INTRODUCTION

Cataract, opacity of lens of the eye, is the leading cause of visual impairment accounting for 51% of the total global blindness [(1).] The prevalence of blindness due to cataract is higher in developing countries and is increasing with population growth and ageing [(2).]

In view of this WHO has initiated a global initiative for prevention of blindness in the "Vision 2020: the Right to Sight".

In India, according to a recent survey in the 'Rapid Assessment of Avoidable Blindness' (RAAB) study; cataract was responsible for 77.5% of avoidable blindness [(3)].

An association between diabetes and cataract is now well established. Cataract is one of the earliest secondary complications of Diabetes mellitus [(4)] [(5)]. There is evidence that the risk of cataract increases with increasing duration of diabetes and severity of hyperglycaemia [(6)].

Incidentally as India also hosts a large number of diabetic populations, the number of people suffering from cataract is on the rise.

The only treatment for cataract is surgery, providing excellent sight restoration. Considering the magnitude of population suffering from cataract, though cost effective, the surgery for sight restoration causes a great economic

burden especially in developing countries. There is thus an urgent need for inexpensive, non-surgical approaches to the treatment of cataract [7]. The present study is planned to find out some means of preventing or delaying cataract formation.

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. Plants have been found to be rich sources of phytochemicals like flavonoids, tannins etc. These phytochemicals are now proven to have great antioxidant and other therapeutic properties.

This study was thus undertaken on selected medicinal plants in an attempt to throw some light on the role of these plants as anticataractous agents. Medicinal plants like Aegle marmelos (bael), Embilica officinalis (amla), Syzygium cumini (jambhul) and Allium sativum (garlic), were chosen and their effect on cataractogenesis with respect to their antioxidant nature and aldose reductase inhibitory effect was studied.

CATARACT:

Cataract, opacity of the lens of the eye is mostly related to ageing, or may develop after an injury, inflammation, disease or occasionally could be congenital. Oxidative stress and diabetes are now considered as the major risk factors for age related cataract.

A) Oxidative Stress in the Lens:

The utilization of oxygen by metabolically active cells produces various free radicals.

Free radicals (FR's) represent any chemical species that have one or more unpaired electrons rotating in their outermost orbit. Various oxygen derived free radicals generated in metabolic pathways are superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet -}$), peroxyl radical ($LOO^{\bullet -}$) [8] [9]. These free radicals are also termed as Reactive Oxygen Species (ROS).

Molecules like Hydrogen peroxide (H_2O_2), Hypochlorous acid (HOCL), singlet oxygen (`O_2) and Ozone (O_3) that behave as oxidants are also converted to free radicals [10].

ROS are generally formed from normal metabolic reactions. Exogenous factors like environmental pollution, ultraviolet radiation, light, drugs, xenobiotics and transition metals can accelerate their production [11].

ROS formed in metabolic pathways are mainly those in the mitochondria during ATP synthesis, β oxidation, activation of P-450 enzyme system or by macrophages and neutrophils as a part of immune response [12].

All cells have their own defense system against free radicals known as Antioxidants. An imbalance between antioxidant defense system and FR generation causes oxidative stress.

Oxidative stress affects and damages biomolecules such as DNA, proteins and lipids, thereby affecting the cellular integrity. Almost all biological macromolecules can be oxidized by ROS; however, lipids and proteins are most liable biomolecules present in the lens.

Lens lipids:

The ROS can act on lipids, mainly PUFA of cell membranes and bring about lipid peroxidation; Lipid peroxidation is particularly destructive, as it develops a self perpetuating chain reaction.

The ROS removes a hydrogen atom from the membrane PUFA and form a lipid free radical. Oxygen is added to this lipid free radical to form fatty acid peroxyl free radical and oxidizes other PUFA, initiating new reactions. This mechanism is facilitated by transition metals (Cu & Fe) and double bonds of PUFA.

As a result of oxidative damage to PUFA, mainly of cellular or sub cellular membrane, the cohesion, fluidity, permeability & metabolic function of the cells is altered.

Lens proteins:

The ocular tissue, mainly lens, has a high percentage of proteins. The proteins in the lens, α and β crystallins are extremely long lived and have less turnover. Thus any alteration on the proteins is very important and can cause significant disturbance in the functioning of the lens [13] [14].

It has been observed that significant amounts of aromatic and sulphur containing amino acids in the protein structure make it more vulnerable to free radical damage. This condition is observed in the lens whose protein composition contains high proportions of tryptophan, tyrosine, phenylalanine, histidine, methionine and cysteine amino acids that can be modified by ROS, producing adducts and aggregation of proteins and altered enzyme function.

The peptide bonds are also susceptible to attack by FR's mainly due to oxidation of proline residues contributing to the peptide bonds.

B) Antioxidants and Lens:

All cells have their own protective mechanisms to counter act the oxidative assault. This is brought about by substances called antioxidants, which remove or minimize the deleterious effects of free radicals.

The lens has a protective system that can be classified into enzymatic and nonenzymatic antioxidants [15].

i) Enzymatic Antioxidants:

Enzymatic Antioxidants catalyze the transfer of electrons from a substrate to ROS, thereby stabilizing the ROS. The substrate or reducing agent used in the reaction is later regenerated to be used again. This regeneration is generally by using NADPH generated in different metabolic pathways.

The main antioxidant enzymes protecting the lens against ROS are Superoxide dismutase (SOD), Catalase, Glutathione peroxidase and Glutathione reductase. Each of these enzymes catalyzes the reduction of a particular type of ROS [16].

Superoxide dismutase (SOD) **-** Catalyzes the dismutation of superoxide radical (O_2^{*-}) intoH_2O_2 and O_2. The enzyme is a metalloprotein and has three isoforms. Cu-SOD and Zn-SOD are located in the cytosol, while Mn-SOD is mitochondrial.

Glutathione peroxidase – The enzyme is a selenoprotein and reduces H_2O_2 and other organic peroxides to water and alcohol. It uses reduced glutathione (GSH) as the electron donor. Glutathione peroxidase serves as an important defence against ROS mediated damage to lipid membranes and other molecules susceptible to oxidation.

***Catalase* –** It is a hemoprotein containing four heme groups. The enzyme is present in peroxisomes, mitochondria and cytoplasm. It catalyzes the conversion of H_2O_2 to H_2O and O_2. Catalase has a higher affinity to H_2O_2 when H_2O_2 is present in high concentration.

All of the above enzymes are found in the lens epithelium.

ii) Non Enzymatic Antioxidants:

They are a heterogeneous group of molecules, which act by donating an electron to a free radical and stabilize the free radical. The most important non enzymatic antioxidants in the lens are Vitamin C (Ascorbic acid), Vitamin E and Glutathione.

***Vitamin C (Ascorbic acid)* -** It is a potent water soluble antioxidant, reduces superoxide (O_2^{*-}), (OH^{*-}) and lipid hydroperoxide into more stable forms. Vitamin C also brings about recycling of α-tocopheryl to α-tocopherol. In the process of reducing ROS, ascorbic acid is converted to dehydroascorbate anion radical, which in turn can be reduced to native state by dehydroascorbate reductase and GSH. Vitamin C is present in the lens in large concentration.

Vitamin E- Serves as a primary defence liposoluble antioxidant in membranes. It converts superoxide (O_2^{*-}), (OH^{*-}) and lipid hydroperoxide radicals into less reactive molecules. To stabilize these ROS, α-tocopherol is converted to α-tocopheryl radical which is stable and does not react with other biomolecules. Through reactions mediated by Vitamin C, GSH and lipoic acid, α-tocopherol is regenerated. The antioxidative ability of Vitamin E thus depends on the concentration of the compounds which keep α-tocopherol in reduced state.

Glutathione (GSH) - It is a tripeptide (γ-glutamyl- cysteinyl- glycine) with sulphydryl (SH) group in its active site. GSH transfers electrons to various ROS like hydroxyl, carbonyl radicals to stabilize them and minimize their harmful effects. GSH also acts as a co-substrate for enzyme Glutathione peroxidase (GPx) in the removal of H_2O_2 and organic peroxides.

As mentioned earlier, GSH is required for the regeneration of α-tocopherol and Vitamin C. GSH also serves to maintain the lenticular proteins in reduced state. In the lens, next to Vitamin C, glutathione serves as an important antioxidant defence mechanism.

C) Hyperglycemia and Lens:

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin.

Prolonged exposure to elevated glucose causes – i) acute reversible changes in cellular metabolism and ii) Long term irreversible changes in stable macromolecules.

The injurious effects of hyperglycemia are characteristically observed in tissues that are independent of insulin for glucose entry, eg- eye lens, kidney etc. These tissues are not capable of restricting glucose entry into cells. With special reference to cataract, the following mechanisms have been proposed to explain how hyperglycemia possibly causes cataract development.

a) Non enzymatic glycation
b) Oxidative stress and
c) Polyol Pathway

a) Non Enzymatic Glycation:

Under hyperglycemic conditions, some of the excess glucose reacts non-enzymatically with proteins or other biological macromolecules.

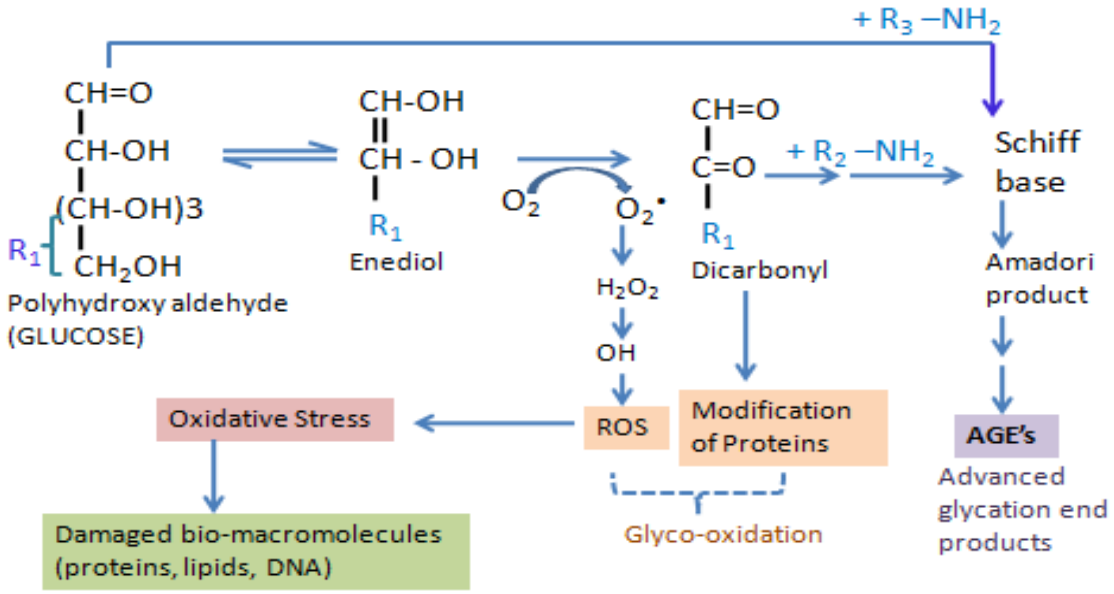
Glucose first undergoes auto oxidation to form unstable enediols. The carbonyl group of glucose attaches to the free amino group of proteins or

amino acids to form a labile Schiff base. This is the beginning of the formation of advanced glycation end products (AGE'^{s}) and the first step of the complex Maillard process. (Figure-1)

Once formed, Schiff base adducts undergo a slow chemical rearrangement over a period of weeks to a more stable, but chemically irreversible-Amadori product.

Immunological and chemical evidence indicates that progressive accumulation of AGE'^{s} in diabetic eye lens contributes to accelerated cataractogenesis in hyperglycemic experimental animals and diabetic humans [17] [18].

Figure 1: Non Enzymatic Reaction of Excessive Glucose in Hyperglycemia



b) Oxidative Stress and Diabetic Cataract:

Diabetes mellitus has been found to be inextricably connected with increased oxidative stress both in diabetic humans and hyperglycemic animals [19] [20].

Increased oxidative stress is now a widely accepted participant in the development and progression of diabetes and its complications.

A hypothesis suggesting that – 'the initial event leading to oxidative stress in hyperglycemia would be enhanced generation of ROS at the mitochondrial level as a consequence of increased intracellular glucose metabolism' has been proposed [21] [22].

It is explained as follows:

- As glucose enters the lens independent of insulin there is increased intracellular glucose metabolism.
- This results in increased entry of reducing equivalents in the mitochondrial respiratory chain, and thus there is an increased production of superoxide radicals and oxidative stress.
- It is recently proposed that increased superoxide radicals can affect the transcription of aldose reductase (AR) gene, resulting in

the upregulation of AR enzyme and thus the increased activity of Polyol Pathway during hyperglycemia [23].

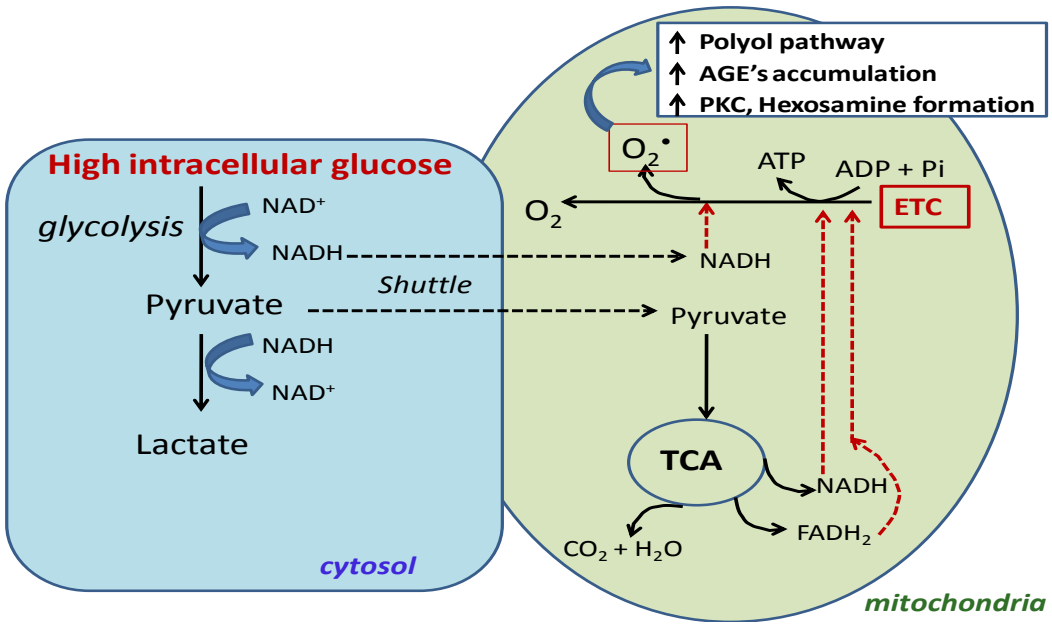


Figure 2: Increased intracellular glucose metabolism enhanced superoxide generation

- Superoxide anion radical is also responsible for inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [24,25,26,27]
- Inhibition of GAPDH is responsible for an increased formation of AGE forming compound methylglyoxal[28,29] which in turn causes production of an activator of endogenous protein-kinase C (PKC) and activation of hexosamine pathway. (Hexosamine pathway converts Fructose-6-P to glucosamine 6-P, an AGE)

- Methylglyoxal formed is also responsible for substrate induced upregulation of AR[30] which may further facilitate development of diabetic complications.

c) Polyol Pathway:

During hyperglycemia, the cellular levels of glucose greatly increase in tissues where glucose entry is independent of insulin, eg: lens, retina, kidney, peripheral nerves etc.

This excess glucose in cells is fluxed via polyol pathway which accounts for 30% of glucose oxidised [31], whereas this pathway accounts for only 3% under normoglycemic conditions [32].

Aldose reductase, the key enzyme of polyol pathway, catalyzes the reduction of glucose into the corresponding sugar alcohol - sorbitol [33], which is then converted to fructose by sorbitol dehydrogenase.

Sorbitol is osmotically active and accumulates in the cells as its penetration through cellular membranes is poor. Also the conversion of sorbitol to fructose by sorbitol dehydrogenase occurs at a very slow rate.

Sorbitol thus accumulates in the cells causing osmotic swelling and changes in membrane permeability. The resulting hyperosmotic stress is postulated to be the primary cause of diabetic cataract.

Polyol pathway induced oxidative stress:

There are 3 potential mechanisms by which the polyol pathway can induce oxidative stress-

i) AR enzyme utilizes NADPH as cofactor for the conversion of glucose to sorbitol. Increased AR activity during hyperglycemia thus depletes NADPH which is also required by Glutathione reductase to regenerate GSH. There is thus a significant decrease in NADPH and consequently decreased GSH.

ii) Oxidation of sorbitol to fructose by SDH causes oxidative stress because its cofactor NAD^+ is converted to NADH. NADH in turn is substrate for NADH oxidase to generate ROS. [34]

iii) The polyol pathway converts glucose to fructose. Fructose and its metabolites fructose-3-P and 3-deoxy-glucosone are more non enzymatic glycation agents than glucose. The formation of AGE is therefore increased. AGE, as well as binding of AGE to their receptors is known to cause oxidative stress. (Figure - 3)

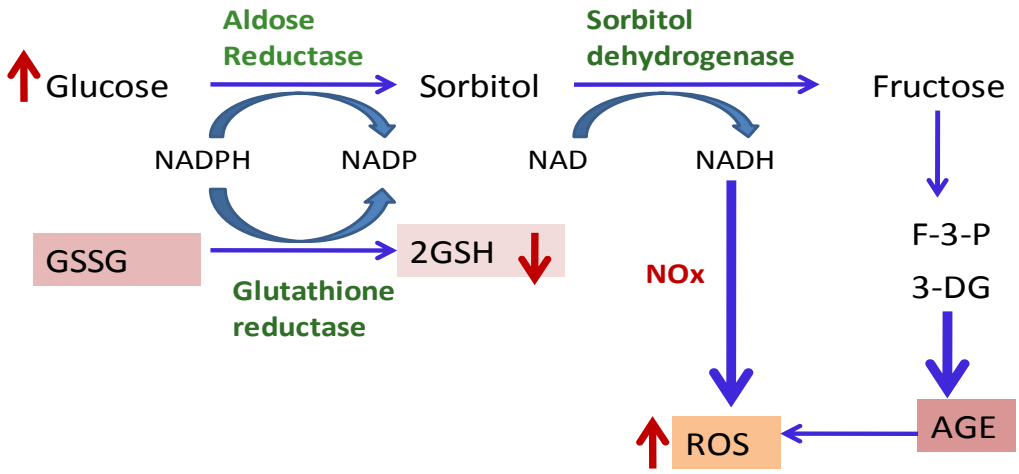


Figure 3: Polyol pathway induced oxidative stress

F-3-P : Fructose 3 phosphate
3-DG : 3-deoxy glucosone
AGE: Advanced glycation end products
ROS : Reactive oxygen species
GSH : reduced glutathione
GSSG : oxidised glutathione

D) Role of Medicinal plants in prevention of cataract:

Diabetes mellitus is a global epidemic affecting various essential biochemical functions and resulting in numerous complications including cataract.

In India, medicinal plants have been used as natural medicine since the days of Vedic glory. Many of these plants have potential anti-hyperglycemic property and thus have a remarkable role in the treatment of diabetes and its complications. Great opportunities are open for scientific investigations of herbal medicines in this field.

Herbs are staging a comeback and herbal renaissance is happening all over the world. Herbal drugs are considered free from side effects and are compatible with normal human physiology.

Phytoconstituents like flavonoids and tannins are effective antioxidants as they serve as free radical scavengers and inhibitors of lipid peroxidation. Plants can thus be beneficial in treating and preventing diseases caused due to free radicals and oxidative stress.

Plant flavonoids are now being considered as potential agents that could reduce the risk of cataract formation by affecting multiple key pathways involved in lens opacification viz. i) non enzymatic glycation ii) oxidative stress & iii) polyol pathway .

The hypoglycemic activity of various medicinal plants has been studied. The details of plants selected for our study is given below-

1) **Aegle marmelos (Bael),*(Family Rutaceae):*** Leaf extract produced hypoglycemic and antioxidant effect in alloxan induced diabetic rats. [35]

2) **Allium sativum (Garlic), *(Family Liliaceae):*** Components of garlic cloves showed potent antioxidant property and also inhibited accumulation of advanced glycation end products (AGE) [36]. Allicin

from garlic was found to be most potent antidiabetic agent in diabetic mice and rabbits. [37]

3) **Embilica officinalis (Amla), *(Family Euphorbiaceae)*:** The fruits are a rich source of vitamin C, a known antioxidant. The tannoids of E.officinalis are potent inhibitors of Aldose reductase enzyme and thus may have an important role in the management of diabetic complications. [38]
4) **Syzygium cumini (Jamun), Syn- Eugenia jambolana *(Family Myrtaceae)*:** Seeds were found to regulate the blood glucose level and also improve glucose tolerance. Seed extract also showed profound antioxidant activity. [39]

In view of the effective hypoglycemic and antioxidant property of these plants, an attempt is made in the present study to evaluate the protective effect of their water extracts against diabetes induced cataract.

The effect of these medicinal plant water extracts on soluble lens proteins, MDA, antioxidant enzymes like Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and key enzyme of polyol pathway- Aldose reductase is studied. The results are compared with that of vitamin C with respect to the same parameters.