REVIEW OF LITERATURE

Cataract- loss of transparency of the lens of the eye is the leading cause of visual impairment accounting for 51% of the total global blindness [(1)]. Cataractogenesis is one of the earliest secondary complications of Diabetes mellitus [(4), (5)]. Reports from WHO indicate that the human population worldwide appears to be in the midst of an epidemic of diabetes [(40)] .

The risk of cataract is known to increase with the increase in the duration and severity of diabetes [(6)]. The only means of sight restoration for cataract patients is cataract surgery, which is expensive and could result in surgical complications, mainly in diabetics. Study of the processes or mechanisms to delay the cataract formation would therefore be beneficial.

Increased oxidative stress is a widely accepted phenomenon in the development of cataract. The etiology of diabetic cataract is multifactorial. It involves various mechanisms of glucose toxicity like oxidative stress, increased polyol pathway and non enzymatic glycation [(41)] .

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 [(40)].

This review deals with the pathophysiology of diabetic cataract with reference to oxidative stress and glucose toxicity. It also reviews the role of selected medicinal plants as beneficial agents in diabetic cataract.

Biochemistry of Cataract:

Cataract: opacity of the lens of the eye, accounts for the leading cause of visual impairment worldwide.

Clinical, epidemiological and basic research studies by Bron et al (1998) [42]; Pollreisz and Schmidt Erfurth (2010) [43] , Obrosova et al (2010) [44] have reinforced the association between diabetes and cataract formation. Diabetic patients have a 60% more likelihood of developing cataract.

Cataract development in diabetics could be at a younger age and with a faster progression rate as compared to non diabetics. This observation was put forth by Bron et al (1998) [42].

Individual studies conducted by Baynes & Thorpe (1999)[28] , Kyselova et al (2004) [4] and Brownlee (2005) [45] suggested that mechanisms related to glucose toxicity such as oxidative stress, enhanced polyol pathway and increased non enzymatic glycation, play a significant role in the development of eye complications such as cataract in diabetic patients.

In a review on pharmacological prevention of diabetic cataract, Kyselova et al (2004) [4] have made a special mention of three mechanisms among several others that they thought could be of importance in diabetic cataract formation. They are: i) Oxidative stress, ii) Non enzymatic glycation and iii) Polyol pathway.

This review further deals with each of the above mechanisms.

I) Oxidative Stress and cataract:

The role of reactive oxygen species has been implicated in various diseases by Halliwel & Gutteridge (1990) [46].

Bhuyan and Bhuyan (1986) [47] observed that oxidation is an early event in cataract formation. They found considerable oxidation of membrane lipids in cataractous lenses as against normal lenses. Significant elevation of H_2O_2 levels in cataractous lenses was also observed in their study. A similar increase in H_2O_2 levels in cataract lenses was observed by Spector and Garner (1981) [48] and Ramchandran, Morris et al 1991[49].

It remained unclear whether increase in H_2O_2 was the cause or a result of the lens pathology. To obtain a better understanding of the role of oxidative

stress and H_2O_2 in the development of cataract, researchers developed model systems for experimental cataract by using lens organ culture technique.

Jernigen et al (1981) [50] proposed that H_2O_2 is the major oxidant causing lens damage. Garner, Garner and Spector (1982) [51], Spector, Wang et al (1993) [52] in their studies subjected lenses in organ culture to elevated H_2O_2 levels and confirmed that H_2O_2 in high concentration could cause cataract.

H_2O_2 induced oxidative stress damages the lens proteins. This was proposed by Mc Namara and Augustyen (1984) [53] when they incubated human lens proteins with H_2O_2 in vitro for 12 weeks. Zigler, Huang and Du (1989) [54] also noted similar results. Their study indicated that OH* formed from Fenton reaction is the cause for much of the protein modification and the finding was in confirmation with the work of Zigler, Jr Jernigan et al (1985) [55].

Garner, Garner and Spector A (1983) [56] also showed that H_2O_2 stress inhibits Na/K pump and thus causes uncoupling of ATP hydrolysis and ion translocation. The intra cellular environment of the lens is thus disturbed. These observations confirm that H_2O_2 can cause cataract.

Influence of various concentrations of H_2O_2 was studied on goat lenses in vitro. Parveen and Bulakh (1996) [57], suggested that incubation of goat lenses for 3 days with 50mM H_2O_2 and 100 mM H_2O_2 respectively, led to cataract formation. A concentration of H_2O_2 of 10mM strength also induced cataract in

goat lenses in vitro. This observation was put forth by Nahid and Bulakh (1998) [58].

Various other models were used to study the oxidative stress induced cataract. Hyperbaric oxygen, X-radiation, selenium and photochemical stress are examples of such models.

The lens is continuously exposed to endogenous and exogenous reactive oxygen species (ROS) which induce oxidative stress. Spector (1995) [59] stated that such chronic exposure of ROS to the lens may contribute to cataract formation by causing biochemical changes that gradually disarrange the lens fibers resulting in loss of transparency.

Diabetic hyperglycemia results in chronic oxidative stress which further results in over production of ROS. This concept was proposed by Hunt, Dean et al (1988) [60]. Sakurai and Tsuchiya (1988) [61], also made a similar mention. Both research teams also suggested that chronic hyperglycemia involves non enzymatic glycation of proteins and formation of products that in turn lead to generation of ROS.

In diabetic individuals, a large number of ROS can be generated through various pathways. The work of Wolf, Dean (1987) [62] and Lin (2003) [63] presented that excess of glucose may lead to superoxide radical ($O_2^{\cdot -}$) production by auto oxidation of glucose and oxygen in the presence of transition metals.

It was also noted by Stitt (2005) [64] that glucose may induce glycation of lens proteins, which can generate $O_2^{\cdot -}$ and simultaneously accelerate the formation of advanced glycation end products (AGE'^{s}). In vitro studies on lens epithelial cells by Hong, Lee et al (2000) [65] had also suggested the role of AGE's in the production of $O_2^{\cdot -}$ and H_2O_2 by reacting with specific cell surface receptors.

In diabetes, there is also an increased utilization of glucose through the polyol pathway. Obrosova (2005) [66] stated that increased sorbitol formed through increased polyol pathway generates oxidative stress. Ookawara, Kawamura et al (1992) [67] had already suggested that oxidative insult in the diabetic lens is aggravated due to the glycation and inactivation of lens antioxidant enzymes like superoxide dismutase.

The work of Reddy, Kasahara et al (2004) [68] and Rolo, Palmeira (2006) [69] also re-establish that increased intracellular glucose levels lead to increased sorbitol formation. There is thus a resultant loss of NADPH and glutathione during this conversion further causing oxidative stress.

To combat the continual assault of free radicals and ROS normally, the cell milieu has a range of antioxidants.

1) Overview of Antioxidants in the lens:

Antioxidant defence mechanisms in the lens include both- enzymatic and non-enzymatic defences.

In an article by Maritim, Sanders and Watkins III (2003) [70] enzymatic and non-enzymatic antioxidants have been reviewed.

Non-enzymatic antioxidants mainly include vitamins A, C and E, glutathione, lipoic acid, Coenzyme Q, bioflavonoids and some minerals (Cu, Zn and Se) and cofactors (folic acid, thiamine, pyridoxine and cobalamine).

Enzymatic antioxidants are superoxide dismutase, catalase, and glutathione peroxidase and glutathione reductase.

1.1) Non-enzymatic antioxidants:

These are a heterogeneous group of substances and act by donating an electron to free radicals in order to stabilize them and make them less harmful.

i) Vitamin C (Ascorbic Acid):

Vitamin C is normally found in high concentrations in the aqueous humor and lens of the eye.

Various studies by Varma, Kumar et al (1997) [71], Hegde, Varma (2004)[72], Devamanoharan, Henein et al (1991) [73] have shown that vitamin C plays a pivotal role in the protection of lens from oxidation. It is found that

vitamin C plays an important role in lens biology as an antioxidant and as a u-v filter when present in aqueous humor.

Heath (1965) [74] stated that the lenses of diurnal animals have a high concentration of vitamin C. This fact is now well recognized for over 60 years. Taylor et al (1991) [75] and Taylor & Nowell (1997) [76] also noted that the lens concentration of vitamin C is many times higher than that of plasma. Vitamin C was thus said to protect the lens from photochemical and oxidative stress.

Dietary deficiency of vitamin C led to the decrease of vitamin C concentration in the lens. This was studied by Ohta, Niwa et al (2001) [77]. Earlier Berger, Shepard et al (1989) [78] had observed that vitamin C concentration in the lens is decreased on aging and also in cataractogenesis.

The antioxidant role of vitamin C as studied by Yu (1994) [79] is by bringing about the reduction of superoxide ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot -}$) and lipid hydro peroxides to more stable forms. They proposed that ascorbate is also involved in the regeneration of vitamin E by converting α-tocopheryl radical to α-tocopherol.

However, studies of Halliwel & Gutteridge (1989) [80], Vander Pols (1999) [81], observed that vitamin C can generate H_2O_2 by reducing molecular O_2 in the presence of metal ions. Halliwel & Gutteridge (1992) [9] and Yu (1994) [79] have also suggested that in excessive concentrations of metal ions like Fe^{+3} and Cu^{+2}, vitamin C can act as a pro-oxidant.

In the study carried out by Sasaki, Giblin et al (1995) [82] it was noticed that the oxidation product of ascorbic acid; dehydroascorbic acid itself could be cataractogenic. The regeneration of ascorbic acid from dehydroascorbate by specific reductase enzyme is thus essential for maintenance of lens transparency.

The work of Spector, Ma & Wang (1998) [83] also showed that ascorbate along with metal ions makes a major contribution to H_2O_2 production.

ii) Vitamin E:

Vitamin E is known to suppress the propagation of lipid peroxidation. Along with vitamin C, it inhibits hydroperoxide formation, inhibits Fenton & Haber Weiss reactions. This property of vitamin E was brought to light by the work of Chow (1991) [84] and Oberley (1988) [85].

Vitamin E scavenges free radicals. As studied by Weber, Bendich and Machlin (1997) [86] vitamin E is a component of the peroxyl radical trapping antioxidant system. It protects membrane lipids from peroxidation by reacting with the peroxyl and superoxide radicals and singlet oxygen.

There are conflicting reports about Vitamin E levels in diabetic animals and human subjects. Asayama, Nakane et al (1994) [87] showed an increase in vitamin E levels in diabetics while Garg et al (1996) [88], Palmer et al (1998) [89],

Cinar et al (2001) [90] observed a decrease in Vitamin E. The levels of this vitamin remained unaltered in a study by Martinoli et al (1993) [91].

α-tocopherol (vitamin E) is the most potent lipid soluble antioxidant which protects the membrane lipids. Powers and Lennon (1999) [92] postulated that α-tocopherol can stop the chain reaction of ROS and thereby protect membrane damage.

Chihuailaf, Contreras (2002) [93] explained that vitamin E stabilizes ROS by converting to α-tocopheryl radical, which is stable and does not react with other biomolecules. α-tocopheryl is regenerated to α-tocopherol by vitamin C, GSH and lipoic acid. Thus the antioxidant ability of vitamin E depends on the concentration of these substances which regenerate vitamin E during oxidative stress.

The role of vitamin E in the prevention of cataract has been confirmed by various animal, epidemiological and clinical studies. Ross (1990) [94] noted that subcutaneous injection of α-tocopherol prevented the ionising radiation induced damage in rat lenses as compared to those rats not supplemented with vitamin E. Other animal studies by Nagata et al (1999) [95], Ohta et al (1999) [96] also confirmed the preventive effect of vitamin E when used as a topical application. Other researchers like Leske et al (1998) [97] have studied that the risk of development of cataract was 57 % less in individuals taking a regular

supplement of vitamin E and 42 % less in subjects with high plasma vitamin E levels.

iii) Vitamin A:

Human lenses are found to contain vitamin A and carotenoids. In a study conducted by Yeum, Shang et al (1999) [98] the concentration of these nutrients was found to be higher in the epithelial region of the lens than that in the cortex / nuclear portion.

A prospective study conducted by Chasen-Taber, Willet et al (1999) [99] showed that the risk of cataract development decreased by 22 % in those individuals with a high intake of carotenoids and vitamin A.

Other cohort studies by Hankinson, Stampfer et al (1992) [100] and Brown, Rimm et al (1999) [101] also showed similar findings. Moeller, Jacques et al 2000[102] hypothesized that protective effect of carotenoids may be due to quenching of reactive oxygen species generated due to uv light Yu (1994) [76] studied that the antioxidant property of vitamin A is due to its chemical structure. The long chains of conjugated double bonds convert superoxide and lipid peroxide radicals to less reactive forms.

iv) Glutathione (Tripeptide – γglutamyl cysteinyl glycine):

In its reduced form, glutathione (GSH) is a major intracellular redox buffer. It functions as a free radical scavenger by virtue of acting as a co-substrate for enzyme glutathione peroxidase in the removal of H_2O_2 and other organic peroxides. GSH transfers electrons to oxidized molecules like $OH^{\cdot -}$ and carbonyls and in turn becomes oxidized (GSSG).

GSH/GSSG functions as the major redox couple, Wu, Fang et al (2004)[103] in their work stated that this redox pair determines the antioxidative capacity of the cells. The glutathione concentration was observed to be decreased in cataractous lenses of chemically induced diabetic animals. This observation was made by Obrosova and Stevens (1999) [104].

In the studies conducted by Sasaki H, Giblin F J et al (1995) [79] GSH has been found in lens epithelium. It was also found to be present in cornea and retina by Kannan, Tang et al (1993) [105]. Taylor, Jacques et al (1995) [106] stated that after vitamin C, glutathione was the next best antioxidant for defense against photo-oxidation in the lens.

The fact that GSH is required for the maintenance of lenticular proteins in a reduced state was brought to light by the work of Zhang, Chai et al (2008)[107].

Various roles of GSH were determined by Sundaram, Kavookajian et al (2007) [108] where the primary role of GSH as a free radical scavenger was mentioned. They also reported that GSH was required for cell proliferation,

protein synthesis, cell cycle regulation, homeostasis of immune system and also utilization of glucose and for other metabolic pathways.

Ganea and Harding (2006) [109] studied that GSH helps to maintain the thiol groups of the lens crystallins in reduced form and thus maintains lens transparency. Low levels of glutathione were reported in aged human lenses by Harding (1970) [110].

George, Jyothi et al (2003) [111] observed decreased GSH levels in human cataractous lenses. Similar findings were noted in diabetic cataract lenses by Donma, Yorulmaz et al (2002) [112]. The work of Doganay, Turkoz (2002) [113] and Doganay, Borazan (2006) [114] showed decreased GSH levels in experimentally induced cataract models.

GSH is also known to participate as an antiglycating agent in the Maillard reaction and thereby prevent glycation of proteins. This role of GSH was described by Ortwerth and Olesen (1988) [115] and Nagaraj, Prabhakaram, Ortwerth (1994) [116]. They showed that the presence of GSH in glycation mixtures in vitro prevented sugar mediated protein crosslinking.

1.2) Enzymatic Antioxidants:

Antioxidant enzymes- superoxide dismutase, glutathione peroxidase and glutathione reductase are discussed in this review. Each of these enzymes is found to neutralize the harmful nature of a particular type of reactive oxygen species.

i) Superoxide Dismutase (SOD) : EC 1.15.1.1

SOD converts superoxide radicals to H_2O_2 and thereby restricts the deleterious interaction of superoxide anion with other biomolecules.

Three isoforms of SOD are located in different compartments of the cell. Cu –Zn SOD is found in the cytoplasm, nucleus and ECF, while Mn-SOD is found in the mitochondria. All isoforms have been identified in the eye.

Cejkova, Stipek et al (2000) [117]; (2004) [118] and Bilgihan, Bilgihan et al (2003) [119] have detected SOD in the cornea. Presence of SOD in lens epithelium was studied by Ozmen, Ozmen et al (2002) [120] and in aqueous humor by Satici, Guzey et al (2003) [121].

Obara (1995) [122] reported that the activities of Cu-Zn SOD decreased in cataractous lenses. He stated that this fall in SOD was due to its utilization for neutralizing the accelerated rate of ROS generated in diabetic cataract.

In an in-vitro study conducted by Khanna,Wang et al (1997) [123] it was observed that SOD mRNA levels decreased in rats exposed to hyperglycemic environment.

The inactivation of SOD by sugars was investigated by Yan and Harding (1997) [124]. It was noted that the antigenic property of the enzyme was lost by incubation with sugars.

The findings of Ozmen, Ozmen et al (2000) [125] also noted the decrease in Cu-Zn SOD in diabetic cataractous lenses.

It was observed by Fridovich (1995) [126] that loss of the cytosolic SOD resulted in superoxide induced cell damage. Behndig, Karlson et al (2001) [127] noted that Cu-Zn SOD is the dominant isoform of SOD in the lens. They observed increased levels of $O_2^{\cdot -}$ in lenses from mice lacking this isoform. Such lenses were more prone to photochemical cataract.

SOD activity in human lenses was found to decrease with age. This was observed by Scharf and Dovrat (1985) [128] and they proposed that this fall in SOD contributed to the formation of senile cataract.

In-vitro studies by Olofsson, Marklund (2005) [129] showed that hyperglycemic stress accelerated cataract formation in lenses from SOD null mice. The same researchers in (2007) [130] showed the increased occurrence of cataract in streptozotocin (STZ) induced diabetic SOD null mice. Their study emphasized the importance of SOD in the prevention of hyperglycemia induced cataract.

ii) Glutathione Peroxidase and Glutathione reductase:

Glutathione peroxidase (GPx) and glutathione reductase (GRx) are found in the cytoplasm, mitochondria and nucleus. Glutathione peroxidase catalyses the conversion of H_2O_2 to water by using reduced glutathione as the hydrogen donor. The oxidized glutathione (Glutathione disulphide GSSG) is then recycled back to glutathione (GSH) by glutathione reductase using NADPH as the cofactor. Glutathione peroxidase system functions effectively only if GSH is

regenerated. Heyningen and Pirie (1953) [131]; showed that glutathione peroxidase is coupled with glutathione reductase.

The lens is highly affected by harmful oxidants like H_2O_2. Increased glutathione peroxidase activity prevents lipid peroxidation by attacking H_2O_2. This explanation was put forth by McCay, Gibson et al (1976) [132].

The research work on cataract by Reddy (1971) [133]; Meloni, Carta et al (1990) [134] explained that glutathione reductase protected the lens and erythrocytes from oxidative damage. They proposed that glutathione reductase maintained the sulphydryl groups of proteins.

Earlier Jedziniak, Kinoshita et al, (1973) [135]; proposed that the activity of glutathione reductase could also decrease due to the deficiency of its coenzyme FAD.

Various researchers like Cejkova, Stipek et al (2004) [118] and Cejkova, Vejrazka et al (2004) [136] have reported the presence of GPx in cornea.

Glutathione peroxidase is also located in the lens epithelium, aqueous humor and retina. These findings were put forth by Satici, Guzey et al (2003)[121].

It was determined by A. Ringvold, E. Anderssen et al (2000) [137] that in cornea the primary antioxidant is vitamin C and SOD, while GPx and GRx have a secondary role. Further the work of Cejkova, Stipek et al (2000) [115] and (2004) [116] explained that when an episode of oxidative stress is triggered, the activity of antioxidant enzymes begins to decrease. GPx is first affected

followed by SOD, resulting in accumulation of H_2O_2 and subsequent damage to cornea.

Godvin and Wohaieb (1988) [138] described that as the activities of antioxidant enzymes glutathione peroxidase and superoxide dismutase decrease in blood and different tissues of diabetic individuals, the levels of ROS increases. Ozmen, Erkin et al (2002) [139] confirmed these findings and also noted that along with decrease in antioxidant enzymes, non-enzymatic antioxidants like GSH also decrease with aging.

Ganea and Harding (2006) [109] explained that failure to regenerate GSH from GSSG using glutathione reductase and NADPH may be one cause of reduced GSH levels.

Glutathione reductase activity in blood was found to be lower in diabetic cataract pateints as compared to senile cataract, whereas the activity of this enzyme in lens of diabetic cataract was higher than that in senile cataract. These observations were presented by Donma, Yorulmaz et al (2002) [112].

II) Non Enzymatic Glycation

During hyperglycemia, excess glucose reacts non-enzymatically with proteins and other constituents in blood. Brownlee (1996) [140] studied the advanced glycation end products (AGE's) in diabetes and aging. The glycation causes chronic irreversible changes in the long lived molecules like crystallins in eye lens, chromosomal DNA, extra cellular matrix etc.

The formation of AGE is initiated by the attachment of glucose carbonyl group to a free amino group of the protein or amino acid. This results in the formation of a Schiff base which is a labile molecule and is the first step in the complex Maillard process.

Monnier et al (1992) [141] explained that the schiff base adducts undergo slow chemical rearrangement to form a stable chemically reversible Amadori product. Complex biochemical cascade reactions like dehydration, condensation, fragmentation, oxidation, cyclization of the Amadori product form advanced glycation end products.

Kowluru, Kern & Engerman (1996) [142]; Wohaieb & Godvin (1987) [143] showed that irreversible AGE's were formed via a sequence of glycation & oxidation reactions. Baynes (1991) [19] also suggested that glycation and oxidation are closely connected and the complex processes are referred to as glyco-oxidation.

The Amadori product formed during formation of AGE's undergoes auto oxidation. It can therefore contribute to oxidative damage of proteins exposed to hyperglycemia. This aspect was presented by Baynes (1991) [19]; Baynes & Thorpe (1999) [28].

The work of Brownlee (1996) [140], Westwood & Thornally (1995) [144] suggested that AGE's participate in glucose derived cross link formation and are responsible for diabetic complications.

Extensive research by various researchers like Araki, Ueno et al (1992)[17], Duhaiman (1995) [18], Lyons Silvestri, Dunn et al (1991) [145], Nagaraj, Sell et al (1991) [146], Shamsi, Sharkey et al (2000) [147] with immunological and chemical evidence indicated that progressive accumulation of AGE's in the lens of diabetic humans and animals contributed to accelerated cataractogenesis.

III) Polyol Pathway

Various hypothesis have been put forth to understand the process of cataract formation.

The aldose reductase osmotic hypothesis is one important hypothesis which suggests that accumulation of polyols initiates lenticular osmotic changes resulting in cataract formation.

The work of Kador, Kinoshita et al (1984) [148], Greene, Sima et al (1992)[149], Obrosova, Faller (1997) [150] have gathered considerable evidence to implicate that activation of sorbitol pathway by glucose is an important component in the pathogenesis of diabetic complications.

Aldose reductase (AR) is a NADPH dependent enzyme and was described by Hers (1956) [151] to have glucose reducing activity.

Van Heyningen in (1959) [152]; subsequently reported that during diabetic and galactosemic cataractogenesis, high levels of AR activity was present in rat

lenses. He also observed that aldose reductase derived polyols, sorbitol and galactitol accumulated in the lenses.

Based on these findings, Kinoshita et al (1968) [153] and Varma and Kinoshita (1976) [154], demonstrated that the use of pharmacological inhibitors of AR ameliorated cataract formation in diabetic rats and galactose exposed rabbits.

These observations further led Varma and Kinoshita (1977)[155], Kinoshita et al (1979) [156] and (1981) [157] to propose that accumulation of sorbitol in lens due to increased AR activity causes osmotic swelling of the lens. The osmotically swollen lens undergoes osmotic imbalance and protein insolubilization resulting in cataract formation. It was also suggested that a similar sequence of events could account for other diabetic complications like retinopathy, neuropathy and nephropathy. The osmotic theory of cataract formation and the study of aldose reductase inhibitors (ARI) have promoted extensive research in the last few decades.

Bhatnagar and Srivastava (1992) [158] suggested that tissue injury in hyperglycemia may be due to increased NADPH utilization as a result of increased AR activity.

Gonzalez et al (1984) [31] proposed that as AR is an NADPH dependant enzyme, a profound increase in AR catalyzed reaction may impose significant strain on NADPH supply. In support of this observation Sheetz and King (2002)[159] further explained that as NADPH is used in important reductive

metabolism processes like detoxification of ROS and hydroperoxides etc a large drain out of NADPH pool could compromise the cells ability to protect itself from oxidative damage.

It was also contended by Gonzalez, Sohor & Mclean (1983) [160], Lee & Chung (1999) [161], Obrosova, Gao et al (1998) [162] that NADPH depletion is combined with significant fall in lenticular GSH levels. GSH is an important intralenticular antioxidant which is maintained by NADPH dependent enzyme glutathione reductase.

In an experimental study Varma, Kinoshita (1974) [163] found that mice which were devoid of lens AR did not develop cataract in hyperglycemic conditions. Lee, Chung et al (1995) [164] demonstrated that polyol (sorbitol) accumulation is responsible for diabetic cataract formation by using transgenic mice which had over expression of AR.

Studies by Cheung, Fung (2005) [165] also showed that a genetic defect in AR in mice prevented the diabetic complications in retina.

Early trials on animal models by Pitts et al (1986) [166], Stribling (1990)[167] and Hotta et al (1990) [168] have demonstrated that aldose reductase inhibitors offered significant protection against diabetic complications.

Through experimental models Kador, Kinoshita et al (1985) [169], suggested that compounds that could inhibit AR could be effective in preventing certain diabetic complications.

These studies thus highlight the susceptibility of the polyol pathway as a target for devising strategies for prevention of diabetic complications.

Prevention of Diabetic Cataract:

Multiple mechanisms have been proposed in the development of diabetic cataract.

Kyselova, Stefek (2004) [4] have claimed that the exact sequence of events which result in lens opacification are not yet clearly defined. The three important and probable mechanisms that may be involved in diabetic cataract formation are:

i) Non-enzymatic glycation of lens proteins

ii) Oxidative stress and

iii) Increased polyol pathway in hyperglycemia.

A definitive pharmacological therapy is not yet available and thus surgical removal of cataractous lens is the only respite to cataract patients.

Various experiments were conducted to study the possible pharmacologic prevention of diabetic cataract based on the above mentioned mechanisms.

i) Prevention of Non-enzymatic glycation of lens proteins:

Researchers focused on the possibility of delaying cataractogenesis by offering protection to the amino groups of long lived proteins. They contended that an efficient inhibitor of non-enzymatic glycation would inhibit formation of advanced glycation end products (AGE's) and protein crosslinking.

Brownlee et al (1986) [170], Khatami, Suldan et al (1988) [171], Lewis & Harding (1990) [172] studied the role of aminoguanidine in the prevention of diabetic complications. It was observed that aminoguanidine could react with compounds at different stages of glycation to prevent the formation of glycation products.

It was also claimed by Swamy, Green et al (1996) [173] that aminoguanidine slowed the progression of lens opacification in rats with moderate diabetes.

Cotlier (1961) [174] had reported the cataract protective effect of aspirin in patients with rheumatoid arthritis and diabetes. Later, several experimental studies by Ajiboye & Harding (1989) [175], Bucala, Manabe et al (1985) [176], Rao & Cotlier (1988) [177], Swamy & Abraham (1989) [178] showed that aspirin protected the lens proteins against a variety of cataractogenic chemicals.

Studies by Crompton et al (1985) [179], Qin, Smith & Smith (1993)[180] asserted the protective action of aspirin and claimed that the protection of lens protein was brought about by the acetylation of their vulnerable groups. The

acetylation of a single lysine in human crystalline was identified by Lin, Barry et al (1998) [181].

Various researchers have shown that aspirin, ibuprofen and paracetamol delay experimental cataracts in laboratory animals. Blackytny & Harding (1992)[182], Gupta et al (1984) [183], Swamy & Abraham (1989) [173] confirmed that aspirin lowered the glycation of lens protein and also helped to maintain glutathione levels in the lens.

ii) Reduction of Oxidative Stress:

The search for possible agents to protect the lens from cataractogenesis continued by way of reducing oxidative stress.

It is well established that antioxidants reduce oxidative stress by eliminating formed ROS by scavenging, trapping and quenching them or by chelating metal ions to inactive forms. Work on the role of various antioxidants has already been discussed in this section earlier.

iii) Inhibition of the polyol pathway:

Development of potential new agents to interfere with cataract formation by inhibition of polyol accumulation in the lens could be another approach in cataract prevention.

A wide range of molecules were synthesized to inhibit the enzyme aldose reductase. Large numbers of clinical trials were started on AR inhibitors, but as stated by Stribling (1990) [162] a lot of problems were encountered. Moreover, most of the trials concentrated on other diabetic complications rather than cataract.

The study of an aldose reductase inhibitor (ARI) ponalrestat on diabetic complications by Crabbe and Goode (1998) [184] showed disappointing results while the work on compounds epalrestat and tolrestat by Yabe, Nishimura (1998) [185] proved to be beneficial. According to Hotta, Kakuta (1990) [168] trials on sorbinil, a leading ARI were withdrawn due to unacceptable adverse effects.

Srivastava et al (2005) [186] in their review article stated that clinical trials with ARI were conducted with little information about the enzyme. There was scanty information available about the involvement of AR enzyme in processes other than glucose utilization. In recent years, apart from glucose oxidation, extensive data has been gathered regarding the role of AR in inflammation, detoxication and growth.

Several studies conducted by Ramana, Dixit et al (2000) [187] and Dixit, Balendiran et al (2000) [188] focused on the structural and kinetic properties of AR. The function of AR to detoxify toxic glutathione conjugates of unsaturated

aldehydes was put forth by their study. The study also showed that AR reduces the aldehydes generated by oxidized phospholipids.

Ruef, Liu et al (2000) [189] suggested the role of AR in inflammation during their study on arterial injury. In another study conducted by Ramana, Friedrich et al (2004) [190] it was found that AR regulated the activation of inflammatory factors in hyperglycemic vascular smooth muscle cells.

In their review Srivastava et al (2005) [186] also mentioned that the adverse effects in the clinical trials on ARI's could be attributed to the diverse and immensely important roles played by this enzyme. The authors further stated that more selective and effective inhibitors of AR needed to be studied which would specifically inhibit the cytotoxic role of AR without affecting its detoxification role. Such inhibitors would be helpful in the management of secondary diabetic complications.

As stated by Kyselova et al (2004) [165], ARI's could definitely have a potential to correct biochemical and metabolic derangements during hyperglycemia, which could further help to prevent irreversible changes of eye lens crystallines and prevent or delay cataract formation. The authors also claimed that development of an anticataract agent in humans would require better understanding of normal lens transparency, pathogenic link between hyperglycemia and lens opacity, mechanisms of better drug penetration. They also proposed for supplementation with an anticataract agent as an adjunct

therapy to prevent the formation of diabetic cataract and confirmed that clinical trials would be required in this area.

Medicinal Plants and Diabetic Cataract

Great strides have been taken to understand and manage diabetes and its related complications, yet the disease has been unabated. Various researchers have urged for an urgent need to identify and develop novel therapies to combat the disease.

Considering that diabetes is a multifactorial disease, Bailey 2000[191] emphasized for a need of range of different agents to address different features of the disease at its different stages.

Tiwari & Madhusudhana Rao (2002) [192] pointed out that it is now agreed by increasing number of researchers and practitioners that traditional medicinal systems are more holistic in their approach. Traditional medicines make use of a variety of herbal and non herbal ingredients that act on multiple targets through multiple mechanisms.

Traditional medicinal plants have been used since ancient times by both physicians as well as laymen to treat various diseases like diabetes, cardiovascular diseases, cancer etc. The active principles and properties of these plants are studied by numerous researchers like Havsteen (1984)[193], Middleton Jr, Kandaswamy et al (2000) [194] to name a few.

A large amount of epidemiological evidence put forth by Taylor (1993) [(195)]; (2000) [(196)] suggests that sufficient intake of fruits and vegetables lower the risk of cataract in humans. This effect may be attributed to the presence of a variety of constituents like vitamins, minerals, fiber and numerous phytochemicals in the fruits and vegetables.

Patil (2009) [(197)], Majumdar et al (2010) [(198)], Kalt et al (2010) [(199)] maintained that important phytochemicals – flavonoids, possibly prevent both age related and diabetic cataract as they are capable of affecting multiple factors responsible for ocular diseases impairing vision.

Micromolar levels of a plant flavonol- quercetin was chosen to inhibit oxidation induced sodium and calcium influx and loss of transparency. This study was conducted in vitro by Sanderson et al 1999[(200)] in rat lens organ culture.

Later Cornish et al (2002) [(201)] also showed quercetin and its metabolites to be an active inhibitor of oxidative damage in the lens. In another in vitro study on organ culture model by Gayathri et al (2010)[(202)]methylated quercetin isolated from leaves of *Cochlospermum religiosum* was confirmed to have lens protecting activity in selenite induced cataract in rats. A similar observation was put forth by Rooban et al (2011) [(203)] using a flavonoid fraction isolated from leaves of *Vitex negundo* (*Nirgudi*).

A few decades ago Varma et al (1977) [155] studied the effect of quercetin in streptozotocin induced diabetic rats. The diabetic animals not receiving quercetin developed cataract in ten days while those receiving quercetin did not develop cataract even after 25 days of onset of diabetes. The hyperglycemia in both the groups was almost similar.

Lu et al (2008) [204] performed a similar study on diabetic rats using isoflavonoid rich soy protein. He observed a marked reduction in the death rate and incidence of cataract in treated diabetic animals. They also argued that low glucose concentration was observed in treated rats.

Lower incidence of cataract in streptozotocin induced diabetic rats was also noted by Nakano et al (2008) [205] when they used a mixture of bioflavonoids as protective agents. Separate studies conducted by Beyer-Meyers & Farnsworth (1979) [206] and Mohan et al (1988) [207] recorded that topical administration of quercetin in galactosemic rats diminished cataractogenesis. They observed that the topical administration of quercetin reduced lens intracellular edema, prevented fluid accumulation in extracellular spaces and maintained integrity of lens fibers.

Significant correction in the eye lens electrolyte disturbances and normalisation of lens proteins was noted by Ramana et al (2007) [208] when they administered quercetin orally to galactosemic rats.

To study the anticatarct effect of various flavonoids, the rat selenite cataract model was extensively used. Shearer et al (1997) [209], Gupta et al (2009) [210] and Kyselova (2010) [211] have reported results on this model. Their results are in confirmation with other researchers.

Lija et al (2006) [212] reported that the flavonoid fraction from the plant Emilia Sonchifolia decreased the maturation of selenite induced catarct more effeciently as compared to quercetin. It was also observed that the activities of SOD, catalase and reduced glutathione were increased while the levels of TBA reacting substances were decreased on treatment of lenses with this flavonoid.

Onion, common food stuff was identified as a rich source of flavonoids. Fossen et al (1998) [213], Miean & Mohamed (2001) [214] found that the flavonoids in onion were mainly quercetin, quercetin-4'-glucoside and quercetin-3,4,diglucoside.

Effect of fresh onion juice by topical application into rat eyes was studied by Javadzadeh et al (2009) [215]. Their study also contended the rise in SOD and glutathione peroxidase activities in selenite exposed rat lenses. The mean total antioxidant levels were higher and prevention of cataract formation was about 75 %.

Several other workers have isolated flavonoids from various plant sources and studied its effect on selenite models. To name a few are

Thiagarajan et al (2001) [216]- green and black tea, Thiagarajan et al (2002) [217] - Ginko biloba; Durukan et al (2006) [218]- grape seeds, Vibin et al (2010) [219]- broccoli.

Ertekin et al (2004) [220] noted that Ginko biloba also offered considerable protection to rat lenses in radiation induced cataract.

Stefek (2011) [221] has reviewed the anticataract potential of flavonoids. Flavonoids could decrease the risk of cataractogenesis by influencing key mechanisms of lens opacification, viz: oxidative stress, non-enzymatic glycation and polyol pathway.

Multi Functional Nature of Flavonoids:

Flavonoids are natural polyphenolic substances found in plants and they efficiently affect multiple key molecular mechanisms in both diabetic and age related cataract.

A] Flavonoids as antioxidants:

The antioxidant nature of flavonoids is the best described biological activity of this group. Abundant literature covering numerous studies on antioxidant property of flavonoids is available and is however beyond the scope of this review.

Work of various researchers including Pietta (2000) [222], Butkovic et al (2004) [223], Boots et al (2008) [224] has pointed out that flavonoid quercetin is the most potent scavenger of ROS, including superoxide, peroxyl, alkoxyl and hydroxyl radicals and also reactive nitrogen species.

Bors & Michel (2002) [225] postulated "Bors criteria"- which mention general structural aspects of flavonoids which are responsible for their effective antioxidant effect / radical scavenging. They are namely – i) presence of a catechol ring B, which can donate H^+ or electrons to stabilize free radicals, ii) 2,3 unsaturation with oxo function in C ring responsible for electron delocalization and iii) presence of 3-OH group in the heterocyclic ring which increases radical scavenging activity.

The property of flavonoids to chelate transition metal ions has been well documented by Nijveldt et al (2001) [226], Pietta (2000) [222], Williams et al (2004) [227].

B] Flavonoids as inhibitors of Advanced Glycation:

Non-enzymatic glycation has already been reviewed as a key mechanism causing diabetic cataract.

In a large study of 62 different flavonoids Matsuda et al (2003) [228] formulated the structural aspects of flavonoids for inhibition of non-enzymatic glycation of proteins. Increase in the number of hydroxyl

groups in the structure were found to strengthen the inhibition of advanced glycation. Methylation or glucosylation of flavones, flavonoids also altered their inhibitory action.

Morimitsu et al (1995) [229] studied four flavonoids from the methanol extract of plant *Thymus vulgaris* and found that levels of AGE's and fructosamines of serum albumin were suppressed in vitro.

The potent antiglycation action of flavonoids from *Vaccinium vitis idaea* berry extract was demonstrated by Beaulieu et al (2010) [230] using bovine serum albumin model. Other natural flavonoids like rutin, lutelin and rutin metabolites were shown to have glycation inhibitory effect by Wu et al (2005) [231].

Nagasawa et al (2003) [232] studied that flavonoids rutin and G-rutin, a water soluble glucose derivative of rutin, decreased the protein glycation in muscle and kidney when exposed to glucose in vitro. They also observed similar effects in vivo in streptozotocin induced diabetic rats.

C] Flavonoids as aldose reductase inhibitors:

The accumulation of sorbitol due to excessive influx of glucose through polyol pathway has been well established as a primary

contributing factor for diabetic cataract formation. Work of various researchers has been considered in the earlier section of this review.

Extensive work by Constantino et al (2000) [233], Miyamoto (2002)[234], Suzen et al (2003) [235], Alexiou et al (2009) [236], Obrosova et al (2010) [237] have confirmed that ARI's represent a potential therapeutic strategy to prevent the onset or progression of diabetic cataract.

The inhibitory effect of active flavonoids isolated from natural products was studied in rat lens or on human recombinant aldose reductase by many researchers. Flavonoids represent an important class of ARI's and since mid 1970's a number of studies by Varma et al (1975)[238], Varma & Kinoshita (1976) [154], Okuda et al (1982) [239], Nakai et al (1985) [240], Jung et al (2002) [241], Matsuda et al (2003) [228], Lee et al (2010) [242] have reported the inhibition of AR by flavonoids.

Apart from flavonoids, plants also contain a large number of other phyto-constituents like alkaloids, tannins, phenols, gallic acid, carotenoids etc. All of these phytochemicals are noted to have antioxidant properties and are said to be beneficial in a large number of diseases including diabetes and its complications.

In an attempt to address the need for development and evaluation of new ARI's based on efficacy, selectivity and safety issues Saraswat, Muthenna et al (2008) [243] evaluated the aldose reductase inhibitory

potential of the aqueous extract of some commonly consumed plant sources using rat lenses. They identified certain dietary agents with definite ARI potential and suggested the inclusion of these in the diet to combat diabetic complications.

Various indigenous medicinal plants are used since ancient times for the treatment of ocular diseases and also diabetes and its complications.

After a careful review, a few medicinal plants were selected for the present study. These plants were well known for either their anticataract, hypoglycemic or antioxidant properties.

Medicinal Plants:

1) Aegle marmelos (Hindi – Bael): Family –Rutaceae

It is a spiny tree - the root, bark, leaves, fruits and seeds of which are valued in Ayurvedic medicine in India.

A.marmelos leaves were selected for the present study. Leaves of the plant are reported to have astringent, laxative, expectorant properties. They are also said to be useful in ophthalmic conditions, diabetes, inflammation etc.

Karunanayake et al (1984) [244] have studied that the aqueous decoction of bael leaves show significant hypoglycemic effect. A similar observation was made by Seema et al (1996) [245]. It was also noted by Das et al (2009) [246] that *A.marmelos* leaf extract also helped in the regeneration of β cells of pancreas in diabetic rats.

The leaf extract was also noted to inhibit various free radicals in vitro by Jagetia et al (2003) [247] who further also proposed that the leaf extract has radio-protective action and can reduce radiation induced sickness. They explained that the radio-protective action could be due to scavenging of free radicals and elevation of glutathione levels, thereby preventing lipid peroxidation.

The hypoglycemic and antioxidant activity of A.marmelos leaves was also evaluated by Upadhya , Shanbag et al (2004) [248]. They treated alloxan induced diabetic rats with bael leaf extract and found that the blood glucose levels in diabetic rats normalised and there was a significant elevation in the levels of glutathione peroxidase and GSH. Lipid peroxidation in terms of MDA was also found to be decreased.

Other studies conducted by Ponnachan et al (1993) [249] also endorsed the hypoglycemic actvity of A.marmelos leaf extract in alloxan diabetic rats.

A recent study was conducted by Narendhirakannan & Subramainan (2010) [250] in which streptozotocin induced diabetic rats were administered the leaf extract orally. They found that the altered activities of key antioxidant enzymes SOD, catalase and G-Px were reverted back to normal and blood glucose levels were also normalized after treatment with the extract. Histopathological studies on pancreas of diabetic rats also revealed protective effect on β cells after A.marmelos administration.

2) Allium sativum (Hindi: Lahsun) Family – Liliaceae

Allium Sativum is a perennial bulbous plant cultivated as an important condiment crop in India.

Garlic is supposed to possess higher nutritive value than other bulbous crops. It is known for its medicinal properties since ancient times. Both Ayurveda and the western world have appreciated the benefits of garlic.

Raw garlic and its medicinal preparations are widely recognized for its cardio-protective, anti-hypertensive and lipid lowering action. It is also known to correct gastric problems, cold, cough, fevers, ocular diseases, arthritis and a number of other conditions. Garlic also has

antibiotic action. The ancient ayurvedic texts mention its use as a 'Rasayana' or rejuvenator.

Garlic cloves are found to contain volatile oils rich in allyl disulphide and diallyl disulphide. Phytochemicals such as allin, allicin are also present in large amounts.

Garlic has been extensively studied and shown to possess a range of medicinal properties like antithrombotic, antibiotic, hypolipidemic, hypoglycemic and hypotensive activities.

Hana Drobiova et al (2009) [(251)] studied the effect of garlic extract on diabetic rats. They administered garlic extract intra-peritoneally for 3 weeks to STZ induced diabetic rats. The deranged antioxidant levels in the diabetic rats recovered after recieving garlic extracts and reached a level above those observed in normal rats. This increase in the antioxidant levels paralled with a decrease in blood glucose level of the diabetic rats, bringing them to euglycemic state. A similar effect in the antioxidant levels was observed by the researchers in garlic treated hypertensive rats with a reduction of 50% in the blood pressure.

Earlier, Anwar and Meki (2003) [(252)] had observed similar results on treating STZ-diabetic rats with garlic oil.

Recently Padiya et al (2011) [(253)] evaluated the effect of garlic extract in fructose induced metabolic syndrome in rats. They elucidated

that oral administration of raw garlic extract increased insulin sensitivity and reduced metabolic complications in diabetic rats. Administration of raw garlic extract also normalized the increased TBARS and decreased GSH levels in the liver of fructose fed diabetic rats.

Another study conducted by Raju et al (2008) [(254)] observed the effect of methanolic extract of garlic in delaying cataract in STZ-induced diabetic rats. It was noted that garlic administration to diabetic rats attenuated the glycemia induced oxidative stress. Garlic administration was found to normalize glucose levels, thereby highlighting its hypoglycemic potential. Their results also testify that the garlic extract suppressed the polyol enzyme and thus mediated in maintenance of osmotic equilibrium and membrane integrity in the lens. The GSH content, antioxidant enzymes were conserved and protein crosslinking and aggregation was also prevented.

Eidi et al (2006) [(255)], Zhaoc and Shichi (1998) [(256)] elucidated that a bioactive compound allicin, present in garlic is responsible for garlic's effective antioxidant potential against changes imposed by hyperglycemia.

Allicin from garlic was also reported to exhibit pronounced hypoglycemia in mild diabetic rabbits in a study by Mathew et al (1973)[(257)].

Garlic extract prepared in solvents like ethanol, ethyl acetate also showed to have antihyperglycemic activity in alloxan induced diabetic rabbits. This observation was put forth by Jain et al (1975) [258].

S-allyl cysteine, a key component in aged garlic was elucidated as a potent antioxidant and inhibitor of AGE's formation by Ahmad et al (2006) [36].

Adachi et al (2006) [259] found another compound- bis oxo vanadium from garlic to be an effective antidiabetic agent in type I diabetic mice.

3) Emblica Officinalis (Hindi: Amla) Family- Euphorbiaceae

The trees are small or medium sized found in deciduous forests and also cultivated in gardens and homeyards.

Pericarp of mature dried fruits is extensively used in Ayurvedic preparations for the treatment of many chronic ailments, including diabetes. It is also holds an important place in Ayurvedic medicine as a "Rasayana" (rejuvenator) and anti ageing drug.

Suryanarayana et al (2004) [38] reported that the aqueous extract of amla inhibited enzyme aldose reductase, a key enzyme in the polyol pathway. This inhibitory effect was suggested to be due to tannoids

present in amla. It was also observed that the formation of sugar cataract in lens organ culture was also prevented. This effect was attributed to the inhibition of AR.

This observation further led Suryanarayana et al (2007) [260] to study the effect of E.officinalis in laboratory animals. They treated STZ-induced diabetic male wistar rats with E.officinalis powder for 8 weeks. They observed no change in the blood glucose levels of diabetic rats after treatment with E.officinalis powder. The progression of diabetic cataract was delayed in treated animals which were attributed to inhibition of AR by the tannoids of *amla* as observed in their previous study. Emblica also prevented the alterations in TBARS and protein carbonyls inspite of the elevated blood glucose levels in the experimental animals. There was also prevention in the lenticular oxidative stress and protection of lens proteins in the rats treated with emblica.

Puppala, Suryanarayana et al (2012) [261], further isolated β glucogallin a major component from the *amla* fruit and studied its role as an ARI. They showed that β glucogallin from *amla* effectively inhibited sorbitol accumulation by 73% under hyperglycemic conditions in lenses of transgenic mice having over expression of human aldose reductase.

In a recent study by Rao et al (2013) [262], a mixture of E.officinalis along with turmeric was evaluated for its antioxidant and antidiabetic

efficacy in diabetic rats. A significant reduction in the plasma glucose level and glycated hemoglobin was observed in diabetic rats after treatment with the mixture. It was also observed that the mixture brought about an increase in glutathione peroxidase activity and restoration of GSH.

In a review article on the medicinal properties of E.officinalis Khan (2009) [263] has cited various studies an its antioxidant and antidiabetic nature.

4) Syzygium Cumini (Eugenia jambolana) (Hindi: Jamun) (Family- Myrtaceae)

It is a large tree found in all forests over a many parts of India. The fruits are oval to elliptical, dark purple, luscious, fleshy and edible. Seeds of S.cumini are widely used to treat diabetes by the traditional systems of medicine since many centuries.

The anti hyperglycemic activity of S. cumini seeds is by now well established. Shrotri et al (1963) [264], Bansal et al (1981) [265], Grover et al (2000) [266], Vikrant et al (2001)[267] have all confirmed the hypoglycemic role of S. cumini seeds through their in vitro / in vivo studies.

The S. cuimini fruit pulp extract was studied for its hypoglycemic & antioxidant effect in STZ induced diabetic rats by N. Rekha et al (2008) [268].Their observations demonstrated that administration of

aqueous extract of S.cumini pulp markedly reduced the blood glucose levels of diabetic rats. There was a significant increase in the levels of SOD, catalase, G-Px & GSH resulting in decreased free radical formation in the liver of diabetic rats. The increased blood levels of TBARS were also reverted to normal after the extract administration.

The authors further claimed that the antidiabetic and antioxidant property may be due to the antioxidant phytochemicals like gallic acid, tannins, vit C, etc present in the fruit.

Ruan et al (2008) [269], evaluated the antiox activity of S.cumini leaves in vitro. Their data indicates that S. cumini leaf extracts contain number of phenolic compounds exhibiting strong free radical scavenging activity.

Although the hypoglycemic property of S. cumini seeds was extensively studied, no studies were conducted on the mechanism by which S. cumini caused hypoglycemia. In view of this, Karthic et al (2008) [270] studied the inhibitory effect of S. cumini seeds extract on porcine α amylase and observed a strong inhibition of 98%. They postulated that the inhibition of α- amylase by S. cumini seeds thereby decreased the rate of starch digestion resulting in decreased post prandial blood glucose levels in diabetic patients.

Purohit et al (2000) [271] observed at 37.17 % decrease in BSL at 6 hrs after an intra peritoneal injection of alcoholic extract of S.cumini

seeds in alloxan- diabetic rats. An enhancement in the insulin secretion was also noted after IP injection.

An increase in the activity of hexokinase and decreased activity of G- 6-Phosphatase in liver was observed by Prince et al (1997) [272] after an oral administration of aqueous seed extract in a dose of 2.5g/kg for 1 month to alloxan diabetic rats.

In a study Bhat et al (2008) [273] suggested that S.cumini is a good inhibitor of glucosidases, showing 44.37% inhibition with chloroform seed extract on intestinal glucosidase and 55.08% on liver glucosidase with methanol seed extract.

The chloroform extract of S.cumini seeds also showed to inhibit porcine α amylase by 22.3%.