1. **GENERAL INTRODUCTION**

Any opacity in the lens or its capsule is known as “Cataract”. Cataracts are described as an opacification (cloudiness) of the lens that leads to the scattering of light entering the eye and a loss of vision. Cataracts, which affect more than 50 million people (Taylor and Davies, 1987), are the most common cause of blindness in the world. There have been significant advances in surgical techniques and refinement of intraocular lens implants which have benefited cataract patients (Zigler et al., 2003). It is one of the major causes of visual disability and blindness throughout the world (Spector, 1974).

Among various types of cataracts, “senile cataract, i.e. opacity due to old age, account for the vast majority of the cases, in which a number of factors superimpose normal aging process. Most forms of cataract are clearly due to changes in the chemical environment of the lens (Bito, 1977). Although it is difficult to identify the initiating cause and precataractous alterations, understanding of the lens structure, metabolic processes and related fluids e.g. aqueous/vitreous humor may represent the changes induced due to opacity, which in turn, might help in determining the possible preventive measures.

In the developing world, where medical services are often unavailable or extremely limited, the situation is worse. In India alone around 30 million people suffer from cataract (Bhat, 1987). Developing anti-cataract agents has been difficult because cataract is not a single disease with a single aetiology. There are three major categories of cataract (nuclear, cortical, and posterior sub-capsule), each of which is multifactorial in aetiology and highly variable in severity and rate of progression (Zigler et al., 2003). In order to study cataracts and possible treatments for cataracts, a
number of *in vivo* animal models have been developed. For example, administration of L-buthionine sulfoximine, a specific inhibitor of glutathione biosynthesis, to preweanling mice (aged ≤ 12-days) provides a model system for the induction of cataracts by depletion of lens glutathione (Calvin *et al*., 1986). The strong sulfhydryl oxidant, selenite, has been used to produce cataract in rats (Shearer *et al*., 1992). This selenite-induced cataract model has been extensively utilized to demonstrate that calpain-induced proteolysis causes truncated crystallins to precipitate and scatter light. Other *in vivo* experimental animal models such as hyperbaric oxygen, and UVA light, have also been utilized to investigate the mechanism of formation of human senile nuclear cataract (Giblin, 2000).

Oxidative stress, defined as an excess of pro-oxidants relative to antioxidants and a key factor in the gradual loss of lens transparency, is implicated in the initiation of maturity onset cataract which appears late in life and is probably not associated with congenital conditions or other diseases, such as diabetes (Spector, 1995). Evidence from epidemiological studies, model systems and human lenses obtained after cataract surgery, has indicated a role for oxidation in this opacification process. This has fuelled interest in the role of diet and dietary supplements in slowing down the progression of cataract. Halliwell & Gutteridge (1999) concluded that dietary antioxidants have a significant impact on cataract development based on the epidemiological evidence. Experimental studies have shown that, pre-treatment of the plant antioxidant, quercetin, at concentrations of 30 μM for 24 h, inhibited hydrogen peroxide-induced oxidation of the rat lens (Sanderson *et al*., 1999; Cornish *et al*., 2002).
1.1 CATARACTS

1.1.1 Definition and Symptoms

The function of the lens is to transmit and focus light on the retina, hence it must be transparent. When oxidative damage to the lens and its proteins becomes extensive, the lens becomes sufficiently cloudy to obstruct vision, and the individual is said to have a cataract. Alternatively, events that cause loss of order and induce abrupt fluctuations in refractive index result in increased light scattering and loss in transparency, commonly called cataract (Bunce, 1994b). Defined as a clinically significant opacity of the lens, cataract has been an area of intense research in the last few decades. The first symptom of cataract is blurred vision. A cataract blocks some light from reaching the retina and distorts the light being focused on the retina.

1.1.2 Types of cataract

There are three major forms of cataracts (nuclear, cortical, and posterior sub-capsule). Cortical cataracts are those opacities that originate in the outer layers of the lens. They typically display over-hydration and eventually liquefaction of the lens fibre cells secondary to electrolyte imbalance (Bunce, 1994b). Nuclear cataract occurs when modification and aggregation of lens structural proteins create light-scattering zones in the central region of the lens (Bunce, 1994b). Posterior subcapsular cataract (PSC) is described as an accumulation of abnormal epithelial cells at the posterior pole of the lens and just inside the capsule (Bunce, 1994b). Instead of differentiating into elongated fibre cells at the equator in the normal manner, the cells assume an irregular form and are displaced toward the posterior pole. This type of cataract probably arises as a result of damage to DNA. Many individuals will develop pure cortical, nuclear,
or PSC cataracts, but mixed cataracts are also quite common (Bunce, 1994b). It has been determined that cataract formation in these three forms is a complex and multifactorial process (Spector, 1984). High molecular weight protein aggregates, accumulation of water insoluble proteins (Harrington et al., 2004) and increase in intracellular calcium content (Marcantonio et al., 1986) have all been implicated in the pathogenesis of cataracts.

1.1.3 Factors Causing Cataract

Truscott (2005) proposed that the lens was analogous to an island because, like an island, the lens was coupled to the rest of the world only via a circulating fluid medium. However, the lens is affected by somatic and developmental factors. For example, although cataract is overwhelmingly a disease of the elderly, developmental impacts at earlier times, such as early childhood may be important. Evans et al. (1998) reported that the birth weight at one year of age was a predictor of later cataract development. They speculated that alterations in lens growth or differentiation were key stages leading to abnormal structural or functional changes that predispose to cataract development. Also, external factors may be important in cataract development. Harding (1991) postulated that diarrhoea may be a risk factor for cataract based on the observation that cataract was more prevalent in India. Age-related cataract is a condition characterized by multiple mechanisms and multiple risk factors including oxidation, osmotic stress, and chemical adduct formation (Bunce et al., 1990). The factors contributing to age-related cataractogenesis are a combination of pathological and normal aging processes. Chronic oxidative stress is widely believed to be a major factor in the aetiology of age-related cataract (Zigler et al., 2003). For example, Anderson et al. (1994) reported that free radicals induce lipid peroxidation that leads to cataract formation. This was supported by several clinical,
basic and epidemiologic studies (Kurusheva et al., 1997). Other environmental, behavioural, physiological, and biochemical variables are also associated with types of cataract (nuclear, cortical, posterior subcapsular, and mixed) (Mohan et al., 1989). Lenses age slowly, a process that begins in the second decade of life and progresses more rapidly in middle and older age. The aging process results in the accumulation of light scattering opacities that interfere with vision; this usually occurs during the sixth or seventh decade of life, but may occur earlier or later depending on the individual (Mares, 2004). Since age is the major risk factor for cataract, understanding changes in the lens with aging may well provide an insight into those processes that are responsible for the onset of cataract in later life. Many alterations take place in the lens with age. These include changes in elastic properties and total ion content; increased fluorescence and nuclear light scattering; and a possible decrease in the refractive index of the nucleus (Truscott, 2005). Also, there are noticeable changes in the composition of membrane proteins with age. Old fibre cell membranes have a very high cholesterol content, which means that these bilayers are very rigid (Broekhuysen, 1981). The alterations of lipid composition consequently decrease the membrane fluidity – older membranes being more like butter than olive oil (Truscott, 2005). Roberts (2001) proposed that young and adult eyes avoid damage by intense ambient light because they are protected by a very efficient antioxidant system. However, after middle age, there is a decrease in the production of antioxidants and antioxidant enzymes. At the same time, fluorescent chromophores (lipofuscin) accumulate to concentrations high enough to produce reactive oxygen species (ROS) (Roberts, 2001). Therefore, during the aging process, the (outer) epithelial cells will be most vulnerablely damaged through modification of DNA, while the (inner) fibre membranes can be photo-chemically damaged through modification of certain amino
acids (such as histidine, tryptophan, and cysteine) (Roberts, 1984) in the main intrinsic membrane proteins and through damage to the lipids (Roberts et al., 1985). Such damage will result in a change in the refractive index of the lens material, leading to aggregation and a predisposition to age-related cataract, as proposed by Benedek in his theory of cataract formation (Benedek, 1971). Sunlight seems to be an important environmental factor causing cataract, which may involve photo-oxidative processes. An increased risk of cataract was found for job locations in the sunlight, leisure time activities in the sunlight and a history of wearing a hat in summertime (Anonymous, 1991). Mohan et al. (1989) found an increased risk of cataract (all types) with decreased cloud cover at place of residence. Leske et al. (1991) further reported that occupational exposure to sunlight was a risk factor for nuclear cataract. Tarwadi & Agte (2004) recognized that photo-oxidative stress from x-ray or UVB radiation of sunlight (wavelength of 290-320 nm) was a cause for cataract. Lifestyle-related factors, such as cigarette smoking, appear to provide an additional oxidative challenge associated with depletion of antioxidants as well as with enhanced risk for cataract formation (Taylor et al., 1995). Leske et al. (1991) also reported that smoking was a risk factor for nuclear cataract. Alcohol consumption appeared to be a minor risk factor (Truscott, 2005). Another recognized cause for cataract is exposure to environmental pollution (Tarwadi & Agte, 2004). Diet also plays an important role in cataract formation. Mohan et al. (1989) reported that diets low in selected nutrients increased risk for posterior subcapsular, nuclear, and mixed cataracts. They also found an increased risk of cataract (cortical, nuclear, and mixed) with the use of cheaper cooking fuels, and with lower levels of an antioxidant index based on red blood cell levels of glutathione peroxidise and glucose-6-phosphate dehydrogenase and plasma levels of ascorbic acid and vitamin E (posterior subcapsular and mixed). Poor
education and lower socioeconomic status are associated with poorer nutritional states and were also significantly related to increased risk for cataract formation (Leske et al., 1991; Taylor et al., 1995). For example, The Italian-American Cataract Study Group (1991) reported an increased risk of all types cataract for persons with less than a high school education. Excessive caloric intake was also a factor contributing to cataract. Diabetes increases the likelihood of cataract three- to four-fold. Leske et al. (1991) reported that diabetes increased the risk of posterior subcapsular, cortical, and mixed cataracts. Obesity, defined as more than 20% overweight, is a major risk factor for non-insulin-dependent, or type II, diabetes and Leske et al. (1991) found that body mass index was a risk factor for nuclear cataract. Higher blood pressure was also found to increase the risk of nuclear and mixed cataract (Mohan et al., 1989). Therefore, weight control can be recommended as a prudent, safe, economic, and effective means of lowering risk probability for diabetes and the associated complication of cataract (Bunce et al., 1990). Certain pharmaceutical substances and possibly acute episodes of dehydration are other recognized causes for cataract (Bunce et al., 1990). For example, aspirin, even when use less than once a month, increased the risk of posterior subcapsular and mixed cataract (Mohan et al., 1989); gout medications increased mixed cataract risk; and oral steroid therapy increased posterior subcapsular cataract risk (Leske et al., 1991). The Italian-American Cataract Study Group (1991) also reported that a history of cortisone use was a risk factor for subcapsular cataract. Family history and use of eyeglasses by age 20 years, which is an indicator of myopia, which increase risk of mixed cataract (Leske et al., 1991). The Italian-American Cataract Study Group (1991) reported that a positive family history of cataract (which may involve changes in gene expression) was a risk factor for posterior subcapsular, cortical, and mixed cataracts and that increased serum
level of uric acid was a risk factor for PSC. However, a decreased risk of cataract was found for persons with a positive history of arthritis (posterior subcapsular, nuclear, and mixed) and increased handgrip strength (mixed). The results support a role for nutritional, medical, personal, and other factors in cataractogenesis.

In Ayurvedic terminology, cataract is termed Linga Nasha or Timira. According to Ayurvedic principles, such a condition develops due to the aggravation of Vayu. Vayu dries up things. Here, aggravation of Vayu dries up the liquid that makes the lens and the retina supple. Hence, Ayurvedic treatments first correct the Vayu composition. Medicinal Plants used in Ayurvedic eye drops formulation are rich source of tannin and tannin like compounds. Antioxidant and antimicrobial properties of ayurvedic eye drops are attributed to the presence of tannins and tannin like compounds. Now-a-days, people increasingly prefer ayurvedic eye drops because it is safe and relatively free from side effect and adverse reactions. Ayurvedic eye drops are known to show anti-inflammatory, antioxidant and antimicrobial activity due to their tannin content in aqueous extract. Ayurvedic eye drops preparation contains aqueous extracts of different herbs. In ophthalmology the area where ayurvedic treatment is potential are early stages of cataract, diabetic retinopathy, myopia, logophthalamos etc. all these diseases are difficult to cure with modern medicines (Bhavana et al., 2010). Cataract remains the leading cause of visual disability, and it contributes 50% blindness worldwide (Mitra et al., 2000). Cataract blindness is the major cause of preventable blindness worldwide especially in the developing countries of Africa and Asia (WHO, 2005). The potential role of antioxidants in preventing various diseases is well documented. There are reports suggesting the beneficial effects of vitamins C and E preventing cataract by virtue of their antioxidant property (Watkins R., 2002). Various herbal drugs such as Osmium sanctum, Emblica officinalis, Ginkgo biloba, and green
tea were shown to delay the onset and progression of cataract development in experimental animals (Van der Pols, 1999; Reddy et al, 2001). The anticataract efficacy of these preparations was mainly attributed to their antioxidant potential.

1.2 MEDICINAL PLANTS WHICH ARE USED IN CATRACT AND OPHTHALMIC PROBLEMS

1. *Emblica officinalis* (Amla) family Euphorbiaceae is used in the formulation Ophthacare which is herbal eye drop preparation containing basic principles of different herbs Clinical trial was conducted in patients suffering from different ophthalmic disorders namely, conjunctival xerosis, conjunctivitis, acute dacryocystitis, degenerative conditions and postoperative cataract patients with an herbal eye drop preparation (Satyarati et al, 1976).

2. *Dhatura metel* (Datura): family Solanaceae has different alkaloids which are responsible for anticatarrhal activities (Khan 2009).

3. *Desmodium gangeticum* (Desmodium): family Fabaceae, the extract of root part is used in anti-catarrhal activities [Thakur et al., 1989.].

4. *Aegle marmelos* (Bael): family Rutaceae, Leaf is used as anticatarrha activities, and ophthalmic. Seed show antimicrobial activities which is used in preparation of eye drops (Warrier 1993).

5. *Eclipta prostrata* (Eclipta): family Asteraceae The alcoholic extract of entire plant has been reported to anticatarrhal activities (Husain 1993).

6. *Curcuma longa* (Turmeric): turmeric and curcumin are effective against the development of diabetic cataract. Further, turmeric may be explored for
anticataractogenic agents that prevent or delay the development of cataract (Anonymous 1).

7. **Ocimum sanctum (Tulsi):** Leaves of Ocimum sanctum delayed the onset as well as the subsequent maturation of cataract significantly in 2 models of cataract i.e. galactosamic cataract in rats and naphthalene cataract in rabbits (Sharma et al., 1998).

8. **Trigonella foenum–graecum (fenugreek) seeds** have Anti-cataract activity in alloxan diabetic rats (Vats et al., 2004).

9. **Pterocarpus marsupium** bark (Fabaceae) extract have anticataract activity in alloxan diabetic rats (Vats et al, 2004).

10. **Aralia elata:** The water extract of *Aralia elata* (*Aralia* extract) has been used in rat lens cultures and a rat model were used to observe anticataract activity treatment with *Aralia* extract at a concentration of 1 mg/ml lowered lens opacity by 36.4 and 31.3% after 24 and 48 h, respectively (Young-Shin Chung et al., 2005).

11. **Cheilanthes glauca (Cav.)** Adiantaceae Doradilla Doradilla extracts exhibited a strong antioxidant capacityOnly rutin was able to partially inhibit cataract formation in high glucose conditions (Edgar et al., 2007).

12. **Allium cepa:** The plant is cultivated throughout India. Onion bulb and leaves are the important part of diet. Ether soluble fraction of onion (0.25mg/kg, orally) has been exhibited potent antioxidant activity which show anticataract properties (Detroja et al., 2008).

13. **Azadirachta indica (Neem):** Aqueous extracts of the plants exhibited anticataract potentials which were evaluated in vitro in rat lenses. AR inhibitory
The activity of the aqueous extract of different plants was calculated considering the AR activity of normal rat lenses as 100%. The concentration of the plant extract that showed maximum AR inhibitory activity was selected to further study its effect on galactose-induced lens swelling and polyol accumulation in vitro (Halder 2003).

14. *Withania somnifera* (Ashwagandha): extracts of the plants exhibited anticataract potentials which were evaluated in vitro in rat lenses (Halder 2003)

### 1.3 OBJECTIVES OF RESEARCH WORK

- To screen selected medicinal plant for its anticataract activity using *in vivo* model.
- Activity guided fractionation leading to a possible isolation of active compound/s and their pharmacological evaluation for anticataract activity.
- To study the possible mechanism of action of the isolated compound/s.

### 1.4 STRUCTURE OF THE THESIS

**Chapter 1**
This chapter describes the introduction related to cataract, its types, factor causing cataract, medicinal plants used for the treatment of cataract objectives of the proposed research work.

**Chapter 2**
Detailed description of the plant selected for the study *Andrographis paniculata*

**Chapter 3**
This chapter describes the materials and methods used for the research work.
Chapter 4
This chapter describes the results of the study of *in vivo* animal model, phytochemical analysis of the plant and biochemical parameters

Chapter 5
This chapter describes the discussion of the results obtained for the anticataract activity of *A. paniculata*, its mechanism of action responsible for its activity.

Chapter 6
This chapter summarizes the outcome of the entire work and conclusions drawn from the results.