SUMMARY AND CONCLUSION

Cataract is the major cause of visual disability throughout the world and accounts for about 51% of all blindness worldwide. In India, cataract was responsible for 77.5% of avoidable blindness. ('Rapid Assessment of Avoidable Blindness' study 2008).

It has been identified and extensively studied that the process of cataract formation is more pronounced and accelerated in patients suffering from diabetes. Cataractogenesis is one of the earliest secondary complications of Diabetes mellitus and the risk of cataract is known to increase with the increase in the duration and severity of diabetes.

An important contributing factor towards cataract formation in diabetes is oxidative stress which is produced as a result of imbalance between production of reactive oxygen species (ROS) and their removal. Secondly, due to increased levels of glucose, there occurs non-enzymatic glycation of lens proteins that is simultaneously accompanied by oxidation and formation of advanced glycation end products (AGE's). Another aspect due to which diabetes influences cataract formation is the osmotic imbalance theory. Increased glucose influx and utilisation by the polyol pathway causes accumulation of sorbitol resulting in an osmotic imbalance which disturbs the electrolyte balance, transport across membrane and also adds to oxidative stress by preventing regeneration of GSH.

In view of this, a ‘multi target’ strategic approach to delay cataract formation would be successful by using substances which could act as antioxidants, aldose reductase inhibitors and antiglycation agents.

Phytochemicals from various traditional medicinal plants are found to have multiple beneficial effects in diabetes and its complications. India has a rich heritage of ancient traditional systems of medicine. Various indigenous plants are used in Ayurveda for the treatment of diabetes induced hyperglycemia and cataract.

Currently, there is also a growing interest in indigenous plants as promising sources of antioxidants. Various plants are thus being studied for their antioxidant properties.

The present study was aimed at elucidation of medicinal plants like Syzygium cumini (Jambhul), Aegle marmelos (Bael), Emblica officinalis (Amla) and Allium sativum (garlic) as anticataractous agents. The role of Vitamin C as an anticataract agent was also studied. The anticataractous property of the plants included in this study and also Vitamin C was analyzed with respect to their role in the intervention of hyperosmotic stress and hyperoxidant stress in lens. The effect of these plant extracts on enzyme Aldose Reductase, and antioxidant enzymes in lens (SOD, Glutathione Reductase, Glutathione Peroxidase) was studied.

Experimental Sugar cataract (glucose induced) was chosen as a model for the present study.

Goat lenses were used for development of cataract. Goat eyeballs were obtained from the slaughter house and transported to the laboratory in an ice-box. Lenses were removed from the eyeballs with the help of cataract knife within one hour, by intracapsular lens extraction method. The lenses were then incubated in tissue culture medium (TC 199) by "Lens Organ Culture Technique".

The lenses were categorized under the following groups:

Sr. No	Group	No. of lenses
1	**Normal lenses:** lenses incubated in TC-199 for 72 hours	30
2	**Experimental diabetic cataract:** lenses incubated in TC-199 + 110mM Dextrose for 72 hours	30
3	**Experimental cataract with plant extracts**: i) Lenses incubated in TC-199 + 110mM Dextrose + 0.25% S.cumini (Jambhul) aqueous extract for 72 hours	30
	ii) Lenses incubated in TC-199 + 110mM Dextrose + 0.25% A.marmelos (Bael) aqueous extract for 72 hours	30
	iii) Lenses incubated in TC-199 + 110mM Dextrose +0.25% E.officinalis (Amla) aqueous extract for 72 hours	30
	iv) Lenses incubated in TC-199 + 110mM Dextrose + 0.25% A.sativum (Garlic) aqueous extract for 72 hours	30
4	**Experimental cataract with Vitamin C:** Lenses incubated in TC-199 + 110mM Dextrose + 0.25% Vit.C for 72 hours	30

At the end of 72 hours of incubation, lenses from each group were removed and 10% homogenate of whole lens was prepared in 0.1M sodium phosphate buffer, pH 7.4 . The homogenate was centrifuged at 10,000 rpm for 30 min at – 4^0C in a refrigerated centrifuge. The supernatant was collected and stored at –20^0C until further use. The lens homogenates from each group were subjected to the estimation of:-

1) Lens proteins (total soluble protein concentration)
2) MDA levels as an index of lipid peroxidation.
3) Specific activity of enzymes -Aldose Reductase (Polyol pathway).
4) Specific activity of antioxidant enzymes- -Superoxide dismutase
   -Glutathione Peroxidase
   -Glutathione Reductase.

The findings of the study are summarised as follows:

A Brief overview of selected medicinal plants and vitamin C on lipid Peroxidation, antioxidant enzymes and Aldose reductase activity in dextrose induced cataract lenses.

Earlier studies have shown that oxidative stress, antioxidants and polyol pathway are all linked in various pathological states as in diabetes. Under glycemic stress, increased influx of glucose through polyol pathway changes the redox status of the cell and thus the activities of antioxidant enzymes are also altered. Activation of the polyol pathway disrupts NADP / NADPH balance inside the cell. The redox status is thus altered and NADPH being unavailable, the NADPH requiring antioxidant enzymes are inhibited.

It is thus desirable to have a holistic outlook on the effect of selected medicinal plants and vitamin C on all the parameters under the scope of this study for delaying the progression of diabetic cataract.

i) S.cumini (Jambhul):

The seed extract showed a remarkable effect on all the parameters under study. (Figure 12)

As compared to dextrose induced cataract lenses, the lenses under glycemic stress when incubated with S.cumini seed extract showed a significant increase in the activities of antioxidant enzymes SOD, GPX and GRx and a marked

reduction in lipid Peroxidation and conservation of lens soluble proteins. The seeds are reported to be rich in flavonoids / polyphenols, due to which they exhibit high free radical scavenging activity, thereby sparing the antioxidant enzymes.

S.cumini extract also significantly decreased the activity of aldose reductase which may be another reason for maintaining the intracellular redox status. A decreased / normalised activity of AR also implies decreased or no accumulation of sorbitol, thereby decreasing the osmotic imbalance and restoring the lens structure and function.

A significant decrease in the activity of AR suggests its inhibition by S.cumini seed extract. The seeds have been used as anti-hyperglycemic agents in diabetes and are studied to have an α-amylase inhibitory effect in laboratory animals. A similar inhibitory effect on AR could be possible; however no literature in this regard is available. Various flavonoids are known to inhibit AR. S.cumini seeds being rich in flavonoids the inhibitory effect on AR could be attributed to the presence of these flavonoids.

An important observation is that S.cumini seed extract proved to be the most efficient among the selected medicinal plants in delaying cataractogenesis. It brought about a maximum preservation of lens soluble proteins, maximum decrease in lipid Peroxidation and maximum increase in antioxidant enzymes SOD, GPX and GRx activities and also a maximum decrease in the AR activity.

ii) A.marmelos (Bael):

When lenses under glycemic stress were treated with the leaf extract of A.marmelos, it was observed that it caused an increase in the total soluble proteins but the increase was not statistically significant.

There was a statistically significant increase in the activities of antioxidant enzymes GPx, and GRx and thus a statistically significant decrease in the lipid Peroxidation as well.

The leaf extract also brought about a marked increase in the activity of enzyme Superoxide dismutase and a decrease in the activity of aldose reductase. However these differences were not statistically significant. (Figure 13)

A.marmelos leaves contain a high amount of flavonoids which act as scavengers of free radicals. This probably results in the decrease in lipid Peroxidation and maintenance of lens proteins. As mentioned earlier, A.marmelos leaf extract was shown to increase the cellular glutathione levels by Jagetia et al (2003) [247]. GRx being an important enzyme in the regeneration of GSH, our observation of an extremely high activity of GRx in lenses treated with bael leaf extract supports their finding.

There could be a possible induction of GRx synthesis by the active components present in bael leaf extract which needs to be further explored.

An increase in the GPx activity caused by bael leaf extract in our study also indicates that its flavonoids either regulate the synthesis of GPx or that the enzyme is spared as the flavonoids themselves scavenge the H_2O_2 as observed by Devi et al (2011) [238].

iii) E.officinalis (Amla):

Lenses incubated with E.officinalis fruit extract showed appreciably favourable changes in the lens profile. (Figure14).

The total soluble proteins were significantly preserved and there was a significant decrease in the lipid Peroxidation. The Polyphenols, mainly tannoids present in amla are shown to have vitamin C like free radical scavenging activity. This may cause a decrease in the lipid Peroxidation [332]. Other studies by Suryanarayana et al (2007) [260] have also mentioned that amla prevents aggregation and insolubilisation of lens proteins caused due to hyperglycemia.

In our study, the amla extract also showed an increase in the activities of lens enzymes SOD, GPX and GRx, but the increase was not statistically significant.

The most significant effect of amla fruit extract was observed on the activity of aldose reductase where it drastically decreased the AR activity. The

role of amla in the inhibition of AR has been well studied by a number of researchers and has been discussed in the earlier section.

E.officinalis could play an important role in preventing / delaying diabetic cataract by virtue of being capable of inhibiting AR, the key enzyme of polyol pathway, thereby maintaining osmotic balance and preventing the excessive formation of advanced glycation end products via this pathway. Amla may hence play an important role in maintenance of lens proteins and its clarity. Moreover, the flavonoids being potential ROS scavengers also decrease lipid Peroxidation induced cell damage.

iv) A.sativum (garlic):

The extract brought about a significant preservation of the soluble proteins in the lens. This effect was in conjunction with the significant decrease in lipid Peroxidation in the lenses despite of hyperglycaemic environment present. Allicin, present in Garlic extract is observed to prevent protein aggregation and protein modification.

The activities of all the antioxidant enzymes were also profoundly increased, which explains the reduction in lipid peroxidation (Figure 15). This observation is in agreement with many previous studies which have shown that garlic reduces lipid Peroxidation in various diseases including diabetes.

In the present study, garlic was found to cause a slight decrease in the activity of enzyme aldose reductase in lenses under glycemic stress. Similar findings were recorded by Tsai et al (2011) [353] in the brain of galactose injected mice.

v) Vitamin C:

By virtue of being a potent aqueous phase antioxidant, Vitamin C increased the activity of SOD, GPx, and GRx in treated lenses as compared to those lenses without treatment. However, when compared to lenses treated with medicinal plant extracts, the rise in the activities of these enzymes was not very high. Vitamin C also caused a decrease in the activity of AR in hyperglycaemic lenses, though the change was not statistically significant.

CONCLUSION

A definite pharmacological therapy for the treatment of cataract does not exist till date and surgical removal of the cataractous lens is the only respite to cataract patients.

Extensive research on animal models and clinical trial is being done for the development or identification of potentially effective anticataract agents. In recent years, there has been considerable focus on the search of phytochemical therapeutics.

In the present study, medicinal plants like Syzygium cumini (Jambhul), Emblica officinalis (Amla), Aegle marmelos (Bael), Allium sativum (Garlic) were investigated for their anticataract potential in experimental diabetic cataract.

- All plant extracts caused a decrease in lipid Peroxidation, increased the activity of antioxidant enzymes and decreased the activity of Aldose reductase.
- Although the overall result on treatment with the plants was delayed progression of experimental cataract; each plant showed a different response pattern.

- Superoxide dismutase activity was maximally raised by S.cumini (Jambhul) and A.sativum (garlic).
- A.sativum, Aegle marmelos (bael) leaves and S.cumini greatly increased in the activities of antioxidant enzymes GPx, and GRx .
- S.cumini and E.officinalis (Amla) brought about a statistically significant decrease in the activity of Aldose Reductase.
- Among the selected medicinal plants in our study, S.cumini emerged as a promising therapeutic agent against glycemic stress induced cataract.
- Studies on the effect of S.cumini in prevention of diabetic cataract are very scarce and the present study is probably one of the few studies of S.cumini seed extract in prevention of diabetic cataract.
- Use of these plant extracts for formulation of eye drops for topical application as anticataract agents could provide great respite to mankind.

Limitations of the study:

- Apart from being inhibitors of glycation, antioxidants and ARI's, it is important that the plant extracts also exhibit adequate penetration into the human lens either systematically or topically.
- Use of these plant extracts to slow the progression of cataract would require their prolonged administration. It is thus important that the plant extracts should exhibit minimum side effects.

Thus, more in-vivo experiments on animal models and clinical trials need to be done to ascertain the benefits of these plant extracts in humans.