# 1

# Introduction

"The eye speaks with an eloquence and truthfulness surpassing speech. It is the window out of which the winged thoughts often fly unwittingly. It is the tiny magic mirror on whose crystal surface the moods of feeling fitfully play, like the sunlight and shadow on a still stream".

– Henry Theodore Tuckerman

Eyes are the only implausible sensory organ through which the vista of green flora, glorious blue sky, magnificent waterfalls, splendid sea and many more exquisite types of scenery around us is interpreted. The loss of vision is like life without a soul. Cornea, sclera, iris, pupil, retina and lens comprise the key components of the human eye. The lens becomes opaque causing loss of visual acuity in cataract which is one of the widespread ocular diseases in the globe today.

Cataract is responsible for 51% of world blindness, which represents about 20 million people (WHO, 2010). One half of the unoperated cataracts belong to developing countries of Asia and Africa (Truscott, 2005) where an outsized economic and social consequences of the blindness is associated with extensive disability and surfeit mortality (Frick and Foster, 2003). According to Brian and Taylor (2001), the prevalence of cataract in India is three times that of the United States. As said by Tabin *et al.* (2008), the extraction of the cataract is the only treatment for cataract that is expensive and not without risks leading to a backlog of unoperated cataracts in the world and also mentioned about a global initiative called 'VISION 2020: the right to sight' that was launched in 1999 by the WHO and the international agency for the prevention of blindness to eliminate avoidable blindness by the year 2020.

Eyes restrain the lens whose unique cellular and molecular architecture enables it to transmit and focus light on the retina to interpret the

view in front of us (Bloemendal *et al*., 2004). The structural adaptations of the lens serve to reduce light scatter, facilitating it to function as 'biological glass' (Bassnett *et al*., 2011). In adult lens, the proliferation occurs exclusively at the equatorial region from a putative stem cell population (Oka *et al.,* 2010). The lens consists of tightly packed fibre cells with a specialized organization (Kuszak *et al*., 2004a).

The cytoplasm of ocular lens fibre cells encloses a concentrated multicomponent mixture of predominantly crystalline proteins (Hoehenwarter *et al*., 2006). The major types of lens crystalline proteins include α, β and γ crystallins that are stable and water soluble proteins accounting for about 90% of the total protein contents (Horwitz, 2000; MacRae, 2000). Due to very low protein turnover in lens, these are more susceptible to accumulate extensive post translational modifications (Hariharapura *et al*., 2013). According to Sharma and Santhoshkumar (2009), α crystallin begins to lose its protective action with aging and forms aggregates of crystallin leading to the accumulation of water insoluble modified crystallins that scatters light and in due course results in cataract.

Cataract is defined as lens opacification resulting in significant variations in the refractive index of the lens over distances similar to the wavelength of transmitted light (Wormstone and Wride, 2011). According to Michael and Bron (2011), age is the major risk factor associated with cataract development. Many studies reveal that cataract is a multifactorial ocular disease as it is associated with several risk factors like aging, hypertension, renal failure (Harding, 1991a), diabetes, sunlight UV exposure, dehydration, diet, oxidation of lens, lipid peroxidation (Kothadia *et al*., 2011), alcohol consumption (Wang *et al*., 2008; Hiratsuka and Li, 2001) smoking (Tan *et al*., 2008), and use of steroids (Klein *et al*., 2001). The development of cataract was also associated with genetic factors (Shiels and Hejtmancik, 2007) and post translational modifications of the ocular lens protein (Virgolici *et al*., 2007). One of the initiating events in the process of cataract formation is believed to be oxidation (Chitkara, 2004; Boulton and Saxby, 2004).

The effective etiological factors in the development of cataract are believed to be the oxidation reaction in the lens that may arise as a consequence to normal aging and those triggered by UV radiation (Abraham *et al.,* 2006). Oxidative stress is involved in the development of cataract that causes the oxidation of lens protein. Diminution of reduced glutathione (GSH), antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) with increase in age were most important factors involved in the generation of cataract (Graw, 2009a). Oxidative damage leads to the modification and degradation of protein, damage to DNA, mitochondria and eventually cell death (Doshna *et al*., 2005). It has been suggested by Fan *et al*. (2006) that the development of cataract is influenced by the ascorbate. According to Jacques *et al*. (2001), there is an increased risk of cataract due to the lack of dietary antioxidants.

The major component found in cell surface of animal cells is glycoproteins which are carbohydrate linked protein macromolecules. The oligosaccharide moieties of glycoproteins hexose, hexosamine, fucose and sialic acid play an important role in the protein stability, function and turnover (Wiese *et al*., 1997). Glycoprotein staining can be used to detect the carbohydrate moiety in the polypeptide. If the reactive oxygen species are not properly controlled, then they can cause severe damage to cellular macromolecules especially DNA (Barzilai and Yamamoto, 2004). Comet assay is performed to detect the extent of DNA damage.

As said by Suryanarayana *et al*. (2005) diabetes causes the increased level of oxidised DNA, proteins and lipids that are also limiting factors in various diabetic complications. Hydrogen peroxide produced through glucose autooxidation at a higher concentration results in cataract formation (Pastene *et al*., 2007; Javadzadeh *et al*., 2009). Aldose reductase and sorbitol dehydrogenase are the enzymes of polyol pathway that catalyse the conversion of glucose to sorbitol and sorbitol to fructose respectively. Osmotic swelling, alterations in membrane permeability, leakage of glutathione, myo-inositol,

generation of free radicals and hydrogen peroxide are due to the osmolyte sorbitol that primarily causes the diabetic complications such as cataract, retinopathy and nephropathy (Jung *et al*., 2011).

Retinal arterioles have anatomical and physiological similarity to cerebral ones. The blood vessels are seen directly only in the eye. The retinal vascular changes are associated with clinical complications namely hypertension and diabetes (Wong and Mitchell 2004; Liew *et al*., 2011; Wang *et al*., 2006). The prevalence of high blood pressure increases with aging and is one of the most common diseases of the world. Cataract surgeries are mostly performed in subjects of above 60 years who are usually affected by hypertension (Lloyd-Jones *et al*., 2009; Guimaraes 2002; Lira *et al*., 2001; Schein *et al.,* 2000a). Mahmood and Iqbal (2008) found that screening for diabetes and hypertension was an essential requirement for cataract surgery among the subjects as these medical conditions were found to be more prevalent in their study population.

Scanning electron microscopy (SEM) enables to study the surface morphology of the cataractous lenses. SEM coupled with energy dispersive X-ray spectroscopy reveals the elemental composition of the specimen. Vrensen *et* al. (1992), investigated the ultrastructure of fibre membranes in human lenses varying in age from premature to 40 years by Scanning and Transmission electron microscopy. According to Krafft and Sergo (2006), various biomedical issues are investigated by Infrared (IR) and Raman spectroscopy which are vibrational spectroscopic techniques that provide information on the chemical composition and molecular structures in cells and tissues.

**Objectives of the study**

With the aforementioned information, the present research work was designed with the following objectives:

- To analyse the changes in soluble protein and insoluble protein in the eye lens of the cataractous subjects

- To analyse any correlation between selected biochemical parameters and insoluble protein content in eye lens
- To study the eye lens architecture in cataractous subjects
- To study post translational changes in eye lens proteins leading to cataract

Accordingly, the study was designed with six groups of apparently normal cataract men (ACM), apparently normal cataract women (ACW), diabetic cataract men (DCM), diabetic cataract women (DCW), hypertensive cataract men (HCM) and hypertensive cataract women (HCW) to analyse the changes in the status of soluble and insoluble proteins. Correlation between selected biochemical parameters and insoluble protein was analysed to show the influence of these parameters with the development of cataract. The biochemical parameters analysed in eye lens included in the study were enzymatic and non enzymatic antioxidants, lipid peroxidation, protein carbonyl, protein sulphydryl, nitrite, marker enzymes of polyol pathway, membrane bound enzymes, proteins, total cholesterol, DNA, RNA, glucose, fructose and glycoproteins. The extent of DNA damage was carried out in six groups of cataractous lenses by comet assay. The histopathological analysis was done in all the six groups of cataractous subjects. Morphology and elemental composition of the cataractous lenses were examined by scanning electron microscopy with energy dispersive X ray spectroscopy (EDAX). Spectral analysis by Fourier Transform Infrared (FT-IR) and Raman Spectroscopy was carried out to identify the functional groups in the lens proteins of the cataractous subjects which might indicate any post translational changes occurring in the cataractous lens.