems to be associated with an earlier age at cataract surgery (VanNewkirk et al., 2002). Systemic corticosteroid use has been strongly associated with posterior subcapsular cataracts, whereas the data for inhaled corticosteroids are mixed (Urban Jr and Cotlier, 1986; Garbe et al., 1998; Carnahan and Goldstein, 2000; Jick et al., 2001).
II Cataract and its mechanism

1. Lenticular defense system

The antioxidant system is a potentially important protective system for ocular tissues, since it is involved in various pathological processes in the eye, including cataract (Bhuyan and Bhuyan, 1984). The balance between the production and catabolism of oxidants by cells and tissues is critical for maintenance of the biologic integrity of the tissue. Ocular tissues contain antioxidants that prevent damage from excessive oxygen metabolites; these either act by decomposing peroxide or trapping the free radicals, leading to interference with the chain of oxidation reactions (Rao et al., 1985). The major protective enzymes of the antioxidant system in the lens are SOD, CAT (Fecondo and Augusteyn, 1983), and Gpx (Dwivedi and Pratap, 1987). In addition, there are several nonenzymatic free radical scavengers, such as glutathione (Ross et al., 1983), ascorbate (vitamin C) (Varma et al., 1982) and vitamin E (Trevithick et al., 1989). SOD, a chain-breaking antioxidant, was first described by McCord and Fridovich (1969) in red blood cells. Varma et al. (1977) first described its occurrence in the lenses of different species. SOD converts superoxide to H$_2$O$_2$. The enzyme exists in two forms, one containing Mn$^{2+}$, restricted to the mitochondria, and a cytosolic form containing Zn$^{2+}$ and Cu$^{2+}$. The occurrence of Gpx in the lens was first shown by Pirie (1965). Gpx is required to check lipid peroxidation, initiated by superoxide in the phospholipid bilayer, for maintenance of membrane integrity. CAT is a hemoprotein that requires nicotinamide adenine dinucleotide phosphate (NADPH) for regeneration to its active form (Russell et al., 1991). The presence of CAT in the lens has been well demonstrated (Bhuyan and Bhuyan, 1970). Both CAT and Gpx catalyse the transformation of H$_2$O$_2$ within the cell to harmless by-products, thereby curtailing the quantity of cellular destruction inflicted by products of lipid peroxidation (Santini et al., 1997).
2. Redox homeostasis

The healthy lens is normally well-equipped with antioxidant enzymes and redox system components, e.g. CAT, Gpx and GSH, which protect lenticular proteins against ROS. Hydrogen peroxide, derived from sources both within and outside the lens, has been implicated as a major oxidant in the pathogenesis of experimental and human cataract. Normally, H$_2$O$_2$ is eliminated by GSH, or through the action of the enzymes Gpx or CAT. However, with age, it appears that these protective mechanisms decrease in activity, resulting in elevated H$_2$O$_2$ levels, ultimately leading to opacification (Spector, 1995). The tripeptide, glutathione, is the principal antioxidant in the lens and its depletion is associated with the onset of age-related nuclear (ARN) cataract (Reddy, 1971; Truscott, 2005). Thus, GSH play a significant role in maintaining the lens in a reduced state. The synthesis of GSH within the lens has been well-documented (Reddy, 1971; Murray and Rathbun, 1990; Rathbun and Holleschau, 1992); in addition, a recent report by Li et al. (2010) has suggested the occurrence in the lens of a mechanism for the uptake of GSH from aqueous humour. Such intracellular concentrations of GSH are regulated by its own feedback inhibition of the rate limiting enzyme, $\gamma$-glutamyl cysteine synthetase ($\gamma$-GCS) (Lash, 2005).

3. Lenticular calcium homeostasis and calpain activity

Precise regulation of calcium homeostasis is essential to the viability of most cells (Berridge et al., 1998). The human lens is no exception. In all human cataractous lenses, total calcium is elevated three to 3000-fold, compared to clear human lenses. The calcium-induced lenticular opacity is a well-characterized in vitro model for cataract (Clark et al., 1980; Hightower, 1985), and may be a valid model since the loss of transparency is associated with elevated lenticular calcium levels in humans (Adams, 1929; Duncan and Bushell, 1975; Jedziniak et al., 1976; Duncan and van Heyningen, 1977; Hightower and Reddy, 1982a; Ringvold et al., 1988; Rasi et al., 1992; Dilsiz, 2000; Tang et al., 2003). Similarly, elevated calcium levels have been reported in
cataracts induced by X-ray (Hightower et al., 1983) or selenite (Bunce et al., 1984); the restoration of calcium homeostasis in such conditions has been reported to maintain transparency (Nabekura et al., 2003; Hightower and Reddy, 1982b; Marcantonio et al., 1986; Shridas et al., 2001; Mathur et al., 2000). The intracellular calcium level is regulated by various regulators such as calcium-ATPase pumps (Hightower and Kinsey, 1980), calcium channels (Cooper et al., 1986; Qu and Zhang, 2003), calcium binding proteins (Pavlovitch et al., 1983; Kluge et al., 1990; Rajini et al., 2001) and sodium-calcium exchanger (Wang et al., 1992; Okafor et al., 2003).

Elevated Ca^{2+} in the lens has been hypothesised to activate calpain, a Ca^{2+}-dependent protease, in young rodent models of cataractogenesis. This activated calpain leads to increased degradation of lenticular proteins, such as crystallins, resulting in an opaque lens (Shearer et al., 1997). Recently, several isoforms of lenticular calpain have been characterized (Reed et al., 2003). Calpains include ubiquitous calpains, such as μ-calpain, m-calpain and calpain-10, and lens-specific calpains, such as Lp82 and Lp85. m-calpain and Lp82 are the two major calpains that have been implicated in normal maturation of the lens (David et al., 1993). Although m-calpain (calpain-2), the predominant calpain isoform in the lens, is found in human and rodent lenses, Lp82 appears to be especially active in the rapidly-forming selenite overdose cataract in young rat pups and is not found in human lenses (Nakamura et al., 2000; Ueda et al., 2002). Little is known about the regulation of calpain activity by endogenous inhibitor action (Ma et al., 1998a). Cataract formation is accompanied by several presumptive biochemical indicators of calpain activation, including increased calcium levels, proteolysis of α-spectrin, and decreased caseinolytic activity for calpains, suggesting calpain activation followed by autolytic degradation (Sakamoto et al., 2002). In one study, rodent lenses that had been cultured in ionophore A23187 revealed extensive activation and autolysis of calpains (Nakamura et al., 2000).
4. Apoptosis

The mechanisms of activation of apoptosis in different physiological or pathological conditions have been extensively studied. An apoptosis-like process occurs in lenticular epithelial cells during their differentiation into fiber cells. Cellular events, such as loss of organelles and degradation of nuclei that take place during terminal differentiation resemble apoptosis (Wride, 2000; Bassnett, 2002; Sanders and Parker, 2002). Some previous studies have shown that apoptosis of lenticular epithelial cells (LECs) plays an important role in the development of cataracts induced by exposure to hydrogen peroxide (Spector et al., 1995) ultraviolet radiation (Li and Spector, 1996; Michael et al., 1998) or selenite (Tamada et al., 2000). Moreover, apoptosis also occurs in the process of wound healing and reepithelialization after extraction of the lens (Kato et al., 1997). Apoptosis of LECs is induced when LECs are cultured in medium containing selenite (Nakajima et al., 2006a). The monolayer of epithelial cells covering the entire core of the body of the lens, filled with fiber cells, is susceptible to apoptosis, because several external cataractogenic factors, such as UV radiation, chemicals, and oxidative stress, induce cellular apoptosis (Michael et al., 1998; Tamada et al., 2000; Yang et al., 2002; Belusko et al., 2003). Maintaining the integrity of the epithelium and survival of epithelial cells during adverse conditions is extremely important in the lens, because the rest of the tissue depends on these cells for the maintenance of transparency. Therefore, it is important to identify the factors that play a role in lenticular epithelial cell survival.

5. Lenticular proteins

Transparency is a fundamental characteristic of the normal lens and distinguishes cells of the lens from all other mammalian cells (Trokel, 1962; Benedek, 1971). The transparency and refractive power of the ocular lens depend on an even distribution of the proteins within lenticular cells on the scale of the wavelength of visible light (Delaye and Tardieu, 1983). Dense opacification results when the proteins
form large insoluble aggregates that approach or exceed the dimensions of the wavelength of light, and produce large fluctuation in the index of refraction that result in increased light scattering (Benedek, 1971; Spector, 1984; Clark, 1994).

The mammalian lens consists mainly of proteins, which account for over 30% of its weight (Benedek, 1971). It is the architecture of the crystalline lattice that is ultimately responsible for lenticular transparency and for the proper focusing of light. The highly concentrated cellular proteins in mammalian lenses consist of two families of water-soluble crystallins, namely, α-crystallin and β/γ-crystallin (Andley, 2007), that are uniformly distributed in transparent lenticular cells. In addition, the lens also contains cytoskeletal proteins, which constitute about 2-4% of lenticular proteins, which include intermediate filaments, microfilaments and microtubules (Alcala and Maisel, 1985; Jaffe and Horwitz, 1992).

Several biochemical alterations in lenticular proteins have been recorded in human cataract: an abnormal increase in the ratio of insoluble to soluble protein (Kramps et al., 1976; Spector, 1984); proteolysis of lenticular crystallins (Duncan et al., 1989; Harding, 1991) and decreased content of cytoskeletal proteins (Ozaki et al., 1985; Tagliavini et al., 1986). Pioneering studies have suggested a functional role for the cytoskeletal proteins, such as actin (Mousa and Trevithick, 1979), vimentin (Ireland and Maisel, 1984) and spectrin (Tagliavini et al., 1986), in the maintenance of the normal structure of transparent lenticular cells. Further, the development of senile nuclear cataract is associated with an increase in insoluble proteins (Haard et al., 1978). The proportion of water-insoluble proteins increases in human lenses with aging, and more so during development of cataract (Clark et al., 1969). It is believed that the age-related water-insolubilization of lenticular proteins might, in turn, be mediated via a precursor complex known as water-soluble high molecular weight proteins (Roy and Spector, 1976; Srivastava et al., 1996). Therefore, changes in the water-soluble-high
molecular weight proteins, leading to water-insoluble proteins, of cataractous lenses, might play a critical role in producing cataract-specific aggregates and cross-linked species.

6. Prevention and treatment

Extensive research has been performed on access to surgical intervention, barriers to surgery, outcomes, and methods to improve the cost-effectiveness of cataract surgery. The development of a simple tool called Rapid Assessment of Cataract Surgical Services has allowed countries worldwide to determine how well a cataract-blind person is able to have access to surgery (Limburg et al., 1999). This reporting tool has been invaluable for the Vision 2020 community to chart the coverage of its mission to reduce cataract as an avoidable cause of blindness. Women, especially face barriers to surgical care. The rates of blindness and visual loss due to cataract are significantly higher in women compared to men in many countries (Abou-Gareeb et al., 2001; Venkata et al., 2005).

Although cataract removal and intraocular lens implantation effectively treats this condition and restores good vision, cataract surgery is not without complications, particularly in an elderly patient. Additionally, the provision of cataract surgery requires trained ophthalmologists, appropriate postoperative follow-up management and refractive correction, and use of expensive operating facilities and microsurgical equipment. As many of these services are not readily available in developing countries, identifying methods to prevent or slow cataract development in the general population will be a major public health achievement (Chong and Wong, 2008).

Prevention would lead to significant monetary savings and to avoiding surgical complications in wealthy countries as well. Over the last two decades, there has been a 3- to 4-fold increase in cataract surgeries performed in the United States, with the
threshold for cataract surgery in many countries now at 20/30 or better (Brian and Taylor, 2001). Projections from the Centers for Medicare and Medicaid Services suggest an annual health care bill of $4 trillion by 2015 in the U.S. alone (Chong and Wong, 2008) with cataract surgery accounting for more than 10% of the annual Medicare budget. Cataract surgery is the most frequently performed surgical procedure covered by Medicare (Steinberg et al., 1993). It has also been suggested that a delay of 10 years in cataract onset would halve the number needing surgery and significantly reduce economic burden (Brian and Taylor, 2001). Hence, there is a need to look into alternative modalities to prevent or delay the formation of cataract.

III Experimental approaches

Animal models are valuable tools to study the human disease. The detailed information available regarding development of the eye in rodents, and the fact that cataracts are easily identified in this animal, make the rodent an ideal model for the study of cataractogenesis. Furthermore, the extensive regions of synteny that exist between the human and rat genomes facilitate comparative mapping, and enable identification of novel candidate loci for human cataract. Thus, studies of the molecular and developmental pathobiology of rat cataract models, could possibly reveal mechanisms underlying human cataract development. A several model systems that involve oxidative stress have been used to induce cataract. They include induction by H₂O₂, UV-radiation, buthionine sulfoximine (BSO) and sodium selenite.

1. Selenite cataract

Various experimental models have been developed to delineate the mechanisms of cataractogenesis and focus on the identification of crucial targets. Selenite-induced cataractogenesis in young rats has been shown to mimic human senile cataract with respect to several morphologic and biochemical changes in the lens. This model, being
reproducible, has been used extensively to evaluate the anticataract potential of different test agents (Shearer et al., 1997).

Exposure of suckling rat pups to an overdose of the essential trace mineral, selenium, is a rapid and convenient means of inducing nuclear cataracts. The ability of selenite to cause cataracts was first described in detail in 1977 (Ostadalova et al., 1978). Selenite cataract is usually produced by a single subcutaneous injection of 19-30 µmoles/kg body weight of sodium selenite (Na₂SeO₃) into suckling rats that are 10-14 days of age. Moreover, repeated injections of smaller doses of selenite (Huang et al., 1992) or oral administration of selenite (Shearer et al., 1983) are also cataractogenic. Selenite is cataractogenic only when administered to young rats before completion of the critical maturation period of the lens (approximately 16 days of age). Severe, bilateral nuclear cataracts are produced within 4-6 days. Precursor stages include: posterior subcapsular cataract (day 1), swollen fibers (day 2-3), and perinuclear refractile ring (day 3). Administration of a single dose of selenite leads to impaired oxidative defense, membrane damage, and cataract formation. Oxidation of the critical sulfhydryl groups of Ca²⁺-ATPase within the lenticular epithelial membrane, influx of calcium from the aqueous humor, activation of calpain, cleavage of N-terminal extensions of β-crystallins of the lens, interaction between exposed charged groups, and the formation of insoluble protein aggregates are some of the steps leading to the development of opacification (Shearer et al., 1997).

**Mechanisms of Selenite Cataractogenesis** - Several biochemical processes occur in the lens during selenite cataractogenesis. These include: altered metabolism of epithelial cells, accumulation of calcium, calpain-induced proteolysis, precipitation of crystallins, phase transition, and loss of cytoskeletal proteins (Shearer et al., 1997).
1.1. **Altered Metabolism in Lenticular Epithelium** – Usually, well before any opacity becomes visible, several important changes in metabolism have been documented to occur in the epithelium of the lens during selenite cataractogenesis. These include suppression of mitosis and entry of epithelial cells into prophase, nuclear fragmentation (Anderson *et al.*, 1986), decreased rate of epithelial cell differentiation (Cenedella, 1987), decreased synthesis of, and increased damage to DNA (Huang *et al.*, 1990), and loss of calcium homeostasis (Wang *et al.*, 1993). These early changes in lenticular epithelium possibly result from oxidative damage caused by selenite, possibly to critical sulfhydryl groups on molecules such as calcium ATPase, or ion channels (Shearer *et al.*, 1997).

1.2. **Accumulation of Calcium** - Most cataracts show an increase in lenticular calcium (Harding, 1991). Selenite cataract is especially interesting because uptake of calcium during formation of nuclear cataract is highest in the nucleus, and the calcium concentration in the cortex remains lower during this period (Hightower *et al.*, 1987). In contrast, concentrations of sodium, potassium, and water do not increase, indicating no appreciable changes in generalized permeability of the whole lens (Shearer and David, 1983). Theoretically, increased lenticular calcium could be due to inhibition of outwardly-directed Ca\(^{2+}\)-ATPase pumps or inhibition of Na\(^+\)/Ca\(^{2+}\) exchange. In one study, lenses from rats injected with selenite showed a 50% decrease in Ca\(^{2+}\)-ATPase activity (Wang *et al.*, 1993).

1.3. **Formation of Insoluble Protein in Selenite Cataract** - Factors promoting formation of the insoluble pellet in the rapidly-forming selenite nuclear cataract may be unusual when compared to other forms of induced cataracts. The pellet in selenite cataract is obviously massive, comprising over 17% of the total protein in the nucleus of the lens of the young rat (David and Shearer, 1984). The tendency is to view such cataractous pellets as covalently-linked, high-molecular weight aggregates of
crystallins. However, currently there is no evidence for covalent associations between crystallins.

1.4. Proteolysis – In an interesting study, the state of crystallins in the selenite cataractous condition and that of isolated lenticular proteins incubated with purified calpain was found to exhibit similarities (David et al., 1993);

1) The patterns of migration of truncated crystallins, observed with sensitive two-dimensional electrophoresis, were similar (Shearer et al., 1995);

2) At least eight of the new proteolytic fragments from β-crystallins from the in vivo and in vitro experiments were found to have the same calpain-like cleavage sites on their N-terminal extensions;

3) The in vivo precipitation of β-crystallins occurring in selenite cataract was found to be mimicked by addition of calpain to total soluble proteins from normal rat lens (David et al., 1993).

1.5. Loss of cytoskeletal proteins: Cytoskeletal proteins may be among the first proteins to be lost in selenite cataract (Matsushima et al., 1997). Thus, degradation of cytoskeletal proteins may be an important mechanism during the early stages of selenite cataract formation. Selenite has been found to accelerate the loss of the cytoskeletal proteins actin, tubulin, vimentin, and spectrin, as well as unidentified nuclear proteins of 49, 60 and 90 kDa. Cytoskeletal proteins, such as the vimentin-actin-tubulin system and the beaded filaments composed of phakinin and filensin are reported to be involved in stabilization of the transparent cell structure (Matsushima et al., 1997). It has been hypothesized that calpain-induced proteolysis of cytoskeletal proteins may disrupt normal interactions between crystallins and the cytoskeleton, leading to opacity of the lens. Moreover, in vitro studies conducted on rat lenses have shown that cytoskeletal proteins serve as good substrates for calpain (David and Shearer, 1984).
1.6. **Comparison between selenite cataract and human senile cataract** - Selenite cataract shows a number of general similarities to human senile cataract such as increased levels of calcium, raised insoluble protein content, occurrence of proteolysis, decreased water-soluble protein content and reduced GSH content. However, there are also several major differences. In contrast to human senile cataract, selenite cataract exhibits no high-molecular-weight covalent aggregates or increased formation of disulfides. In addition, selenite cataract seems to be dominated by rapid, calpain-induced, proteolytic precipitation of crystallins, while human senile cataract may be caused by oxidative stress over a long period of time (Shearer *et al.*, 1997). It may be relevant to state that the selenite cataract is a useful *in vivo* rodent model for drug testing, although important differences do exist between human senile cataract and selenite cataract. Therefore, the selenite-induced nuclear cataract model has been used for evaluation of the anticataractogenic potential of ellagic acid in this study.

2 Other models of cataract

2.1 Diabetic cataract

The lens is one of the worst-affected tissues in diabetes mellitus. Glucose is transported into the lens via facilitated transport (Kuck, 1962; Levari *et al.*, 1961) independent of insulin; hence, in diabetes, glucose over-utilization occurs in the lens (Gonzalez *et al.*, 1978). The primary source of energy production in the lens is the glycolytic pathway (Chylack and Cheng, 1978). However, in the presence of elevated glucose concentrations excessive glucose is fluxed through different metabolic pathways such as the polyol pathway and pentose phosphate pathway. The polyol pathway comprises the enzymes aldose reductase and sorbitol dehydrogenase, which catalyze the conversion of excessive glucose to sorbitol, and sorbitol to fructose, respectively; this pathway is activated in the diabetic cataractous lens (Lee and Chung, 1999). This accumulation of sorbitol and fructose, owing to failed diffusion out of the lenticular cells, is held responsible for increased oxidative stress, which triggers a series
of events that finally culminate in the development of cataract in long-term diabetes mellitus (Varma et al., 1979; Chung et al., 2003).

2.2 **Buthionine sulfoximine (BSO)-induced cataract**

The lenticular epithelium is known to have the highest concentration of GSH in the lens (Reddy, 1971). Among other functions, GSH acts as an antioxidant, detoxifies xenobiotics and has been reported to decrease the reactions of lenticular proteins with sugars, thus limiting the damage due to glycation and carbamylation reactions (Harding, 1991). The importance of GSH stems from the finding that this tripeptide is decreased in most types of cataracts (Reddy, 1971; Harding et al., 1996; Geraldine et al., 2006). BSO, an inhibitor of GSH biosynthesis, can induce cataracts in pre-weanling mice (Calvin et al., 1986) and in early postnatal rats (Martensson et al., 1989). This model was suggested as a potential model for obtaining new information about the role of GSH in maintaining transparency of the lens. In the presence of reduced levels of GSH, newborn rats suffer extensive damage to the cytosolic proteins and membrane lipids, leading to clouding of the lens (Spector, 1991; Jacques et al., 1994; Taylor and Nowell, 1997). This model of BSO-induced cataractogenesis has been utilized by free-radical biologists to test the efficacy of antioxidants in reducing oxidative damage to the lens, thereby preventing cataractogenesis (Martensson et al., 1989; Abe et al., 1994).

2.3 **Ultraviolet Radiation (UVR)-induced cataract**

Epidemiologic studies have shown a correlation between cataract and solar ultraviolet radiation (Bergmanson and Soderberg, 1995; West et al., 1998; Delcourt et al., 2000). Case reports support the relationship between UVR and cataract in humans (Baum and Pitts, 1997; Muller-Breitenkamp et al., 1997). Experimental studies have shown that UVR wavelengths of approximately 300 nm are the most harmful to the lens (Pitts et al., 1977; Merriam et al., 2000) and that 300 nm UVR penetrates a
short distance into the lens (Dillon et al., 1999; Lofgren and Soderberg, 2001). Generation of reactive oxygen species (1O₂, O₂⁻, H₂O₂ and 'OH) is a well-documented route for UVR damage (Goosey et al., 1980; Andley, 1987). Increased levels of oxidants disturb the fine balance between generation of free radicals and their elimination by the oxidant scavengers. This, in turn, may lead to further alterations of the biological processes in the lens. As shown in many studies, significant changes in enzyme activities and metabolic concentrations have been detected after UVR exposure (Hightower and McCready, 1992; Cejkova and Lojda, 1996; Reddy and Bhat, 1999; Lofgren and Soderberg, 2001).

3. Prevention by antioxidants

The reliability and extensive characterization of selenite cataract make it a useful rodent model for rapid screening of potential anticataract agents (Shearer et al., 1997). Various natural and synthetic compounds, 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), a procyanidin-rich extract of grape seeds (Yamakoshi et al., 2002), lycopene (Gupta et al., 2003), α-ketoglutarate (Varma and Hegde, 2004), Ocimum sanctum (Gupta et al., 2005), an extract of the oyster mushroom Pleurotus ostreatus (Isai et al., 2009), and an extract of Cineraria maritima (Anitha et al., 2010), have all been found to prevent cataract formation by maintaining normal levels of antioxidant enzymes and by preventing alteration of proteins in the lens. However, other compounds need to be evaluated as dietary supplements to prevent or retard the progression of cataractogenesis. Ellagic acid (EA) is considered to be one such compound merit ing evaluation.
4. Ellagic acid

It is now well established that a diet high in fruits and vegetables is associated with a reduced risk of oxidative-stress mediated diseases such as cancer, cardiovascular and neurodegenerative diseases (Halliwell, 1994). The beneficial effects of fruits and vegetables on health are attributed to their high levels of a wide variety of phytochemicals, of which phenolics constitute the greatest proportion. Polyphenolic compounds are widely distributed in the vegetable kingdom and are often encountered in daily life, being contained in food stuffs such as wine and fruits (Rice-Evans et al., 1996). Ellagic acid is a phenolic compound present in fruits and nuts, including blueberries, blackberries, raspberries, strawberries and walnuts (de Ancos et al., 2000; Anderson et al., 2001; Sellappan et al., 2002). Ellagic acid has a variety of biological activities including anti-oxidant (Priyadarsini et al., 2002), anti-inflammatory (Iino et al., 2002), anti-fibrotic (Thresiamma and Kuttan, 1996) and anti-cancer (Narayanan et al., 1999; Khanduja et al., 1999) properties. Ellagic acid is found to protect against ischemia/reperfusion-induced gastric injury (Iino et al., 2002) and carbon tetrachloride-induced liver fibrosis (Thresiamma and Kuttan, 1996). The anti-carcinogenic properties of ellagic acid include induction of cell cycle arrest and apoptosis (Narayanan et al., 1999), and inhibition of tumor formation and growth in vivo (Khanduja et al., 1999). Although the molecular mechanisms responsible for these effects remain largely unknown, the potent scavenging action may be due to the involvement of both superoxide anion and hydroxy anion (Priyadarsini et al., 2002; Iino et al., 2002).

In view of the antioxidant properties of ellagic acid, and since oxidative stress has been implicated in cataractogenesis, it was hypothesized that ellagic acid can prevent cataractogenesis. This hypothesis was assessed in the present study by evaluating the putative mechanisms governing the in vivo ability of ellagic acid to prevent or retard lenticular opacification in the well-known model of selenite cataract.
The efficacy of the compound was tested by evaluating various biochemical, and molecular parameters.

Hence, the following five-pronged approach was adopted:

**Chapter 1:** To assess the preventive effect of ellagic acid on selenite-induced cataractogenesis based on morphological and biochemical parameters.

**Chapter 2:** To study the regulatory effect of ellagic acid on the components of the redox system in selenite-induced cataractogenesis.

**Chapter 3:** To evaluate the effect of ellagic acid on lenticular calcium homeostasis in selenite-induced cataractogenesis.

**Chapter 4:** To evaluate the effect of ellagic acid in the prevention of selenite-induced apoptosis in epithelial cells of the ocular lens

**Chapter 5:** To analyze the efficacy of ellagic acid in the prevention of protein alterations in selenite-induced cataractogenesis.