DISCUSSION

Cataract is the leading cause of blindness world over; with a greater prevalence in developing countries [2]. There is a well-established association between Diabetes mellitus and cataract; cataract – being one of the earliest secondary complications of Diabetes mellitus [4, 5].

The risk of cataract advances with years and severity of diabetes. India hosts a large number of diabetic populations and appears to be in the midst of an epidemic of diabetes. So far, surgical removal of the cataractous lens is the only respite to cataract patients. However, cataract surgery is expensive and also involves surgical complications, more so in diabetic individuals.

The study of processes or mechanisms to delay the process of cataractogenesis is thus important. Increased oxidative stress, increased polyol pathway and non-enzymatic glycation are important mechanisms in the etiology of diabetic cataract.

Researchers have suggested a ‘multi-target’ approach by using substances which could act as antioxidants, aldose reductase inhibitors and antiglycating agents. Phytochemicals from various medicinal plants have been found to be beneficial in diabetes and its complications.

This study was undertaken to evaluate the antioxidant and anticataract effect of selected medicinal plants like Syzygium cumini (Jambhul), Embilica

officinalis (Amla), Aegle marmelos (Bael), Allium sativum (garlic) on the lens in experimental diabetic cataract. The effect of these plants on the activity of Aldose reductase was also studied.

The role of Vitamin C as an antioxidant in the lens under glycemic stress and its effect if any on lens AR activity was compared with that of each medicinal plant.

The following aspects are considered under the scope of this discussion:-

A) Biochemistry of Experimental Dextrose induced Cataract Lenses with respect to-
   a) Total Soluble Proteins and Lipid Peroxidation.
   b) Antioxidant defence enzymes
   c) Aldose reductase (Polyol pathway)

B) Possible mechanisms for delaying the process of cataractogenesis

C) Effect of selected medicinal plants on dextrose induced cataractous lenses with reference to-
   a) Total Soluble Proteins and Lipid Peroxidation.
   b) Antioxidant defence enzymes
   c) Aldose reductase (Polyol pathway)

A) Biochemistry of Experimental Dextrose induced Cataract Lenses :-

a) Total Soluble Proteins and Lipid Peroxidation:

In the present study, cataract was induced in goat lenses where lenses were incubated in tissue culture medium (TC-199) and 110mM dextrose using 'Lens organ Culture' technique.

In our study, it was observed that there was a statistically significant decrease ($p < 0.0001$) in the amount of total soluble proteins in dextrose induced cataract lenses as compared to normal control lenses. The MDA levels as an index of lipid Peroxidation were also greatly increased in these lenses (Table 1).

These observations are similar to the findings of earlier researchers like Truscott and Augusteyn (1977)[280], who observed a decrease in the total soluble proteins in cataractous lenses by about 40%. Earlier in 1968 Charlton and van Heningen [281] had demonstrated that decrease in lens proteins was due to leak of soluble proteins from cataractous lens, while Sheridan and Zigman (1971) [282] , showed it to be due to conversion into insoluble proteins.

Hunt, Dean et al (1988)[60] and Sakurai and Tsuchiya (1988)[61], suggested that chronic hyperglycemia caused non-enzymatic glycation of lens proteins, causing their aggregation and loss of solubility.

Hyperglycemia induces oxidative stress through various pathways [283]. It is found that this in turn causes oxidation of the lens crystallins as well as membrane proteins resulting in the formation of insoluble protein aggregates.

Lens Proteins are long lived and their synthesis in mature lens is very insignificant. Moreover, the concentration of proteins in the lens is very high. Thus any alteration on the protein has a significant impact on the clarity and transparency of lens.

Chihuailaf et al (2002) [284] studied that presence of large amounts of aromatic and sulphur containing amino acids in a protein structure make the protein more susceptible to free radical damage. The lens proteins have a high percentage of aromatic amino acids- tryptophan, tyrosine and phenylalanine and also large amount of sulphur containing methionine and cysteine that are easily attacked by reactive oxygen species producing altered protein aggregates.

Apart from formation of ROS, hyperglycemia also increases the formation of Advanced Glycation End products. Brownlee (1996) [140], Heidland and Sebekova (2001)[285] observed that non-enzymatic glycation of lens proteins caused protein crosslinking and formation of insoluble protein aggregates resulting in accelerated cataractogenesis in diabetes.

Sundaram et al (2007) [108] observed that the oxidation of thiol groups on lens crystallins causes formation of disulphide adducts that cause protein aggregation and cataract formation.

Increased disulphide formation also results in increased cysteine units in the lens crystallins, rendering them to become insoluble and cause lens opacification.

Lipid Peroxidation:

The involvement of oxidative stress in the progression of diabetes and its complications in now well established [19, 28].

The Reactive oxygen species damage biomolecules such as DNA, proteins and lipids, where in the lens, the membrane lipids and lens proteins are particularly most labile [8].

The PUFA's of membrane lipids undergo a self destructing chain reaction and form cytotoxic compounds like MDA as the end products of lipid peroxidation. Measurement of MDA as an index of lipid peroxidation is widely reviewed [11].

The present study demonstrates that there is a significant elevation ($p < 0.0001$) of MDA levels in experimental dextrose induced cataractous lenses as

compared to control lenses (Table 1). These results are in good agreement with reports of Varma (1991) [15], Fukaya (1988) [286], Nahid & Bulakh (1998) [58] and Jain & Bulakh (2003) [287].

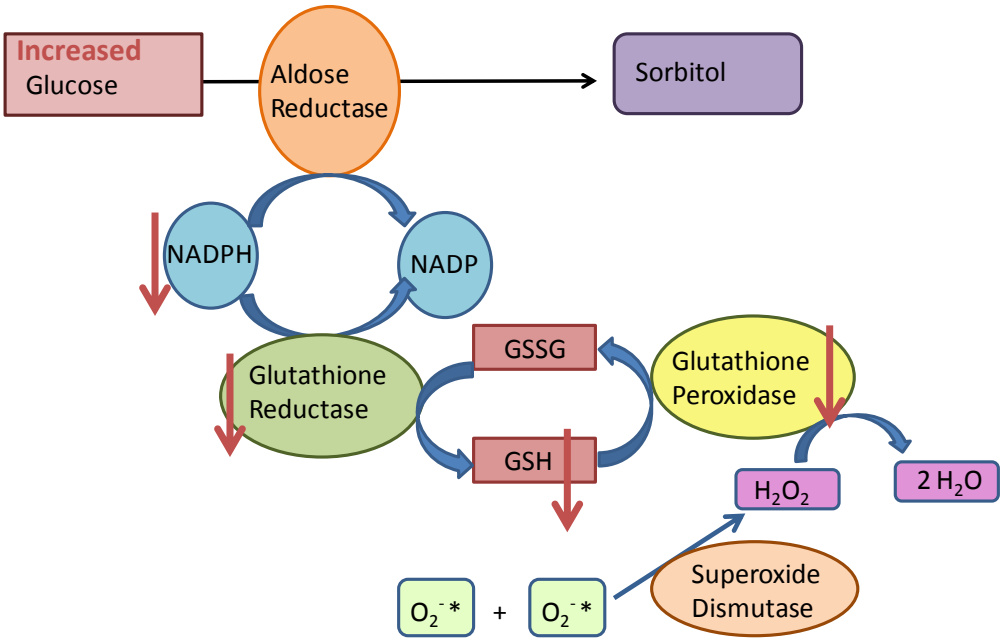
Oxidation is said to be an early event in cataract formation [47] and the single epithelial cell layer in the lens could be the primary site of oxidative damage. In diabetic cataract, glucose oxidation is considered to be the main source of free radicals. Glucose is converted to its enediol form which is oxidised in a transition metal dependant reaction to an anion radical which further forms superoxide anion radicals which damage the membrane lipids [62].

Persistent hyperglycemia also causes excessive interaction of glucose with proteins resulting in the formation of AGE's which in turn promote further formation of free radicals and consecutive oxidative stress [19, 28].

In hyperglycemic conditions, excessive glucose is alternatively increasingly oxidised through sorbitol pathway. The enzyme aldose reductase of this pathway is a NADPH utilising enzyme which catalyses the 1st and rate limiting step of the polyol pathway. Increased utilisation of glucose through this pathway thus causes increased utilisation of NADPH by enzyme aldose reductase. A significant strain is thus imposed on the supply of NADPH. NADPH being an important requirement for several critical metabolic reactions such as detoxification of ROS by glutathione peroxidase - reductase system, the

capacity of the cell to protect itself from oxidative stress is decreased resulting in increased lipid peroxidation.

Figure 17: Hyperglycemia induced oxidative stress



The increased formation of lipid peroxides in dextrose induced experimental cataract lenses thus indicates that oxidative stress and lipid peroxidation could be an integral part of diabetic cataractogenesis.

b) Antioxidant defence system in the lens:

Like other tissues, the lens also has an effective antioxidant protection system to combat the continuous assault by free radicals. These antioxidant defences can be classified as enzymatic and non-enzymatic antioxidants.

Important antioxidant enzymes like superoxide dismutase, Glutathione peroxidise and Glutathione reductase are known to decrease in cataractogenesis. The activities of these enzymes in dextrose induced experimental cataract were thus studied in the present study.

It was observed that the activities of SOD, GPx and GRx were significantly decreased ($p < 0.05$) in dextrose induced cataract lenses as compared to normal lenses (Table 2). This observation is in conformation with the results of various studies by Ozmen & Ozmen (2000)[288], Obara (1995) [122] etc.

i) Superoxide Dismutase: As explained by Obara (1995)[122], the fall in SOD activity could be due to its utilisation during the neutralisation of ROS in hyperglycaemic cataract. Also, H_2O_2 produced during oxidative stress is an inhibitor of enzyme SOD, which could be another cause of reduced SOD activity.

It is noted that Cu-Zn SOD is the dominant isoform of SOD in the lens [127]. The catalytic activity of this enzyme is sensitive to non-enzymatic glycation [289] and the decreased activity of SOD in hyperglycemia may mainly be due to glycation induced inactivation of the enzyme. SOD is also said to loose its antigenic property due to glycation by sugars [124].

The study reported that Fructose and Glucose 6 Phosphate were found to be potent glycation agents than glucose itself. However, greater concentrations of these sugars, in a time dependant manner were required to cause inactivation of the enzyme, suggesting that SOD may be resistant to glycation. This could explain other contrasting results where there was no change in the activity of SOD in tissues of diabetic animals [290].

Inactivation of the enzyme and loss of the antigenic property due to glycation could be - a) simultaneous process as the active site of the enzyme and antigenic site may be similar, or - b) there could be a glycation induced conformational change in the enzyme structure resulting in loss of activity and antigenicity as well.

Khanna et al (1997) [123] observed that the Cu-Zn SOD mRNA decreased in rat lenses exposed to a hyperglycaemic environment of 50 mM glucose. Decreased expression of mRNA of SOD decreased the

expression of these proteins as well as affected the enzymatic activity (291).

Sen, Packer (1996)[292] found that oxidative stress causes oxidative modification of transcription factors and thus alters their translocation into the nucleus. Studies also show that oxidative stress could cause destabilisation of mRNA [293, 294].

Oxidative stress greatly varies the response of antioxidant enzymes in different tissues. Koya, Hayashi et al (2003)[295] and Bhor, Raghuram et al (2004)[296], did not find any significant change in the mRNA expression of Cu-Zn SOD in the glomeruli and small intestine respectively of diabetic rats, despite the altered activities of the enzymes. Some studies have shown raised activity of SOD in liver tissues of diabetic rats but the gene expression was not studied [297].

ii) Glutathione Peroxidase and Glutathione Reductase:

Both Glutathione peroxidase and Glutathione reductase are important enzymes of the redox cycle.

Glutathione peroxidase converts the toxic H_2O_2 to water, using glutathione as the hydrogen donor. Glutathione reductase regenerates the glutathione using NADPH. Thus, activity of GPx depends on the availability of reduced glutathione and more so on activity of GRx.

The activities of both GPx and GRx are decreased in experimental cataractous lenses as compared to normal lenses. Our results are in conjunction with the reports of other researchers [57, 287, 298 and 299]. Oxidative stress is omnipresent in pronounced hyperglycemia. It is studied that when an episode of oxidative stress is triggered, it causes a fall in the antioxidant enzymes [117, 118]. The enzyme to be affected first is GPx which results in accumulation of H_2O_2 thereby subsequently inhibiting SOD.

The decrease in GPx activity in oxidative stress was also noted by Ozmen, Erkin et al (2002) [139], where they noted that along with decrease in GPx, there is a concomitant decrease in GSH as well.

The otherwise relatively high concentration of GSH was reported to be reduced in diabetic lens even in earlier studies by Lou, Dickerson et al (1998) [300]. The decrease in GSH could be due to the failure of the lens to concentrate amino acids required for glutathione synthesis as a result of the hyperosmolar stress in the hyperglycaemic lens.

Failure to regenerate GSH from GSSG by GRx using NADPH could be another cause of reduced GSH level. The hyperglycaemic environment in the lens causes increased glucose utilisation through the polyol pathway, due to which there is increased utilisation of

NADPH by enzyme Aldose reductase, making NADPH less available for GSH regeneration by GRx.

Several studies have reported contrasting findings about GPx activity. Increased GPx activity in the lens was attributed to the rise in lipid Peroxidation by Rao and Bhat 1989 [301].

Lou (2003) [302] observed an increase in GPx activity in diabetic lens which was explained to be a compensatory mechanism to detoxify hyperglycemia induced H_2O_2. Increased activity of GPx and an increase in the mRNA expression of GPx was observed in RBC's, liver and skeletal muscle tissues of streptozotocin induced diabetic rats [303]. Sadi and Guray (2009) [304] also reported that the mRNA expression of GPx was increased in the liver tissue of diabetic rats.

The contrasting results related to the activities of the antioxidant enzymes and their mRNA expressions possibly suggest that oxidative stress may affect these enzymes in an organ specific manner, having different outcomes in different tissues.

Studies also indicate that the ROS may alter signal transduction or translocation of redox specific transcription factors and hence affect mRNA expression of the enzymes [305].

The reduced activity of both enzymes of glutathione redox cycle could also be because of the oxidation induced inter and intra

molecular crosslinking of proteins occurring during hyperglycaemic oxidative stress.

c) Polyol pathway and Aldose reductase in the lens:

The polyol pathway consists of two enzymes-

1) *Aldose reductase:* catalyses the first step of the pathway to convert glucose to sorbitol using NADPH as its cofactor and
2) *Sorbitol dehydrogenase* (SDH): converts sorbitol to fructose.

Aldose reductase is a widely expressed enzyme responsible for the metabolism of aldehydes. The utilization of glucose through the polyol pathway catalysed by AR has been linked to the development of secondary complications in Diabetes mellitus.

The AR catalysed reduction of glucose to sorbitol causes accumulation of sorbitol in the lens which in turn leads to osmotic swelling, ionic imbalance and insolubilisation of the lens proteins [155, 156, 157, 306].

It was first reported by van Heyningen 1959[152] that during diabetic and galactosemic cataractogenesis the activity of AR was very high. Similar observation was made by Kinoshita et al (1981) [157] and (1988) [306] followed by various other researchers.

The activity of AR in experimental diabetic cataract lenses was also studied in the present study and was found to be significantly increased ($p < 0.0001$) as compared to normal lenses (Table 2). Our findings are in good correlation with the findings of earlier researchers mentioned above.

Gonzalez et al (1983) [160] noted that the accumulation of sorbitol and fructose increased with the duration of diabetes which emphasised on the increased activity of AR. Previous studies have shown that mice have much lower levels of AR as compared to humans [307] due to which they are resistant to develop diabetes [164].

Thus an increased activity of AR in diabetic lenses observed in our present study lays emphasis on its role in diabetic cataractogenesis.

In an in-vitro experiment using pancreatic duct cell culture, an increase in the glucose concentration gradually from 5.5mM to 110mM resulted in a concentration dependant rise in AR activity and also AR mRNA expression [308].

Aldose reductase has a high Km for glucose, roughly around 200m, which is never met by the glucose concentration in any diabetic human lens [309]. Yet sorbitol is found to accumulate in the diabetic human lens. The reason as explained by Cheng (1979) [310], is that Hexokinase

activity is significantly decreased with age in the human lens. A high AR to HK (hexokinase) ratio coupled with hyperglycemic stress accounts for increased polyol production.

Implications of Polyol Pathway in Diabetic Cataract:

The polyol pathway, through aldose reductase is responsible for contribution to oxidative stress by three potential mechanisms as mentioned earlier:-

i) Increased AR activity causes depletion of NADPH. As NADPH is also required for the regeneration of GSH by enzyme GRx, unavailability of NADPH induces oxidative stress. In hyperglycemia more than 30 % of total glucose is utilised through polyol pathway causing oxidative stress.

ii) Further, the sorbitol formed by the polyol pathway is oxidised to fructose by sorbitol dehydrogenase which makes use of NAD as cofactor converting it to NADH during the reaction. The enzyme NADH oxidase acts on NADH to generate ROS.

iii) Fructose formed in this pathway and its metabolites are potent agents for non-enzymatic glycation, which increase the formation of AGE's. It is known that AGE formation also causes oxidative stress.

Apart from increasing oxidative stress, the polyol pathway is also responsible for inducing osmotic stress in the lens by causing increased formation of osmotically active sorbitol.

B) Possible Mechanisms of Delaying / Preventing Cataract Formation:

To date there is no definite pharmacological therapy to cataract. The only respite to cataract patients is surgical removal of cataractous lens.

Extensive research on animal model and clinical trial is being done for the development or identification of potentially effective anticataract agents. These agents could make use of one or more possible pharmacologic mechanisms for delaying cataract like –

i) Antioxidants

ii) Inhibitors of non-enzymatic glycation and AGE formation or

iii) Aldose reductase inhibitors

A variety of substances acting as antioxidants, antiglycating agents or AR inhibitors have proved to have great potential of delaying or preventing cataract in animal models, but studies on their role in preventing diabetic cataract in humans is still premature. Some examples are given below –

i) Antioxidants: - Vitamin C [15, 81, 311, 312]

-Vitamin E [15, 97, 313, 314]

-Lipoic Acid [15, 315]

- Pyruvate [316, 311, 317, 318]

- Carotenoids [313, 319]

- Quercetin [200] etc.

ii) Anti glycation agents: - Aminoguanidine [140, 311]

-Aspirin, Ibuprofen, Paracetemol[177, 180, 311, 320],

- Pyruvate [317, 318, 321,] and

- Lipoic acid [162, 322, 323]

iii) Aldose reductase inhibitors:-

Epalrestat[185, 324, 325], Zenarestat[326], Sorbinil [327]

Apart from being inhibitors of glycation, antioxidants and ARI's, it is important that these compounds also exhibit adequate penetration into the human lens either systematically or topically.

Use of these substances as anticataract agents in humans could slow down cataract formation but their prolonged administration would also be required. It is therefore important that these substances exhibit minimum adverse effects.

Thus, in recent years, there has been considerable focus on the search of phytochemical therapeutics.

C) Role of Selected Medicinal Plants:

Epidemiological evidence suggests that a sufficient intake of fruits and vegetables decreases the risk of cataract in humans. Also, phytochemicals like flavonoids from traditional medicinal plants are now being identified as potential molecules showing multiple beneficial effects against different diseases including diabetes and its complications like cataract.

Various in-vitro and in-vivo studies on animal models have shown that plant flavonoids are protective against eye lens opacification. Flavonoids are found to reduce the risk of diabetic cataract formation by affecting the earlier mentioned multiple key processes / pathways in cataractogenesis. The role of different plant flavonoids in each of these processes has been reviewed earlier.

A variety of medicinal plants have been used since ancient times for the treatment of diabetes and even ocular diseases. 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. The effects of each of

these plants as anticataract agents in experimental diabetic cataract were also compared with that of Vitamin C.

Effect of each of these medicinal plants was studied in lenses under glycemic stress for their effect on – i)Total soluble proteins and lipid peroxidation, ii) Antioxidant enzymes- SOD, GPx and GRx and iii) Enzyme of the polyol pathway- Aldose reductase. The results are discussed below.

a) Total Soluble Proteins and Lipid Peroxidation:

In the present study it was observed that as compared to dextrose induced cataract lenses, the concentration of lens total soluble proteins was significantly high ($p < 0.001$) when lenses were incubated with each plant extract inspite of the hyperglycaemic stress in their environment (Table 3).

A statistically significant decrease ($p < 0.001$) in the MDA levels was also observed in lenses incubated with plant extracts and Vitamin C respectively in presence of glucose induced stress as compared to dextrose induced experimental cataractous lenses without plant extracts or vitamin C. (Table 4)

This preservation of Total soluble proteins and decrease in lipid peroxidation (MDA) in lenses incubated with plant extracts can be attributed to the large amounts of Polyphenols in plants.

Lens proteins- crystallins are long lived and have a slow turnover. They are thus greatly prone to accumulation of glycation induced damage (AGE's), which is greatly accelerated in hyperglycemia.

Flavonoids and other Polyphenols from plants are shown to have antiglycation activity and thereby decrease lens protein aggregation. These compounds also exhibit free radical scavenging activity and thus serve to be potent antioxidants as well. It is suggested that the antiglycation activity as inhibitors of AGE'S may well be attributed to their antioxidant property.

Jang et al (2010) [328] reported that other polyphenol quinic acid and its derivatives prevented AGE formation and protein crosslinking and thus also prevented cataractogenesis.

i) Allium Sativum (Garlic): `

Garlic contains S-allyl cysteine, which is its key component and acts as a potent antioxidant. It is also found to inhibit the formation and accumulation of AGE's.

Another bioactive compound from garlic – Allicin, also serves as an efficient antioxidant and was observed to prevent protein aggregation and decrease TBARS in streptozotocin induced diabetic rats. Garlic extract is

known to scavenge transition metal ion generated H_2O_2 and thereby prevent protein modifications mediated through metal catalysed reactions [36, 355].

ii) Aegle marmelos (Bael):

The phenolic compounds present in the plant are natural antioxidants and have a high free radical scavenging and metal chelating activity. Krushna et al (2011) [329], clearly demonstrated in their study that the plant extract also has a membrane stabilising property which probably prevents the osmotic imbalance related protein damage. It is also noted that the leaf extract inhibits variety of free radicals by scavenging them and thereby prevents lipid peroxidation [247].

iii) Syzygium cumini (Jambhul):

The seeds are reported to be rich in flavonoids and other phenolics which accounts for a high free radical scavenging activity [330]. Afify et al (2011)[331] also reported that S.cumini fruits have a very strong free radical scavenging property due to which it could serve as a great antioxidant as well as an anticancer agent.

iv) Emblica officinalis (Amla):

Amla prevents aggregation and insolubilisation of proteins caused due to hyperglycemia resulting in delayed progression of cataract [260]. A similar finding was noted in our study. The lenticular oxidative stress is decreased and lens proteins are protected.

The fruits of E.officinalis are rich in tannins and other phenolic compounds and exhibit antioxidant property. The antioxidant nature of this fruit has been studied in various diseases including diabetes. The potent antioxidant nature is attributed to the presence of hydrolysable tannins having ascorbic acid like action [(332)].

v) Vitamin C:

Vitamin C is present in unusually high concentrations in mammalian lens. Under physiological conditions, vitamin C predominantly exists in its reduced form and serves as a powerful antioxidant quenching ROS and reactive nitrogen species [(333)]. Vitamin C has well been shown to reduce diabetes induced oxidative stress and lipid Peroxidation [(334)].

Our study is in good confirmation with other studies. Various in-vitro experiments on animal models have shown that vitamin C serves as a protective agent against damaging oxygen radicals, thereby emphasizing its anticataract nature [(335)].

In our study it was observed that the highest conservation of total soluble protein under conditions of hyperglycaemic stress was brought about by S.cumini leaf extract. The extract also showed a maximum reduction in the lipid Peroxidation levels as compared to other plant extracts and vitamin C.+

b) Effect on Antioxidant enzymes (SOD, GPx and GRx):

In the living system antioxidant enzymes SOD, GPx and GRx work in conjunction with each other. SOD can effectively exhibit its free radical scavenging activity only when enzymes like GPx and / or catalase and further GRx are available in appropriate concentrations and are working efficiently [336].

The effect of chosen medicinal plants and vitamin C on the antioxidant enzymes (SOD, GPx, GRx) was studied on dextrose induced cataract lenses. It was observed that there was a statistically significant increase ($p < 0.001$) in the activities of all 3 above mentioned enzymes in lenses incubated with plant extracts / vitamin C in presence of dextrose as compared to those without plant extract / vitamin C. (Table 5,6,7)

These results are in consensus with the results of various other researchers.

i) Emblica officinalis (Amla):

The Emblica officinalis fruit extract was found to augment the activities of SOD, GPx & GRx in vitro in other tissues like brain of rats [337].

ii) Syzygium cumini (Jambhul):

The extract is suggested to have a free radical scavenging activity due to which it has a protective effect on SOD & GPx[268]. This is evident due to the increased activities of these enzymes in the lens.

iii) Aegle marmelos (Bael):

Leaf extract was studied to have increased cellular glutathione levels and reduced lipid peroxidation in vitro [247]. In cardiac tissue of rats where myocardial infarction was induced, treatment with A.marmerlos fruit extract showed a significant increase in GSH and SOD, GPx, suggesting its antioxidant property [329]. Similar observation is found in our study on hyperglycaemic goat lenses.

A large number of active compounds were identified from leaf of A.marmelos. Most compounds showed protection against H_2O_2 induced cell damage/ apoptosis [338].

iv) Allium Sativum (Garlic):

In the past two decades, previous studies on garlic were aimed at studying its antithrombotic, antilipemic and anticancer actions. The anticataract role of garlic is now recently being tapped. Allicin an organo sulphur compound is an important constituent of Garlic. Allicin derivative allyl metacaptocatopril formed by combining allicin and captopril was studied to have anticataract

property [339]. It was observed that allyl mercaptocaptopril brought about a restoration of GSH and decreased MDA levels in selenite treated rat lenses. This compound also prevented protein insolubilisation, maintained lens clarity and positively modified the antioxidant enzymes in the lens.

Another organosulphide- diallyl sulphide from garlic oil is found to prevent acetaminophen induced cataract [256]. Diallyl sulphide when combined with N-acetyl-L-cysteine, stimulated GSH synthesis and completely prevented cataractogenesis.

It was observed that intraperitoneal injection of garlic extract almost completely prevented cataractogenesis in selenite treated rats [340]. This effect could be correlated to the rise in GSH levels GPx, SOD activities and a fall in MDA levels observed in these animals.

A similar effect was observed in our study where lenses under hyperglycaemic stress when incubated with garlic extract showed decreased MDA levels and significantly increased activity of SOD, GPx and GRx, with the greatest effect being shown on GPx activity.

v) Vitamin C:

The role of vitamin C as the most potent aqueous phase non enzymatic antioxidant is already discussed earlier. The protective effect of vitamin C on antioxidant enzymes has been presented earlier by Garg and Bansal (2000) [341].

It was observed that vitamin C increased the activity of SOD in the liver of diabetic rats [291]. Another finding was that though the activity of SOD increased, the mRNA for Cu-Zn SOD did not show any statistically significant change which indicated that there could be a possibility of increased Mn-SOD gene expression accounting for increased SOD activity.

The present study shows that the activity of SOD, GPx, and GRx were increased on treatment with vitamin C 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.

c) Effect of medicinal plants / vitamin C on Aldose reductase:

The osmotic hypothesis for formation of diabetic complications due to the accumulation of sorbitol has given rise to an avenue for research on Aldose reductase inhibitors. For more than 3 decades various drugs have been tested for their A-R inhibiting capacity, eg- sorbinil, tolrestat, zopolrestat etc.

In animal models, these drugs were found to offer protection against glucose and galactose induced cataracts [153, 154]. They also offered respite in diabetic nephropathy [342, 343] and neuropathy [344, 345, 346].

It was reported that the synthetic AR inhibitors not only inhibited AR, but could also inhibit other aldehyde reductases [347]. Moreover, it was also noted

that polyol accumulation is not the only cause of cataractogenesis. Use of antioxidants greatly reduced cataractogenesis even when polyol levels were high [348]. This observation brought to light that the activation of Aldose reductase causes oxidative stress and other metabolic imbalance, mainly due to overutilization of NADPH. (Figure18)

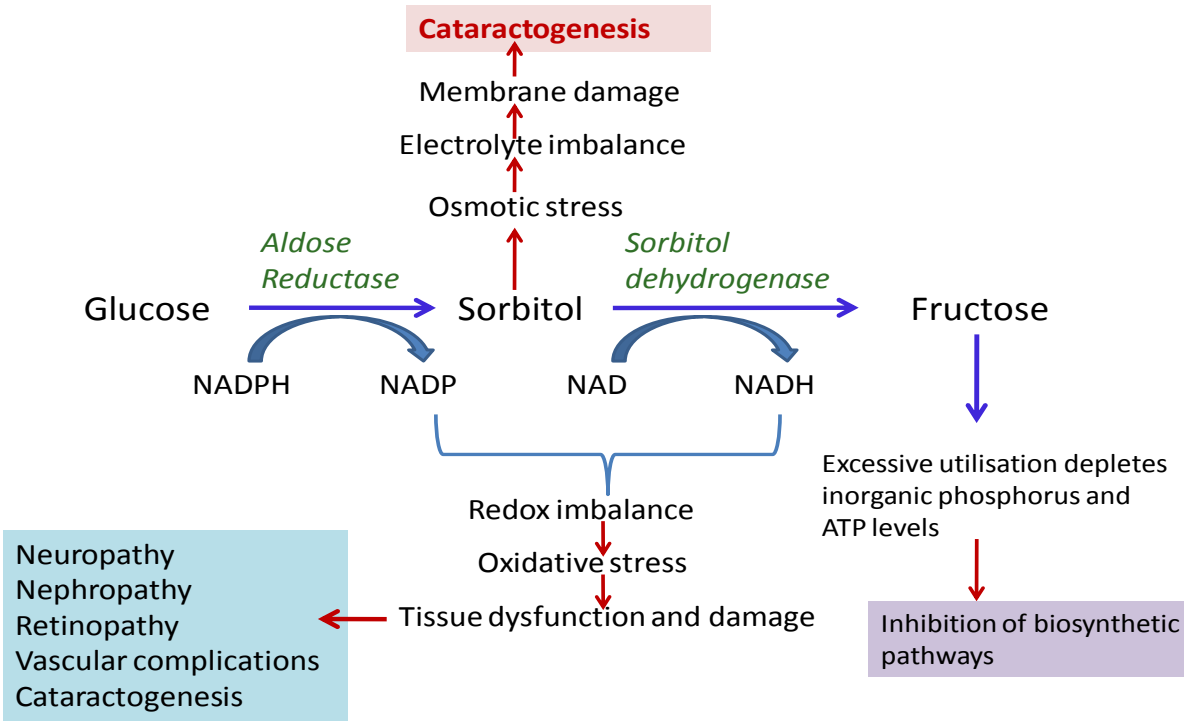


Figure 18: Involvement of polyol pathway in diabetic complications.

Further studies have shown that apart from glucose, AR has the ability to reduce wide variety of aldehydes, including membrane phospholipids and other aldehydes generated during lipid Peroxidation. This property is suggestive of the antioxidant and protective role that AR may also be involved in [349, 350].

Aldose reductase is also shown to be involved in regulation of inflammation and cell growth [351]. Thus, there is a need of identifying more selective and efficient AR inhibitors that could curb the cytotoxic role of AR like cell signalling and does not affect its detoxification function.

In recent years there has been more focus on the identification of natural sources of AR inhibitors which could be beneficial against diabetic complications and also free from side effects. In the present study, the effect of selected medicinal plants and vitamin c on the AR activity was studied in lenses under glycemic stress.

It was observed that all the selected medicinal plant extracts and vitamin C significantly decrease AR activity. E.officinalis and S.cumini showed the greatest efficacy in lowering aldose reductase activity ($p < 0.05$), followed by A.marmelos, vitamin C and garlic (A.sativum) (Figure 11).

i) Emblica officinalis:

In Ayurveda, the plant has been extensively used against many chronic ailments, including diabetes. Suryanarayana et al (2004) [38], assessed the AR inhibitory potential of amla both in vitro and in lens organ culture. They reported that the tannoids present in amla are potent inhibitors of rat lens as well as human recombinant AR. This action of tannoids from amla was compared with that of another natural flavonoid- quercetin, well known for its ARI

potential and it was observed that tannoids from amla are more potent in their inhibition.

In the same study, kinetic studies on the nature of inhibition by tannoids showed that tannoids inhibited AR by uncompetitive inhibition. No inhibitory effect of tannoids was observed on enzymes like G6PD and GRx.

Based on these observations, Puppala et al (2012) [261] further reported that β-glucogallin is the most prominent tannin found in amla and showed that it is a potent and selective inhibitor of human AR (AKR1B1). Through computational molecular docking studies, they indicated that β-glucogallin favourably binds to the active site of the enzyme. The glucose moiety present in the structure of this molecule binds to the active site of the enzyme and appears to mimic the natural substrate (Figure 19). It forms hydrogen bonds with the key residues required for catalytic action. The phenolic moiety in the structure of β-glucogallin inhibits binding of free glucose to the active site of AKR1B1 (Human AR), thereby preventing sorbitol accumulation and diabetic cataract.

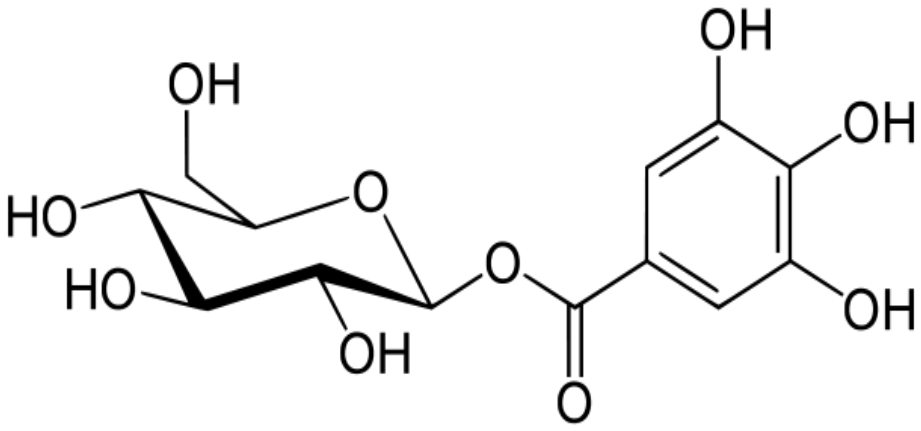


Figure 19: Structure of β-glucogallin

ii) Syzygium cumini (Jambhul):

Seeds of S.cumini have been used in the treatment of diabetes since ancient times. In the past few decades, lot of research has been done on its anti-diabetic nature. The seeds are rich in polyphenols and thus also have free radical scavenging and antioxidant activity.

The hypoglycaemic effect of S.cumini is proposed to be due to the inhibitory effect it has on α-amylase. Karthic et al (2008) [270], observed that the S.cumini seeds had a strong inhibitory effect of 98% on porcine α-amylase activity. They further isolated different flavonoids from S.cumini seed extract and found that these flavonoids inhibited porcine α-amylase. They also noted that the inhibition was of non-competitive type. Earlier Kotowaroo et al (2006) [352] had also reported the α-amylase inhibitory action of S.cumini.

However, there is no literature available on the inhibitory effect of S.cumini seed extract on Aldose reductase. Our study showed a significant decrease in AR activity in lenses treated with S.cumini extract, suggesting its role in AR inhibition.

Literature shows that various flavonoids like quercetin, tannoids etc have AR inhibitory activity. The role of S.cumini seeds in the inhibition of AR could possibly be attributed to the presence of flavonoids. As the flavonoids from the seed extract are already studied for α-amylase inhibition, there could be a

possible inhibitory effect on the activity of AR as well. The AR inhibitory role of individual components from the S.cumini seed extract and the kinetics of inhibition need to be further studied.

iii) Allium Sativum (Garlic):

In our study we observed a mild fall in the aldose reductase activity in hyperglycaemic lenses when incubated with garlic water extract, but the decrease was not statistically significant.

There are studies indicating that S-allyl cysteine, an important component of garlic was found to lower AR activity, sorbitol accumulation and formation of advanced glycation endproducts in the brain of galactose injected mice [(353)].

Studies on the antioxidant role of garlic and its effect on antioxidant enzymes are plenty, but the role on modulation of AR activity needs to be further studied.

iv) Aegle marmelos (Bael):

In the present study it was observed that the activity of Aldose reductase in lenses under glycemic stress decreased when lenses were incubated with

A.marmelos (bael) leaf extract. However, the decrease in AR activity was not statistically significant.

A.marmelos leaves are used in Ayurvedic, Unani and Siddha systems of Indian medicine as antidiabetic agents. The role of this plant in the treatment of secondary diabetic complications is not well studied.

It was shown by Gacche and Dhole (2011) [354] that A.marmelos had a low AR inhibitory potential but it was a very potent antioxidant.

Sankeshi et al (2013) [355] have recently reported that the ethyl acetate extract of A.marmelos leaves inhibited the rat lens AR activity. They also observed that there was a dose dependant inhibition of AR and delayed progression of cataract in streptozotocin induced diabetic rats fed with ethyl acetate.

v) Vitamin C:

The role of vitamin C in eliminating reactive oxygen species is well established. It was shown that vitamin C reduces the diabetes induced lipid peroxidation. Vitamin C supplementation has also shown to increase SOD and CAT activities [334].

In our study, apart from acting as an antioxidant we also observed a fall in the activity of AR in hyperglycaemic lenses incubated with vitamin C. Though the AR activity decreased, the change was not statistically significant. There are not many studies in literature to defend or oppose our finding.