# 4

# Results and Discussion

Cataract is one of the leading causes of visual impairment prevailing worldwide. Currently, prevention of cataract is feasible only by means of surgery (Lin *et al.,* 2013). Cataract removal by surgery has improved with the development of new technologies. But this type of modern surgery seems to be very expensive and may impose a challenge for the economic stability of health care systems (Rao *et al.,* 2011). Due to this, reverberations with regard to surgery and a rapid rise in elderly population may probably cause a backlog of unoperated cataracts in the world (Tabin *et al.,* 2008; Weikel *et al.,* 2013).

Lens opacification burgeons in persons with diabetes mellitus which occurs much earlier when compared to senile cataract and results in increased rates of surgery at a young age (Jeganathan *et al.,* 2008; Murtha and Cavallerano, 2007). Hypertension is a risk factor for transience in elderly people that affects about 70 percentage of this population (McDonald *et al.,* 2009) and high blood pressure is found to be associated with cortical cataract (Leske *et al.,* 1999).

The role of oxidative stress in the pathogenesis of cataract is a crucial factor (Truscott, 2005). Many *in vitro* studies have reported the efficacy of antioxidants in diminishing oxidative damage and lipid peroxidation that are considered to be significant intermediary steps in the development of cataract (Fernandez and Afshari, 2008; Lin *et al.,* 2005). According to Moemen *et al.* (2014), free radicals activate the generation of advanced glycation end products (AGEs) and progression of cataract that are considered to be slow processes. But accumulation of these AGEs is observed to be hastened in diabetes. Hashim and Zarina (2006), opined that cataract is a repercussion of backlog of damaged lens proteins. The reduced state of lens protein is imperative for the proper functioning of the eye lens and these proteins are maintained in the reduced state due to the generation of reducing power (Halliwell, 1997). The unique structure and solubility of ocular lens proteins should be retained throughout the life of an individual so as to preserve the transparency of ocular lens (Moreau and King, 2012).

Hence, in the present investigation an effort was taken to compare the status of proteins and other biochemical parameters in the eye lens extracted from cataractous subjects with and without clinical complications. The outcome ofthe present study are presented in five phases under the following sub titles:

## Phase I

### 4.1. Demographic Characteristics and Life Style of the Selected Cataractous Subjects

4.1.1. Demographic Characteristics of the Selected Cataractous Subjects

4.1.2. Life Style of the Selected Cataractous Subjects

## Phase II

### 4.2. Profile of the Selected Cataractous Subjects

4.2.1. Age

4.2.2. Height, Weight and Body Mass Index

4.2.3. Systolic and Diastolic Pressure

## Phase III

### 4.3 Biochemical Assessment and their Correlation with Cataract Development

4.3.1. Weight, Thickness and Diameter of Cataractous Eye Lens

4.3.2. Status of Proteins in Cataractous Eye Lens

4.3.3. Activity of Enzymatic Antioxidants in Cataractous Eye Lens

4.3.4. Status of Glutathione System in Cataractous Eye Lens

4.3.5. Status of Non-Enzymatic Antioxidants in Cataractous Eye Lens

4.3.6. Lipid Peroxidation Status in Cataractous Eye Lens

4.3.7. Nitrite Levels in Cataractous Eye Lens

4.3.8. Status of Protein Carbonyl and Protein Sulphydryl in Cataractous Eye Lens

## Phase I

### 4.1 Demographic Characteristics and Life Style of the Selected Cataractous Subjects

#### 4.1.1 Demographic Characteristics of the Selected Cataractous Subjects

Several studies indicated that low socioeconomic status were associated with increased risk of cortical cataract (Hennis *et al.,* 2004; Delcourt *et al.,* 2000; Hiller *et al.,* 1986). In a study conducted by Krishnaiah *et al.* (2005), they observed that the incidence of any type of lens opacities noticeably increased in the population with extremely low socioeconomic status. Rim *et al.* (2014) observed an association of lower income grade with that of pure cortical and mixed type of cataracts.

The present investigation was carried out in 280 cataractous subjects who were categorised into six groups. The demographic characteristics include socio economic status, marital status and employment status which is presented in table 9. Among the selected 280 subjects for the study, 270 of them were married. In diabetic cataract men (DCM) group all the subjects under investigation were married. Two of them from each group of apparently normal cataract men and women were observed to be unmarried. Two and three of the subjects from hypertensive cataract men and women were found to be unmarried respectively. But the results seemed to have no influence of marital status on cataract development.

Influence of unemployment in cataract progression was observed in all the subjects in the present investigation and the results are presented in table 9. Maximum unemployment was observed in apparently normal cataract men group (31.92%) and least was observed in apparently normal cataract women group

(13.04%). Among 50 subjects each from DCM and DCW groups, 8 and 10 of them were found to be unemployed respectively. 8 out of 43 subjects and 11 out of 44 subjects from HCM and HCW groups respectively were observed to be unemployed.

Economic status of cataractous subjects is indicated in table 9. The family income of less than Rs.5,000 indicated 19.57, 25.53, 28, 30, 27.91 and 29.55 percent respectively for 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). In the category of Rs.5,000 – Rs.10,000 scale, apparently normal cataract women (ACW) showed the least percent (38.3) of family income. It was observed that the family income obtained showed lesser percentage among the last grade of greater than Rs. 10,000. This indicated a lower economic status among cataractous subjects as the percentage was much higher in the first two categories when compared to the category of greater than Rs.10,000. Diabetic cataract men (DCM), diabetic cataract women (DCW), hypertensive cataract men (HCM) and hypertensive cataract women (HCW) recorded only 4, 8, 11.62 and 6.82 percentages respectively in the category of greater than Rs.10,000.

### 4.1.2 Life Style of the Selected Cataractous Subjects

The results of several research works have indicated a relation between diet and cataract progression. The diet designed by the researchers in many studies exclusively comprised of antioxidant vitamins A, C, E lutein and zeaxanthin (Zhao *et al.,* 2014; Klein *et al.,* 2008; Rautiainen *et al.,* 2010; Zheng *et al.,* 2013).

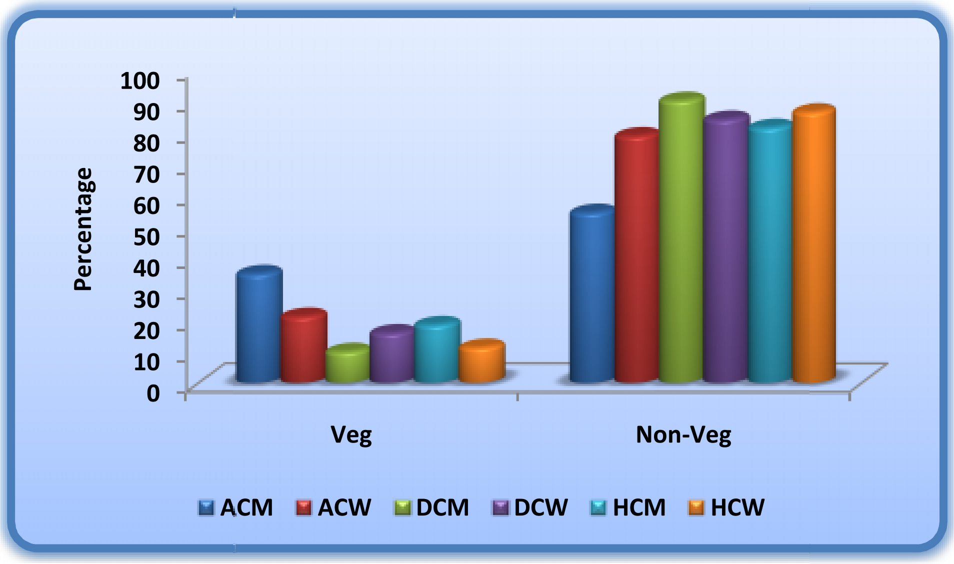
Figure 14 depicts the diet style of cataractous subjects. It is evident that most of the cataractous subjects were non vegetarians. The percentage of vegetarians in apparently normal cataract men (ACM), apparently normal cataract women (ACW), diabetic cataract men (DCM), diabetic cataract women (DCW), hypertensive cataract men (HCM) and hypertensive cataract women

(HCW) groups were 34.78, 21.28, 10, 16, 18.6 and 11.36 percent respectively. The percentage of non vegetarians in 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) were 54.35, 78.72, 90, 84, 81.40 and 86.36. The percentage of vegetarian diet was found to be more among apparently normal cataractous subjects when compared to other groups.

The consumption of fruits and raw vegetables in daily and weekly duration was also recorded among the cataractous subjects and is illustrated in figure 15. Vegetables and fruits are rich sources of antioxidants and vitamins. It can be inferred that the consumption of fruits and raw vegetables were greatly decreased in case of diabetic cataract men (DCM) and diabetic cataract women (DCW) when compared to apparently normal cataract men (ACM), apparently normal cataract women (ACW), hypertensive cataract men (HCM) and hypertensive cataract women (HCW). Only 2% and 6% of the subjects from DCM group consumed fruits and raw vegetables daily whereas 41% and 26% from ACM group consumed fruits and raw vegetables daily. According to Liu *et al.* (2014), individuals with more lutein and zeaxanthin levels in blood may stick onto a healthier diet which is inversely associated with the development of age related cataract. Dietary caloric restriction probably delays age related telomere shortening of lens epithelial cells and protect these cells from hydrogen peroxide damage as opined by Li *et al.* (1998) and Pendergrass *et al.* (2001).

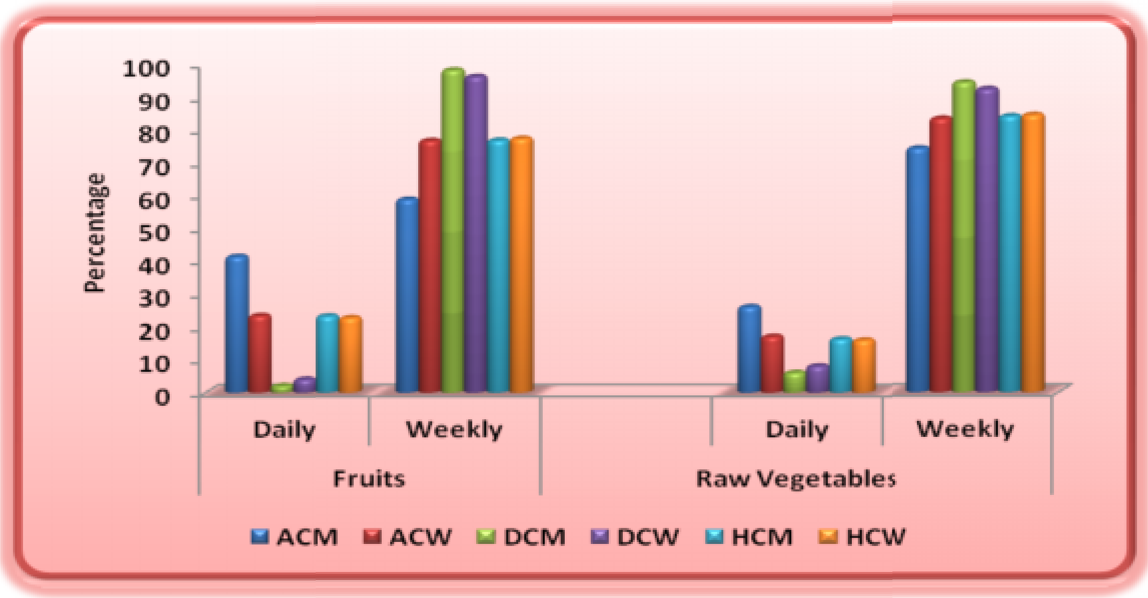
## Figure 14

### Dietary habit of the selected cataractous subjects




## Figure 15

### Consumption of fruits and raw vegetables by the selected cataractous subjects

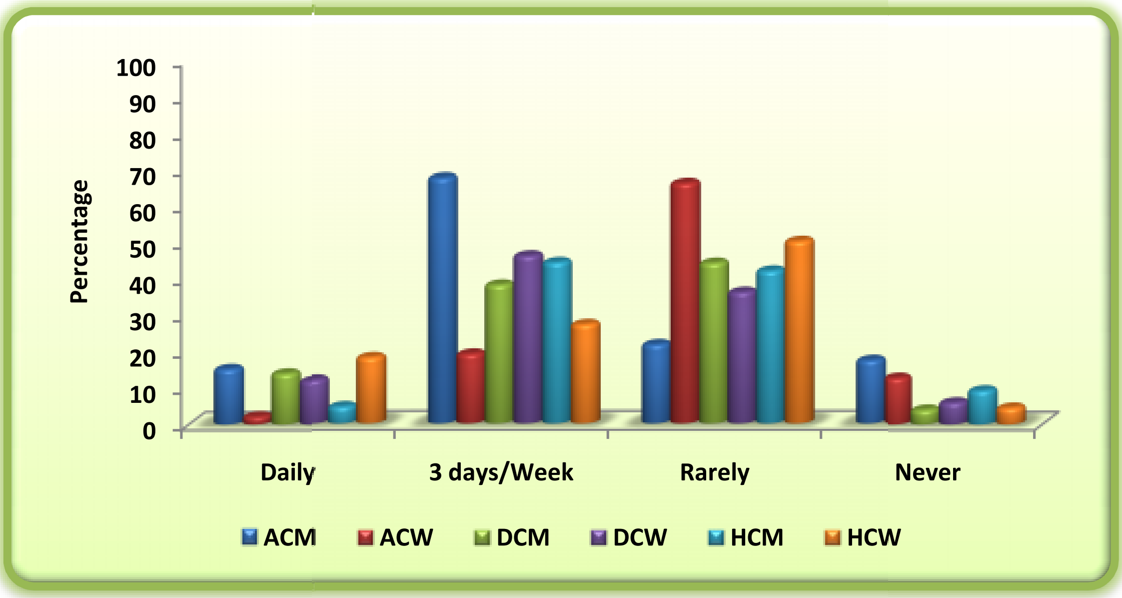



ACM : Apparently Normal Cataract Men
ACW : Apparently Normal Cataract Women
DCM : Diabetic Cataract Men
DCW : Diabetic Cataract Women
HCM : Hypertensive Cataract Men
HCW : Hypertensive Cataract Women

Figure 16 represents the duration of exercise among cataractous subjects. The physical activities performed by most of the subjects were predominantly walking. 67.4% of subjects from ACM group were found to perform exercise 3 days/week whereas only 19.2% from ACW group performed exercise for the same duration. Thirty eight percent of diabetic cataract men (DCM) performed physical activity 3 days per week while 46% percent of diabetic cataract women (DCW) performed physical activity 3 days per week. 9.3% and 4.6% of subjects from HCM and HCW groups reported physical inactivity.

**Figure 16**

**Duration of exercise by the selected cataractous subjects**

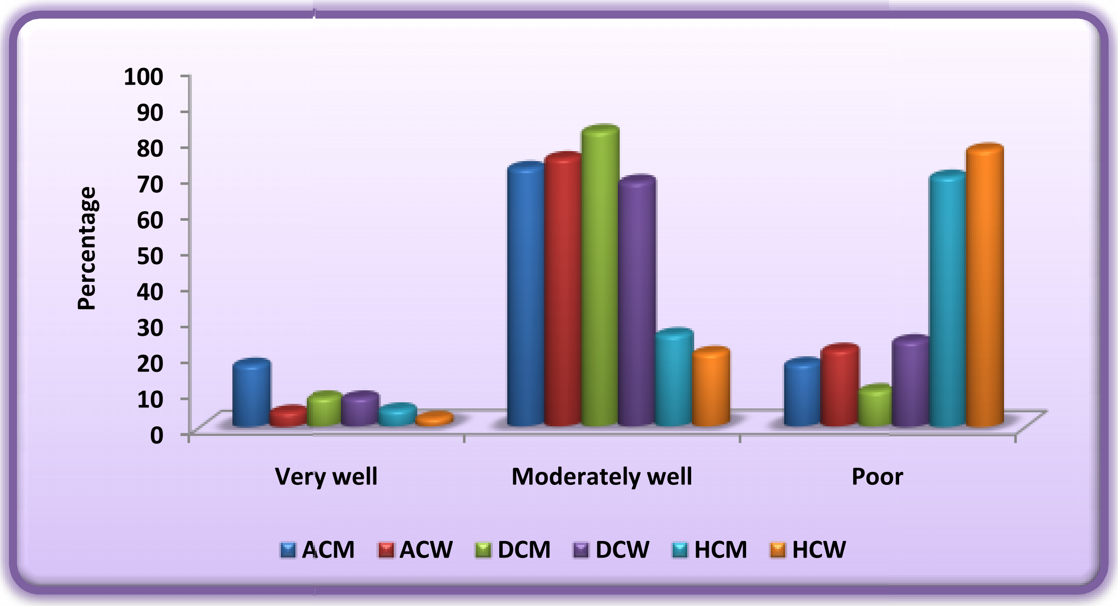



ACM : Apparently Normal Cataract Men
ACW : Apparently Normal Cataract Women
DCM : Diabetic Cataract Men
DCW : Diabetic Cataract Women
HCM : Hypertensive Cataract Men
HCW : Hypertensive Cataract Women

Stress handling by cataractous subjects of different groups is depicted in figure 17. Hypertensive cataract men (HCM) (69.8%) and hypertensive cataract women (HCW) (77.3%) reported to handle stress very poorly. 71.4% of apparently normal cataract men (ACM), 74.5% of apparently normal cataract women (ACW), 82% of diabetic cataract men (DCM) and 68% of diabetic cataract women (DCW) were found to handle stress moderately well. Among all the groups, 17.4% of apparently normal cataract men were found to handle stress very well.

**Figure 17**

**Handling stress by the selected cataractous subjects**




ACM : Apparently Normal Cataract Men
ACW : Apparently Normal Cataract Women
DCM : Diabetic Cataract Men
DCW : Diabetic Cataract Women
HCM : Hypertensive Cataract Men
HCW : Hypertensive Cataract Women

The health behavioural risk variables like exercise and stress did not show any significant association with the incidence of cataract (Rim *et al.,* 2014). Rahman and Salek (2009), suggested that performing regular and different kind of exercises has a good response towards the health of an individual especially to control hypertension. Paunksnis *et al.* (2006), in their study indicated the role of physical activity in cataract development where less active subjects had a greater probability for cataract than the active patients. They also observed that with a decrease in physical activity there occurs an increase in cortical cataract of the left eye. Similar trend was observed in the study conducted by Williams (2009), where physical activity was inversely associated with cataract.

## Phase II

### 4.2 Profile of the Selected Cataractous Subjects

#### 4.2.1 Age

The profile of the selected cataractous subjects in the present investigation is presented in table 10. Subjects from diabetic cataract men (DCM) and diabetic cataract women (DCW) groups were found to develop cataract significantly at an earlier age as compared to subjects from apparently normal cataract and hypertensive cataract groups indicating early incidence of cataract in subjects with clinical complications. The age of apparently normal cataract subjects was found to be significantly higher than diabetic cataract subjects and hypertensive cataract subjects.

Bron *et al.* (1998) reported that individuals with diabetes are likely to develop mature cataract about 10 years earlier. In the present investigation, diabetic and hypertensive cataractous subjects were from sixth decade whereas subjects from apparently normal cataract belonged to seventh decade. This suggested that cataract subjects with clinical complications seemed to develop cataract earlier than those of senile cataract. Krishnaiah *et al.* (2005) suggested a positive association between age and development of cataract which was found to be more prevalent among women.

Several studies have reported that opacification in eye lens occurs with increase in age and is considered the major risk factor for cataract (Wang *et al.,* 2004; Vinson, 2006; Hashim and Zarina, 2007). Since age is the most common factor for the development of cataract, it is often referred to as senile cataract (Taylor *et al.,* 1995) and can be an aging marker for an organism (Harding, 2002). Chen *et al.* (2011), indicated the association of cataract with older age especially ≥ 75 years in men and ≥ 70 years in women. Sobti and Sahni (2013), reported that prevalence of cataract greatly increased with the rise in age. They recorded an increase from 5% in fourth decade age group to around 77% in those greater than and equal to eighty years.

As an individual grows older, he is liable to suffer from exposure to many confounding factors namely oxidative damage and chronic exposure to radiations. Mixed types of lens opacities are found to be most probable in an elderly person (Rim *et al.,* 2014). Congdon *et al.* (2004) observed that 17 percent of Americans with age greater than 40 years had cataract and 5 percent of them had removed cataract.

According to Harocopos *et al.* (2004) age was a crucial factor for cataract development and they suggested that at younger age, the probability of exposure to oxidative stress and repercussion of liquefaction of vitreous body for an individual may be more.

**4.2.2 Height, Weight and Body Mass Index**

Table 10 indicates the height, weight and Body Mass Index (BMI) of the cataractous subjects. The height and weight of 280 cataractous subjects were documented in cm and kg respectively. According to WHO Expert Consultation (2004), the individuals were grouped as underweight who had a BMI < 18.5kg/m$^2$, normal whose BMI ranged between 18.5kg/m$^2$-25kg/m$^2$, overweight with a range 25kg/m$^2$–30kg/m$^2$ and obese for whom BMI was > 30kg/m$^2$. All the groups exhibited a BMI in the range from 24.95 to 26.92 indicating that they were borderline to normal and overweight. None of the groups showed a low BMI. A relation of high BMI to that of cortical lens opacity was observed in many studies (Sabanayagam *et al.,* 2011; Younan *et al.,* 2003), whilst low BMI was found to be associated with nuclear cataract (Kuang *et al.,* 2005).

Williams (2009), proposed a decreased risk of cataract with lower BMI in men But in the present study none of the cataractous groups showed low BMI. In a study conducted by Sobti and Sahni (2013), an inverse association was found between BMI and cataract. Increased height and abdominal adiposity in men with cataract were believed to act as independent risk factors for the progression of cataract (Schaumberg *et al.,* 2000).

A significant difference in height, weight and BMI were observed in apparently normal cataract men (ACM) and apparently normal cataract women (ACW). A similar trend was also documented in diabetic cataract men (DCM) and diabetic cataract women (DCW). In the present investigation, a significant decrease in height, weight and BMI were observed in subjects from HCW group when compared to those subjects from HCM group.

### 4.2.3 Systolic and Diastolic Pressure

Chen *et al.* (2011) hypothesised in their study that diastolic pressure had an indirect influence on the cataract development because a significant difference was observed in diastolic pressure between people with and without cataract owing to confusion between age related changes in diastolic pressure. Many epidemiological studies revealed the influence of blood pressure on cataract progression (Tavani *et al.,* 1995; Klein *et al.,* 1995; Hiller *et al.,* 1986; Chen *et al.,* 1988).

Table 10 presents systolic blood pressure and diastolic blood pressure of cataractous subjects with and without clinical complications. The systolic and diastolic pressure were significantly increased in subjects from hypertensive cataract men (HCM) and hypertensive cataract women (HCW) groups when compared to those of apparently normal cataract men (ACM), apparently normal cataract women (ACW), diabetic cataract men (DCM) and diabetic cataract women (DCW). There was no significant difference among apparently normal cataract men (ACM) and apparently normal cataract women (ACW), diabetic cataract men (DCM) and diabetic cataract women (DCW).

## 4.3 Biochemical Assessment and their Correlation with Cataract Development

### 4.3.1 Weight, Thickness and Diameter of Cataractous Eye Lens

The weight, diameter and thickness of the lens are essential to be maintained in order to maintain their shape and they tend to change with advancement of age. The property of accommodation is unique to the eye lens for which the shape of the lens is necessary to be maintained. The dimensions of the lens were thus noted to know if any changes occurred among cataractous subjects with and without clinical complications.

The dimensions of lenses extracted from cataractous subjects are recorded in table 11. The lens extracted from diabetic cataract men showed a significant increase ($p<0.05$) in diameter and thickness when compared to HCM and HCW groups whereas lens weight was found to be significantly increased ($p<0.05$) in both DCM and DCW when compared to hypertensive cataract groups. Apparently normal cataract groups indicated a significant decrease ($p<0.05$) in lens diameter when compared to DCM and DCW groups whilst lens thickness showed a significant variation ($p<0.05$) between ACW and DCM group. However, there was no significant difference observed in lens dimensions among men and women of apparently normal and clinically complicated cataract groups. The above observation may indicate that the lens dimension varies significantly among groups with co-morbidities.

Assia and Apple (1992), revealed in their study that the human ocular lens specified a diameter of 9.5 mm and a thickness of 4.5 mm. The investigation carried out by Dorairaj *et al.* (2002), suggested the lens diameter and lens thickness to be 0.85 cm and 0.45 cm respectively. The record of lens dimensions might help in resolving the least feasible incision size needed for the cataract surgery of an individual (Ayaki *et al.,* 1993). In an animal study conducted by Obrosova and Stevens (1999), no significant change in the lens weight of a normal and diabetic group was observed.

### 4.3.2 Status of Proteins in Cataractous Eye Lens

The levels of total protein, insoluble protein and soluble protein in eye lens tissues among cataractous subjects are presented in table 12. In the present study, the levels of total protein and soluble protein in cataractous eye lens were decreased in clinically complicated groups, compared to apparently normal cataractous groups whereas an inverse trend was observed in the levels of insoluble protein. Total protein and soluble protein levels in cataractous ocular lens tissue were significantly decreased ($p<0.05$) in DCM when compared to ACM and ACW groups. The eye lens extracted from DCW group indicated a significant increase ($p<0.05$) in the levels of insoluble protein as compared to men and women of apparently normal cataract groups but DCM group showed significant increase ($p<0.05$) only compared to ACM group. Even though cataractous lens from HCM and HCW groups showed an increase in the levels of insoluble protein when compared to apparently normal cataract men and women groups, there was no significant difference between them. But HCW group showed a significant decrease ($p<0.05$) in the total protein levels and soluble protein levels compared to ACW group and DCM group respectively.

Lens proteins are believed to undergo photooxidation causing their damage, aggregation and precipitation in the eye lens leading to opacification of lens (Taylor, 1993). Massin and Kaloustian (2007), opined that cataract lenses are characterised by a substantial increase in water insoluble protein which are formed of protein polymers. Brown colour and fluorescent appearance in cataract lenses may be due to these protein polymers. Protein insolubilization and opacification of lens may be because of sulphydryl oxidation that causes the cross linking and molecular aggregation of long lived lens protein (Takemoto, 1996; Takemoto, 1997). This suggests that during opacification, the water insoluble protein fraction would be found in more concentration in the eye lens than soluble protein fraction. In the present investigation, the levels of proteins were estimated to compare the extent of opacification in ocular lens among clinically complicated cataract groups and apparently normal cataract groups.

Chitra *et al.* (2009), reported a significant decrease in the total protein and water soluble protein and a higher concentration of insoluble protein in the lens of selenite induced lenses where selenite induced cataract is considered as a convenient model for the study of cataractogensis. A noticeable decrease in the soluble fraction of lens protein was observed in diabetic cataract (Kyselova *et al.,* 2005a).

### 4.3.3 Activities of Enzymatic Antioxidants in Cataractous Eye Lens

The activities of superoxide dismutase and catalase enzymatic antioxidants in the eye lens are recorded in the table 13. The activities of superoxide dismutase and catalase were found to be significantly decreased ($p<0.05$) in all other five groups when compared to ACM group. The activity of superoxide dismutase in eye lens tissue removed after surgery from DCW, HCM and HCW groups were observed to be significantly decreased ($p<0.05$) as compared to ACW group whereas catalase activity did not show a similar trend. A significant decrease ($p<0.05$) in the activity of catalase was observed in DCM group compared to hypertensive cataract groups. There was a significant variation ($p<0.05$) in the catalase activity of cataractous eye lens tissue between DCW and HCW groups. Activities of both the enzymatic antioxidants did not show significant variation among men and women of diabetic cataract groups and men and women of hypertensive cataract groups but showed a significant decrease ($p<0.05$) in ACW when compared to ACM.

Oxidative stress is supposed to be associated with the causes of cataract and diabetes (Ozmen *et al.,* 2002; Vinson, 2006; Suryanarayana *et al.,* 2003). Ocular lens tissue also possesses defense system to prevail over the oxidative imbalance. This defense system is generally the antioxidant enzymes such as superoxide dismutase and catalase which were found to have a decreased activity in human cataractous lens samples (Hashim and Zarina, 2006; Donma *et al.,* 2002). As the fibre cells of the lens are not replenished, they tend to last throughout the life span and they become more vulnerable to oxidative insult. This damage may cause the degradation of proteins eventually leading to cataract (Zheng *et al.,* 2010). The repercussion of hyperglycemia in diabetes mellitus is implied to be oxidative stress (Baynes and Thorpe, 1999).

Superoxide dismutase and catalase are antioxidant enzymes that fight oxidative stress by decomposing superoxide radical and hydrogen peroxide respectively (Awasthi *et al.,* 1996). In the eye lens, copper-zinc superoxide dismutase is the most commonly found isoenzyme (Behndig *et al.,* 2001). When there is a higher concentration of hydrogen peroxide in eye lens, the cells utilize catalase for their degradation (Reddy *et al.,* 2001). Thus, these enzymes are found to play a defensive role for the human eye against oxidative stress.

In an animal study conducted by Biju *et al.,* (2007), they reported a decreased activity of SOD in the selenite administered groups. Several studies have delineated that senile cataracts are associated with the diminished levels of antioxidants like SOD and CAT (Fujiwara *et al.,* 1992; Rieger and Winkler, 1994; Reddan *et al.,* 1996). There are many studies that have suggested the harmful outcome of hydrogen peroxide which provokes the generation of reactive oxygen species in lens causing severe injury to lens epithelium and ultimately leading to cataract (Spector, 1995). Wolf *et al.* (2005), indicated the delay in cataract development by artificial targeting of catalase to mitochondria, thus suggesting that scavenging the mitochondrial hydrogen peroxide is important for maintaining the eye lens in a healthy condition.

### 4.3.4 Status of Glutathione System in Cataractous Eye Lens

Glutathione system comprising of glutathione peroxidase, glutathione reductase, glutathione-S-transferase and reduced glutathione in the human ocular lens possesses an important role in controlling the oxidative stress. Their activities in the eye lens tissue among cataractous subjects with and without clinically complicated groups are presented in table 14. The activities of glutathione-S-transferase, glutathione reductase and the levels of glutathione were found to be significantly decreased ($p<0.05$) in ACW as compared to ACM group. When a comparison was made between diabetic cataractous groups and hypertensive cataractous groups in the status of glutathione system, they showed a significant variation ($p<0.05$) in their activities.

Activity of glutathione peroxidase was observed to be least among ACM group and their activity was significantly decreased ($p<0.05$) compared to other five groups. Lens extracted from hypertensive cataract groups showed a significant decrease ($p<0.05$) in GPx activity when compared to diabetic cataract groups.

Cataractous lens tissue from diabetic subjects exhibited a significant decrease ($p<0.05$) in GST activity as compared to HCW group. A significant variation ($p<0.05$)in the levels of reduced glutathione was observed among men and women of diabetic cataract groups as well as between DCW and HCW groups. On the whole, cataractous lenses operated from diabetic groups exhibited a decreased activity of GST and GR but an increased activity of GPx was observed in these groups.

Glutathione system plays an important role in defending the oxidative stress in eye lens. A high concentration of reduced glutathione and an active glutathione redox system are present in the eye lens especially in lens epithelium and help in detoxifying dehydroascorbate and hydrogen peroxide (Reddy, 1990; Giblin *et al.,* 1990). They also protect the lens from oxidative damage by maintaining the proper functioning of structural proteins and enzymes and also by detoxifying xenobiotics (Mannervik and Danielson, 1988).

Many studies have suggested diminished level of enzymes of glutathione system in the human cataractous lens (Hashim and Zarina, 2006; Donma *et al.,* 2002). Any alteration in the activity of glutathione peroxidase may lead to the development of cataract (Spector *et al.,* 1993a,b). Glutathione-S-transferase is a group of multifunctional enzyme that plays a prominent role in preserving ocular lens from detrimental effects of lipid peroxidation (Awasthi *et al.,* 1996). Many studies support the involvement of glutathione reductase and glutathione peroxidase as chief enzymes that help in removing peroxide from the cells (Jones *et al.,* 1981; Fecondo and Augusteyn, 1983; Giblin *et al.,* 1990).

Glutathione is a vital cellular antioxidant which protects the cells from damage caused by oxidative stress (Tirmenstein *et al.,* 2000). Glutathione assists glutathione peroxidase in detoxifying hydrogen peroxide, scavenges numerous ROS, operates as an intracellular reservoir of cysteine, has an essential role in organising the activities of various crucial antioxidants (Adams *et al.,* 1989) and acts as a major reducing agent which is revived incessantly through several metabolic reactions and ascorbic acid (Reddy *et al.,* 1990). Several studies specified the diminution of glutathione which has a direct impact on the activity of glutathione reductase. GR is a glutathione dependent enzyme and has a crucial role in maintaining glutathione homeostasis (Barker *et al.,* 1996).

According to Rao *et al.* (1983), there was decreased activity of glutathione- S-transferase in the cataractous lenses when compared with those of normal lenses. Several animal studies revealed that decreased concentration of glutathione may cause opacification of lens in rats that may be due to uninterrupted oxidation of lens proteins and membrane lipids (Spector, 1995; Dethmers and Meister, 1981; Taylor and Nowell, 1997). Carey *et al.* (2011), observed a decrease in the levels of glutathione, GPx, GR and GST in cataract induced Wistar rat pups.

As suggested by Freel *et al.* (2003), many evidences imply that depletion of glutathione may be due to its oxidation to oxidised glutathione which increases drastically during cataractogensis. Besides this, cataractogensis may also be caused by hyperbaric oxygen which is exemplified by the depletion of GSH and protein sulphydryl group in addition to insolubilization of protein. In the present investigation, an increase in glutathione peroxidase activity was observed among cataractous subjects that may be due to the oxidation of glutathione to its oxidised form. Zhang *et al.* (2008), demonstrated a decrease in certain antioxidant enzyme activities.

### 4.3.5 Status of Non-Enzymatic Antioxidants in Cataractous Eye Lens

Vitamin A, Vitamin E and Vitamin C are non enzymatic antioxidants which generally facilitate the prevention of damage caused by free radicals. Their mean concentration in the ocular lens tissue from cataractous subjects with and without co morbidities is recorded in table 15. Vitamin A did not show significant variation among cataractous groups except for a significant decrease ($p<0.05$) in their levels in DCM group while comparing it to apparently normal cataract groups. In case of vitamin C and vitamin E, a significant decrease ($p<0.05$) in their levels in the eye lens tissue were observed in all cataractous groups when compared to ACM group.

The levels of vitamin C in lenticular tissue from DCM group indicated a significant decrease ($p<0.05$) in comparison with men and women of hypertensive cataract groups whereas similar significance was not observed in the levels of vitamin A and vitamin E. Thus, these comparisons among cataractous groups in the present study indicate that the levels of non enzymatic antioxidants significantly differed in diabetic cataractous groups in comparison to all other cataractous groups.

In a healthy developing eye lens, oxidative insult is prevailed over by the involvement of copious amount of enzymatic antioxidants and exclusively large amount of ascorbate and glutathione (Halliwell, 1999; Ganea and Harding, 2006). The lens is rich in vitamin C which acts as a powerful antioxidant to combat oxidative stress developed due to various causes and has a defensive approach against numerous diseases **(**Mayer *et al.,* 2001; Hegde and Varma, 2004; Simsek *et al.,* 2005).

Eye lens fibres and membranes are believed to have an ample concentration of vitamin E that plays a prominent role in slowing down cataract progression by reducing lens lipids photo-peroxidation and stabilizing cell membranes of ocular lens (Varma *et al.,* 1982; Libondi *et al.,* 1985; Ohta *et al.,* 1996; Karslioglu *et al.,* 2004). The lenses in hyperglycemic environment showed a diminution of antioxidants namely vitamin C and vitamin E (Mitton *et al.,* 1993; Mitton *et al.,* 1997). There are several studies which delineate the significance of vitamin C oxidation in generation of advanced glycated end products (Fan *et al.,* 2006; Linetsky *et al.,* 2008; Ortwerth *et al.,* 1994). The non enzymatic antioxidants namely vitamin E, vitamin C and vitamin A are nutritional antioxidants that were observed to slow down the progression of cataract in experimental animals (Chitra *et al.,* 2009).

### 4.3.6 Lipid Peroxidation Status in Cataractous Eye Lens

Lipid peroxidation may result due to the generation of free radicals in lenticular cells due to many confounding factors. Their status in eye lens among cataractous subjects is depicted in figure 18. The status of lipid peroxidation in cataractous lenses from the groups DCM, HCM and HCW showed a significant increase ($p<0.05$) in comparison to men and women of apparently normal cataract groups. Lenses operated from diabetic cataract women group exhibited a significant increase ($p<0.05$) in the levels of lipid peroxidation with only ACM group and not with ACW group.

However, there were no significant changes in lipid peroxidation status among ACM and ACW, DCM and DCW and between HCM and HCW groups. The results of present investigation were in accordance with the results of Hashim *et al.* (2009), where increased levels of lipid peroxidation was observed in diabetic cataract subjects when compared to senile cataract subjects.

The eye is exposed to various kinds of surrounding factors that may create space for the formation of ROS in the lens. This condition may lead to the development of many biochemical alterations inside the lens eventually causing an unorganised appearance of lens fibres that may hinder the transparency of lens (Spector, 1995). ROS and free radicals are formed in the lens leading to oxidative stress which is one of the major causes of cataract (Ottonello *et al.,* 2000; Vinson, 2006). Mahmoud *et al.* (2014), demonstrated an increased amount of lipid peroxidation products at accelerated rate in diabetes. Lipid peroxidation may occur due to the reaction of free radicals with polyunsaturated fatty acids (Memioeogullari and Bakan, 2004; Karthikesan *et al.,* 2010).

High levels of lipid peroxidation cause damage to membrane function and fluidity that may alter the activities of membrane bound enzymes (Arulselvan and Subramanian, 2007). Malondialdehyde (MDA), which is a byproduct of lipid peroxidation, will reveal the extent of peroxidation in the tissue (Mahmoud *et al.,* 2012). Individuals with diabetes and myopia show elevated levels of lipid peroxidation products in lens and vitreous portion of the eye (Grattagliano *et al.,* 1998; Buyan *et al.,* 1986; Altomare *et al.,* 1995; Micelli-Ferrari *et al.,* 1996) and lipid peroxidation may possibly act as an initiator of cataract in such patients (Ziegler and Hess, 1984). Reactive oxygen intermediates cause peroxidation of membrane lipids and damage the proteins that may create an adverse effect (Micelli-Ferrari *et al.,* 1996). In a study conducted by Chitra *et al.* (2009), an increased levels of MDA was observed in selenite induced cataract groups when compared to normal lenses in rats

### 4.3.7 Nitrite Levels in Cataractous Eye Lens

Nitrite levels in eye lens from selected cataractous subjects are illustrated in figure 19. Cataractous lenses of HCM and HCW groups showed a significant increase ($p<0.05$) and significant decrease ($p<0.05$) in lenticular nitrite levels in comparison to apparently normal cataract groups and diabetic cataract groups respectively. The higher concentration of nitrite was observed in diabetic cataract men and women when compared to other groups.

Nitrite is a metabolite of nitric oxide which is likely to cause changes in the proteins of lens as was observed in the age related nuclear cataract (Paik and Dillon, 2000). An increase in nitrite levels was observed in the cataract lenses of rats (Ito *et al.,* 2001). Many studies have reported an elevated level of nitric oxide in aqueous humor and plasma of diabetic subjects (Chiou *et al.,* 1999; Aydin *et al.,* 2001).

According to Ornek *et al.* (2003), nitrite levels were found to be highest in posterior subcapsular cataracts that are usually linked to diabetes, retinitis pigmentosa and use of various drugs. According to them, nitric oxide which has a short half life plays a crucial role in vasodilation, inflammation, immunity and neurotoxicity. They also suggested that hypertension and diabetes that occur commonly in elderly population are supposed to cause pathology in posterior portion of the eye. In accordance to their study, an increased level of nitrite was observed in diabetic subjects.

### 4.3.8 Status of Protein Carbonyl and Protein Sulphydryl in Cataractous Eye Lens

The levels of protein carbonyl and protein sulphydryl in eye lens tissue among cataractous subjects are presented in table 16. A significant increase ($p<0.05$) and a significant decrease ($p<0.05$) were noted in the levels of protein carbonyl and protein sulphydryl in cataractous lens extracted from diabetic cataract groups in comparison to ACM group and hypertensive cataract groups respectively. This indicated that the highest levels of protein carbonyl were observed in diabetic cataract subjects as compared to all other groups. In converse to the levels of protein carbonyl, the levels of protein sulphydryl were found to be significantly decreased ($p<0.05$) in lenses from diabetic cataract men and women in comparison to ACM, ACW and HCM groups.

Cataract progression mainly depends on the extent of oxidative damage caused to the lens proteins (Boscia *et al.,* 2000; Stadtman, 1992). Oxidative stress causes oxidation and precipitation of lens proteins which may eventually lead to opacification of the eye lens (Meloni *et al.,* 1990; Chandrasena *et al.,* 2008). Evaluation of protein carbonyls is a key index for measuring protein oxidation in the tissues (Dalle-Donne *et al.,* 2003). Increased amount of protein carbonyls are associated with the development of senile and diabetic cataract (Chevion *et al.,* 2000; Vendemiale *et al.,* 1999). Diabetes and myopia are supposed to hinder protein redox status and alteration in this redox status may hinder the transparency of lens. Evaluating the content of protein carbonyl and protein sulphydryl in the lens may represent a valuable index of protein redox status (Altomare *et al.,* 1997).

Protein carbonyl and protein sulphydryl act as direct measure and indirect measure respectively of protein oxidation (Stadtman, 1992; Garland *et al.,* 1988). Many studies have revealed the hindrance of cataract progression by thiol containing molecules that decline the oxidation of protein in lens (Maitra *et al.,* 1995; Elanchezhian *et al.,* 2007). Protein carbonyl levels were found to be elevated in diabetic cataract animals in comparison to diabetic animals (Kyselova *et al.,* 2005a). During the pathogenesis of diabetic cataract, the alterations in protein sulphydryl levels may be due to oxidative stress induced by hyperglycemia and their levels are observed to be decreased in diabetic and senile cataract (Duhaiman, 1995; Hum and Augusteyn 1987).

### 4.3.9 Activities of Marker Enzymes of Polyol Pathway in Cataractous Eye Lens

Polyol pathway is feasible only with the involvement of two major marker enzymes namely aldose reductase and sorbitol dehydrogenase. Their activities in the cataractous lens removed after surgery from the cataract subjects with and without clinical complications are presented in table 17. The activity of aldose reductase in the lens removed from diabetic cataract groups indicated a significant increase ($p<0.05$) in comparison to ACM, ACW, HCM and HCW groups. A significant variation ($p<0.05$) in the activity of AR was observed between apparently normal cataract men and women groups.

Sorbitol dehydrogenase is required in the conversion of sorbitol to fructose and their activity in cataractous lenses from diabetic groups was found to be significantly decreased ($p<0.05$) in comparison to ACM and ACW groups. This enzyme also showed a significant variation ($p<0.05$) in their activity in ocular lens among men and women of cataractous subjects with and without co morbidities. As indicated by the above comparisons, diabetic cataractous subjects showed major significant changes in the activities of polyol pathway marker enzymes.

In diabetes, there is more possibility for the entry of glucose into the lens through polyol pathway where glucose gets converted to sorbitol and gets accumulated in the lens that may create an osmotic stress. This osmotic stress in lens is considered to be a major causative factor for diabetic cataract (Lee and Chung, 1999). Backlog of sorbitol in the ocular lens may result in extreme hydration, alteration in sodium and potassium ions owing to an elevation in intracellular ionic strength (Kinoshita, 1990). Suryanarayana *et al.* (2007), suggested the contribution of hyperosmotic stress due to oxidative tension in the pathogenesis of diabetic cataract. Lee *et al.* (1995) demonstrated that with increase in aldose reductase and sorbitol levels in the eye lens, cataract development also increases that confirm the involvement of polyol pathway in progression of diabetic cataract. Depletion of antioxidants may also result due to the involvement of polyol pathway (Obrosova, 2005).

### 4.3.10 Activities of Membrane Bound Enzymes in Cataractous Eye Lens

Membrane bound enzymes include sodium potassium ATPases, calcium ATPases and magnesium ATPases which are involved in maintaining the membrane fluidity. Their activities in cataractous eye lens are presented in table 18.

The activities of $Na^{+}K^{+}$ATPases, $Ca^{2+}$ATPases and $Mg^{2+}$ATPases in cataractous eye lens were found to be significantly decreased ($p<0.05$) in diabetic cataract and hypertensive cataract groups in comparison to ACW group. A significant decrease ($p<0.05$) in the activity of $Na^{+}K^{+}$ATPases was observed in HCW as compared to DCW. Activity of $Mg^{2+}$ATPases in cataractous lens extracted from diabetic and hypertensive groups indicated a significant decrease ($p<0.05$) as compared to both ACM and ACW.

Alteration in calcium ion homeostasis is concerned with almost all kinds of cataract (Duncan *et al.,* 1994; Duncan *et al.,* 1993) which is maintained by membrane calcium pumps (Galvan and Louis, 1988) and endoplasmic reticulum calcium pumps (Shearer *et al.,* 1997). An elevated level of $Ca^{2+}$ uptake is observed to be involved in selenite induced cataract (Hamakubo *et al.,* 1986). In a study by Biju *et al.* (2007), the levels of $Ca^{2+}$ATPase were observed to be significantly decreased in selenite induced rats which developed cataract. Similar trend was observed in the present investigation also.

The differentiation of lens fibre cells along with other process requires calcium (Wride, 1996). Alteration in homeostasis of eye lens due to calcium ions is known to be involved in cataract development (Gupta *et al.,* 2004). Liu *et al.* (2002) suggested that the levels of $Ca^{2+}$ are preserved by the action of $Ca^{2+}$ATPase. There are many studies which imply that this membrane bound enzyme is liable to oxidation and their activity is thus reduced when they undergo oxidative damage and result in the higher levels of $Ca^{2+}$ in the eye lens (Ahuja *et al.,* 1999). Several studies indicate a decreased activity of $Ca^{2+}$ATPase owing to high affinity of lens lipid to bind $Ca^{2+}$ that may ultimately lead to increased levels $Ca^{2+}$ (Borchman *et al.,* 1993; Tang *et al.,* 2003).

Chitra *et al.* (2009), reported high levels of sodium ions and calcium ions but low levels of potassium ions in the lenses of animals that were induced with selenite. $Na^{+}K^{+}$ATPases help in the maintenance of ionic strength in the eye lens and changes in their activity may lead to an increase in sodium ions and decrease in potassium ions resulting in the hydration and swelling of the lens fibres that may result in cataract development (Chylack and Kinoshita, 1969). The oxidation of thiol groups of this membrane bound enzyme also results in the above condition (Yuregir *et al.,* 1989). A change in the ratio of sodium and potassium ions was found to damage the lens protein which may increase the levels of insoluble proteins (Shinohara and Piatigorsky, 1977). Decreased activities of $Na^{+}K^{+}$ATPases were reported by Shetty *et al.* (2010).

### 4.3.11 Status of Basic Biomolecules in Cataractous Eye Lens

The levels of sugars and nucleic acids in the eye lens among cataractous subjects with and without clinical complications are presented in table 19. Glucose levels in cataractous eye lens from diabetic cataract patients indicated a significant increase ($p<0.05$) when compared to other groups. A significantly decreased ($p<0.05$) levels of fructose in the eye lens tissue from diabetic cataract subjects was revealed in comparison to ACM, ACW, HCM and HCW.

The ocular lenses from clinically complicated cataractous subjects showed a significant decrease ($p<0.05$) in the levels of fructose when compared to apparently normal cataract groups. Levels of DNA and RNA in cataractous eye lens from DCM and DCW were observed to be significantly decreased ($p<0.05$) as compared to ACM group. ACW group exhibited a significant increase in the levels of RNA when compared to DCM, DCW and HCM groups. There was no significant variation observed among men and women in the levels of nucleic acids in cataractous eye lens.

Oxidative stress causes many changes to cellular components like proteins, lipids, cytoskeletal molecules and may result in the lens opacification and nuclear cataract (Giblin *et al.,* 1988; Giblin *et al.,* 1995). Many animal studies have revealed that exposure of the experimental animals to more oxygen would cause the oxidation of proteins, thiols and lipids in the nucleus of lens (Heinecke *et al.,* 1993; Padgaonkar *et al.,* 1999).

ROS generated in the eye lens may damage biomolecules like nucleic acids, proteins and lipids that may lead to apoptosis (Conlon *et al.,* 2003; Huang *et al.,* 2008). This may eventually be involved in the development of cataract (Yao *et al*., 2008; Zhang *et al.,* 2010). Kyselova *et al.* (2004) suggested that increased amount of glucose in hyperglycemic condition might tend to react non enzymatically with proteins leading to the formation of advanced glycated end products (AGEs). These AGEs are believed to accumulate in lens during diabetes. In the presence of transition elements, increased amount of glucose reacts with oxygen to produce superoxide radical which is supposed to be an autooxidation reaction (Lin, 1997; Wolff and Dean, 1987).

Total cholesterol levels in the eye lens tissue operated from cataractous subjects are depicted in figure 20. The levels of total cholesterol in eye lens tissue showed a significant decrease ($p<0.05$) in ACW group when compared to ACM group. There was a significant decrease in total cholesterol levels in lenses from DCW group as compared to ACW group. Kim and Kim (2006), reported no change in the levels of total cholesterol in serum of subjects with and without cataract. Inhibition of cholesterol synthesis has an important association with the development of cataract as opined by Cenedella (1996).

**4.3.12 Status of Glycoproteins in Cataractous Eye Lens**

Glycoproteins namely hexose, hexosamine, fucose and sialic acid levels in lens removed from cataractous subjects with and without clinical complications are depicted in figure 21, 22, 23 and 24. Levels of all the four glycoproteins in the lens tissue extracted from diabetic cataract subjects and hypertensive cataract subjects were found to be significantly increased ($p<0.05$) in comparison to ACM group. Hexosamine and sialic acid levels were observed to be significantly increased ($p<0.05$) in ACW as compared to ACM.

Lenses from DCM and DCW groups exhibited a significant increase ($p<0.05$) in the levels of hexose and fucose when compared to ACW group. Fucose level was found to be significantly increased ($p<0.05$) in the lens tissue extracted from DCM group and DCW group in comparison to HCM group and HCW group respectively. These comparisons among cataractous subjects suggest that diabetic cataract subjects showed an increased concentration of glycoproteins when compared to hypertensive cataract subjects and apparently normal cataract subjects.

Kyselova *et al.* (2004), suggested that lens crystalline proteins may undergo non enzymatic glycation that causes conformational changes with exposure of their thiol groups to oxidation and cross linking. Memon *et al.* (2008)

reported increased levels of serum hexosamine and serum sialic acid in diabetic patients with or without cataract in comparison to control subjects and these glycoproteins were also increased in diabetic subjects when compared to non diabetic subjects with cataract.