CHAPTER - 1

GENERAL INTRODUCTION
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1.0. Diabetes mellitus and its complications

Diabetes mellitus is a complex metabolic disorder associated with severe insulin dysfunction in concurrence with gross abnormalities in glucose homeostasis and lipid metabolism. Diabetes is characterized by hyperglycemia, glucosuria, hyperlipidemia, polyuria, polyphagia, polydypsia, negative nitrogen balance and sometime ketonemia. According to World Health Organization (WHO), 1999 and Indian Council of Medical Research guidelines, fasting plasma glucose levels > 125 mg/dL is considered diabetic condition. Some of the initial characteristic symptoms of diabetes include thirst, polyuria, blurring of vision and weight loss. Diabetes, a global public health problem is now emerging as a pandemic. It is predicted that by the year 2025, three-fourths of the world’s 300 million adults with diabetes will be in non-industrialized countries and almost a third in India and China alone (Mohan, 2004). The number of people with diabetes will increase by 42% (from 51 to 72 million) in industrialized countries between 1995 and 2025 and by 170% (from 84 to 228 million) in industrializing countries. There is a rapidly increasing epidemic of type II diabetes in India and other countries. The International Diabetes Federation estimates that the number of diabetic patients in India more than doubled from 19 million in 1995 to 40.9 million in 2007. This is projected to increase to 69.9 million by 2025. Considering the large population and the high prevalence of diabetes, the burden of diabetes in India would become enormous. Diabetes is currently growing rapidly throughout the world and is the one of major cause of global mortality and the onset being comparatively at a younger age in developing countries, making it a serious health problem.

Classification of diabetes mellitus:

Diabetes mellitus falls into 2 major subgroups. The first group is insulin-dependent diabetes mellitus (IDDM) also referred to as ‘juvenile-onset diabetes’ or Type I diabetes. Type I diabetes occurs due to idiopathic destruction of insulin producing beta cells in the
islets of Langerhans in the pancreas resulting in inability to produce endogenous insulin which is vital for the control of blood sugar and other metabolic functions. The second group is referred to as non insulin-dependent diabetes mellitus (NIDDM), which is also termed ‘maturity-onset-diabetes' or Type II diabetes. This tends to occur in obese persons, although thin persons may also develop NIDDM. NIDDM is much more common than IDDM and accounts for about 85% of patients with diabetes. Type II diabetes is a chronic and multifactorial disease characterized by hyperglycemia as well as insulin resistance in the liver and peripheral tissues and impaired insulin secretion from pancreatic beta-cells.

WHO-classification of diabetes:
1. Type 1 diabetes mellitus (IDDM)
2. Type 2 diabetes mellitus (NIDDM) – (a) Non-obese and (b) Obese
3. Malnutrition-related diabetes mellitus
4. Other types of diabetes mellitus (associated with specific conditions and syndromes)
5. Gestational diabetes mellitus

Complications of diabetes

The long term consequence of hyperglycemia includes progressive development of complications of retinopathy with blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6-fold increased risk of coronary heart disease and ischaemic brain damage. Almost half of those diagnosed as diabetic before the age of 31 years die before they reach 50, largely as a result of cardiovascular or renal complications, often with many years of debilitating disease beforehand. Patients with both NIDDM and IDDM are prone to the development of complications that represent a major cause of morbidity and
mortality. Together, macro and micro vascular diabetic complications are an ever-increasing burden to healthcare authorities in developed nations (Alan, 2003).

The complications of diabetes fall into two major categories:

1. **Acute complications**: The acute complications of diabetes mellitus are ketoacidosis, nonketonic hyperglycemic coma, and hypoglycemic reactions. These complications can be readily attributed to alterations in the metabolism and in the level of blood glucose.

2. **Chronic complications**: The major chronic complications include retinopathy, cataract, nephropathy, neuropathy, arteriosclerosis and lower-extremity amputation - are a significant cause of increased morbidity and mortality among people with diabetes.

### 1.1. Diabetic cataract

Diabetic cataract is one of the most common and earliest secondary complication of diabetes. The loss of transparency or opacification of the eye lens is termed cataract. Diabetic cataract is the leading cause of blindness worldwide. Since glucose uptake at eye lens is insulin independent, it is easily exposed to hyperglycemia induced toxicity. Human lens consists around 33% of protein and over 90% of this is made up of alpha, beta and gamma crystallins. These are long lived and appear to be specific to the lens. The transparency and refractive index property of lens is mainly due to this high concentration of crystallin proteins. The lens fibre arrangement in order, the sparseness of the cellular organelles, the peripheral location of the fibre nuclei away from the optic axis, the small extracellular space (Yorio et al. 1976) and the narrowness of the fibre membranes also impart transparency to lens. The normal young human lens is capable of transparence 90% of incident light in the wavelength range 500-1000 nm. Usually lens transparency decreases with age. The loss of transparency or opacification of lens in diabetes may be due to swelling and breakdown of fibre, expansion of the extracellular space or aggregation of proteins mainly crystallins. It is reported that lens thickness is greater in diabetics than normal subjects (Brown and Hungerford, 1982). Steepening of the front and back curvatures of the lens and shallowing of the anterior chamber also
takes place during diabetes (Sparrow et al. 1992). Loss of refractive index and transference will take place in diabetes.

**Pathogenesis of diabetic cataract**

Multiple mechanisms have been proposed with respect to the development of cataractous lens such as non-enzymatic glycation, oxidative stress and polyol pathway (Ohia et al. 2005; Kyselova et al. 2004). Hyperglycemia induced free radicals take major part in developing diabetic cataract. The healthy lens is rich in antioxidants – vitamin C, vitamin E and reduced glutathione (GSH) and antioxidant enzymes mainly superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase which may counter toxic influence of free radicals. Increased oxidative stress under hyperglycemia diminishes primary antioxidant defense leading to progression of development of cataract. Increased superoxide radical (’O$_2^-$) is responsible for inhibition of glyceraldehyde-3-phosphate dehydrogenase (Rivera-Nieves et al. 1999; Salvemini and Cuzzocrea, 2002), resulting in an increased formation of the advanced glycation end products (AGEs) forming compound, methylglyoxal (Baynes and Thorpe, 1999; Nishikawa et al. 2000), for an elevated production of the activator of endogenous protein kinase C (PKC), diacylglycerol (DAG) (Koya and King, 1998), and the activation of the hexosamine pathway, conversion of fructose-6-phosphate into glucosamine-6-phosphate by the glucosamine-fructose-amidotransferase (Schleicher and Weigert, 2000). Oxidative stress can also affect the transcription of the aldose reductase-gene resulting in the up-regulation of the rate-limiting aldose reductase (ALR) enzyme of polyol pathway (Seo et al. 2000; Spycher et al. 1997), which is coupled with depletion of GSH leading to further enhancement of the oxidative stress. There is a body of evidence which suggests that hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (’OH) are the most reactive and damaging free radicals that contribute to cataract formation. When compared with normal eyes, significantly higher amounts of these free radicals are found in the cataractous lens and in the aqueous humour. Additionally, glutathione reductase activity was found to be inversely proportional to the severity of cataract formation (Ohia et al. 2005).
In the polyol pathway, glucose is converted to sorbitol by ALR. Sorbitol thus produced does not cross cell membranes easily and accumulates in the cells, causing a disturbance in homeostasis. Intralenticular accumulation of polyols has long been suggested to be a major factor in acute models of sugar cataract. Elevated amounts of sorbitol by its osmotic property lead to swelling of lens and alteration in membrane permeability. An altered membrane permeability and with increased lenticular sodium and decrease in lenticular potassium, GSH, myoinositol, adenosine triphosphate (ATP) and free amino acids contributes to cataract formation. These biochemical changes are accompanied by morphological changes which include an initial swelling of the lens epithelial cells and those in the central lens region increase in height and display aberrant vacuoles and dilution of cell contents followed by swelling of the superficial cortical fibre, which eventually rupture to form visible vacuoles (Kador et al. 2000). As lens fibre degeneration progresses, the entire cortex becomes opaque, and eventually nuclear opacity occurs along with liquefaction of the cortical regions. Further formation of AGEs under hyperglycemia lead to alteration and dysfunction of lens crystallins and other proteins. Studies have documented that progressive accumulation of AGEs in diabetic eye lens contributes to accelerated cataractogenesis in hyperglycemic experimental animals and diabetic humans (Shamsi et al. 2000).

1.2. Diabetic retinopathy

Diabetes is considered to be the primary causative factor in the development of diabetic retinopathy. The disease can be broadly categorized into three stages: background diabetic retinopathy, pre-proliferative diabetic retinopathy and proliferative diabetic retinopathy. In the first stage of diabetic retinopathy, hyperglycemia initiates thickening of capillary basement membrane and causes death of pericytes that support the vessel wall. Following this, microaneurysms and vascular leakage take place leading to blockage of retinal capillaries and induction of local hypoxia. Subsequently, endothelial cells die resulting in closure of capillaries and increased areas of nonperfusion. Pre-proliferative diabetic retinopathy is identifiable by the areas of increased retinal hypoxia and multiple haemorrhages because of loss of vascular patency. Increased areas of non-
perfusion stimulates the generation of angiogenic factors leading to the formation of new blood vessels, a characteristic feature of proliferative diabetic retinopathy. Subsequently retinal detachment may take place, causing vision loss or blindness. Hyperglycemia and hypoxia are the two principal factors in the initiation and progression of diabetic retinopathy. Production of a variety of local agents in the ocular tissues, such as vascular endothelial growth factor (VEGF), prostaglandins, cyclooxygenase-2 (COX-2) and nitric oxide (NO) is indicated in the process, all of which contribute to vascular permeability and angiogenesis (Singh et al. 2008). Hyperglycemia is the major factor to activate polyol pathway. The potential detrimental influence of polyol pathway includes sorbitol induced oxidative stress, a decrease in cytosolic nicotinamide adenine dinucleotide phosphate reduced (NADPH) and an increase in nicotinamide adenine dinucleotide reduced/nicotinamide adenine dinucleotide (NADH/NAD+), decreased Na+/K+ ATPase activity, activation of PKC, decreased GSH and altered other antioxidant defense. Also, hyperglycemia contributes to altered retinal function through production of free radicals and formation of AGEs.

1.3. Diabetic nephropathy

Kidney is the one of the major organs that gets injured easily by hyperglycemia. Diabetic nephropathy is a major microvascular complication of diabetes mellitus. About 30-40% diabetic individuals suffer from diabetic kidney problems after 20–25 years of appearance of diabetes. Diabetic nephropathy, a condition characterized by continuous excretion of albumin in urine, an increase in the arterial blood pressure, a relentless decline in renal function. Diabetic nephropathy represents cause of mortality and morbidity in diabetic patients by leading to end-stage renal disease worldwide. Diabetic nephropathy is also a major risk factor for cardiovascular disease. Untreated diabetic nephropathy increases 40-fold risk of early death due to cardiovascular disease. The pathological factors influencing the development of diabetic nephropathy are complex and still not clear. Initial pathological alteration of kidney in diabetes includes renal hyperfiltration, hyperperfusion, and increasing permeability to macromolecules such as albumin. These are accompanied by ultrastructural changes including glomerular
basement membrane thickening, glomerular hypertrophy, and mesangial expansion with the later development of glomerulosclerosis and tubulointerstitial fibrosis.

Severity of diabetic nephropathy depends on duration of diabetes and level of hyperglycemia. Multiple biochemical pathways converge to cause diabetes nephropathy. The sustained high concentration of glucose may induce toxicity by altering cell growth and gene protein expression and increasing extracellular matrix and growth factor production (Raptis and Viberti, 2001). Glucose indirectly influences through the formation of metabolic derivatives such as sorbitol and glycation products.

Several studies have demonstrated that oxidative stress plays a major role in diabetic nephropathy. The major source of reactive free radicals in kidney includes mitochondria, glycolysis, uncoupling of nitric oxide xanthine oxidase, NADPH oxidase, and advanced glycation. Hyperglycemic condition, cytosolic enzyme ALR converts glucose to sorbitol using NADPH as cofactor. Since sorbitol does not cross cell membranes, its intracellular accumulation results in osmotic stress. Accumulation of sorbitol in renal mesangial and proximal tubule cells has been proposed as a mechanism for altered cellular myoinositol level (Haneda et al. 1990) and reduced Na⁺/K⁺-ATPase activity (Cohen and Klepser, 1988), each with a potentially detrimental effect in diabetes. However, in the cells of the inner medulla, sorbitol may function together with betaine (Burger-Kentischer et al. 1999) and glycerophosphorylcholine (Burg, 1996), as part of the organic osmolyte defense against extracellular solute fluctuations. Also osmotic stress increases cellular cytosolic generation of H₂O₂. Reported administration of osmotic diuretics protects proximal tubular cells from reactive oxygen species (ROS) mediated apoptosis (Pingle et al. 2004). Depletion of NADPH through polyol pathway inhibits replenishment of GSH, which is required to maintain glutathione peroxidase activity. This would ultimately decrease cellular antioxidant activity. Subsequently, sorbitol is oxidized to fructose via sorbitol dehydrogenase with NAD⁺ reduced to NADH, providing increased substrate to complex I of the mitochondrial respiratory chain. Since the mitochondrial respiratory chain is thought to be a major source of excess ROS in diabetes, provision of additional
electrons for transfer to oxygen-forming superoxide would augment mitochondrial ROS production.

AGEs are well known heterogenous compounds formed nonenzymatically through an interaction of reducing sugar with free amino group of proteins, lipids and nucleic acids. Increased production and accumulation of AGEs have been implicated in diabetic nephropathy. In kidney glomerular basement membrane, mesangial cells, and renal tubules show high level of these adducts (Miyata et al. 1998). Elevated levels of AGEs in kidney can induce transforming growth factor-β (TGF-β) expression and associated upregulation of various extracellular matrix messenger-ribonucleic acids (mRNAs) (Yang et al. 1994). The changes may mainly contribute to glomerular hypertrophy, basement membrane thickening, and mesangial extracellular matrix expansion, which is associated with significant proteinuria and albuminuria as glomerulosclerosis (Vlassara et al. 1994). Further AGEs exert their effects through its receptors, termed receptor for advanced glycation end products (RAGE). In diabetic mice, over-expression of RAGE shows increased albuminuria, serum creatinine, renal hypertrophy, and glomerulosclerosis when compared to nondiabetic littermates (Yamamoto et al. 2001). Binding of AGEs on the tubular surface has been implicated in the pathogenesis of tubular cell injury, potentially via NADPH oxidase (Thallas-Bonke et al. 2008). Binding to RAGE activates PKC-α-mediated activation of NADPH oxidase and nuclear factor-κβ (NF-κβ), which in turn induces production of various inflammatory cytokines and generation of mitochondrial ROS results in aggravating oxidative stress in the kidney (Simm et al. 1997).

1.4. Diabetic neuropathy

Neuropathy is a common complication of diabetes and is associated with a wide range of clinical manifestations. Approximately 50% of patients who have had diabetes for >25 years will develop neuropathy and will have pain as a symptom of neuropathy (Boulton et al. 2005). Diabetic neuropathy affects all peripheral nerves including pain fibre, motor neurons and the autonomic nervous system. Therefore all organ systems may be involved including diabetic foot, cardiovascular, gastrointestinal and urogenital systems and may
be associated with other autonomic dysfunctions. Occasionally, patients with diabetes can develop focal and multifocal neuropathies that include cranial nerve involvement and limb and truncal neuropathies. This neuropathic pattern tends to occur after 50 years of age, and mostly in patients with long-standing diabetes mellitus. Hyperglycemia is the core dysfunction, but neuropathy maybe mediated by numerous mechanisms which may involve genetic predisposition, osmolyte accumulation, oxidative stress, ischemia, neurotropic factor deficiency and immunologic molecular interplay.

Hyperglycemia induced changes in polyol pathway causes accumulation of osmolytes (sorbitol, taurine, glycerophosphoryl choline, high ALR), which reduce Na\(^+\)/K\(^+\) ATPase activity, resulting in Na\(^+\) retention, cellular edema and cell lysis. Local nerve ischemia induces thickened basement membrane, endothelial cell proliferation, vessel contractility anomalies, hypoxia and occlusion. The redox status of cell is reduced (NADPH, GSH) whereas reactive oxygen species is increased (oxidative stress). AGEs promote auto-oxidation of glucose, endothelial changes, macrophage alterations, nitric oxide quenching and further increase in free oxygen generation. Deficiency of DGLA (dihomo-'γ-linolenic acid) and N-acetyl carnitine with increased mitochondrial electron chain II complex, accelerate hyperglycemic damage. Microvascular insufficiency of endoneural and perineural vessels and decreased blood flow with loss of ionic charges has been correlated with neuropathy. Further elevated level of PKC modulates genetic mRNA expression of basement membrane matrix proteins, glycosylation enzymes (CORE 2 GlcNAc transferase), contractile proteins including actin, myosin, caldsman, and increases oxidative stress. Glycemic control, aldose reductase inhibitors (ARIs), gangliosides, AGE product inhibitors, antioxidants, endothelial blockers, etc. have found use in amelioration of diabetic neuropathy. Out of these strategies, a large number of ARIs reported to protect nerve function and structure in diabetic animals (Cameron et al. 1996; Cameron et al. 1997). But clinical trials of ARIs have produced inconsistent results on diabetic neuropathy or adverse effects have led to the interruption of the trials (Foppiano and Lombardo, 1997).
1.5. Diabetes and cardiovascular disease

Diabetes is a most important risk factor for cardiovascular disease (CVD). People with diabetes have a 2–4 fold greater risk than do nondiabetic individuals of developing atherosclerosis and its complications, which include stroke, myocardial infarction, and peripheral vascular disease. Myocardial infarction is 3–5 times more common in diabetic patients and is the leading cause of death in patients with type 2 diabetes. Diabetes also affects the heart muscle, causing both systolic and diastolic heart failure. The incidence of gangrene of the feet in diabetics is 30 times that in age-matched controls. The factors responsible for its development, in addition to peripheral vascular disease, are small vessel disease, peripheral neuropathy with loss of both pain sensation and neurogenic inflammatory responses, and secondary infection. Several conditions have been proposed to explain the acceleration of vascular alterations in diabetes, including hyperglycemia, accelerated formation of AGEs, increased oxidative stress, hypertriglyceridemia, a high low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio, hyperinsulinemia, and genetic variables.

In diabetic condition, dyslipidemia is highly correlated with pathogenesis of atherosclerosis, and up to 97% of patients with diabetes are dyslipidemic (Fagot-Campagna et al. 2000). A characteristic pattern of increased triglycerides and decreased HDL cholesterol is found in the plasma of patients with diabetes. In diabetes, the predominant form of LDL-cholesterol is the small, dense form. Small LDL particles are more atherogenic than large LDL particles because they can more easily penetrate and form stronger attachments to the arterial wall, and they are more susceptible to oxidation. Thus, oxidized LDL produces several abnormal biological responses such as attracting leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of leukocytes, endothelial cells, and smooth muscle cells (Chan, 1998), all of which are steps in the formation of atherosclerotic plaque. In patients with diabetes, LDL particles can also become glycated in a process similar to the glycation of the protein hemoglobin.
Glycation of LDL lengthens its half-life (Napoli et al. 1997) and therefore increases the ability of the LDL to promote atherogenesis.

Diabetes contributes to defects in the autonomic nervous system, the endothelium, and local metabolism, all of which can result in microvascular disease. Diabetic autonomic neuropathy (DAN) is one factor associated with impaired autoregulation of blood flow in a variety of vascular beds, including the skin and the heart. Patients with DAN have increased rates of sudden cardiac death as well as a higher overall cardiovascular mortality rate. Diabetes decreases nitric oxide bioavailability because of either insulin deficiency or insulin resistance in endothelial cells (Brownlee, 2001). Hyperglycemia also acutely inhibits the production of nitric oxide in arterial endothelial cells (Williams et al. 1998). Decreased amount of nitric oxide results in decreased blood flow to respective tissue leading to impaired homeostasis of the tissue containing the vascular bed. It is reported that reduced production of vasodilator nitric oxide and increased secretions of vasoconstrictor endothelin-1 under metabolic disorder not only enhances vasoconstriction, but are associated with the release of pro-inflammatory cytokines (Woods et al. 1999). Pro-inflammatory cytokines cause or exacerbate injury by a variety of mechanisms including enhanced vascular permeability, programmed cell death (apoptosis), recruitment of invasive leukocytes, and the promotion of ROS production (Chung and Barnes, 1999). Further, numerous studies have demonstrated increased generation of free radicals, activated leukocytes and hypercoagulability states play a crucial role in the pathogenesis of cardiovascular complications in diabetes.

1.6. Oxidant stress in diabetes

There is considerable evidence that oxidative stress play a major role in the development and progression of diabetes and its complications (Ceriello, 2000; Baynes and Thorpe, 1999). The term ‘oxidative stress’ refers to the condition of serious disturbance in the production of free radicals and antioxidant balance in favor of the free radicals, resulting in potential tissue damage (Halliwell, 1995). Free radical generated under physiological conditions are essential for host defense mechanisms as seen with
neutrophils, macrophages and other cells of the immune system and shear-stress induced vasorelaxation. On the other hand, excess free radicals cause tissue injury and cell death (Halliwell, 1996).

Hyperglycemia associated with excessive levels of free radicals, results with damage of cellular proteins, membrane lipids and nucleic acids, and eventually cell death. The increase in the level of free radicals in diabetes could be due to their increased production and/or decreased destruction by endogenous antioxidant defense system. Multiple mechanisms have been suggested to contribute to the formation of reactive oxygen free radicals during hyperglycemic conditions. A large amount of data emphasize non-enzymatic source such as glucose autooxidation, polyol pathway, AGEs and mitochondrial respiratory chain and enzymatic source such as endothelial and vascular smooth muscle cell NADPH oxidase, cyclooxygenase, etc. are mainly involved in free radical production and oxidative stress in diabetes (Fig.1). Elevated free fatty acid can cause oxidative stress due to increased mitochondrial uncoupling (Carlsson et al. 1999) and oxidation ends with the increased generation of ROS (Yamagishi et al. 2001; Rao and Reddy, 2001).

Glucose auto-oxidation

Glucose auto oxidation is also one of the most important sources of free radicals during diabetes. Many studies reported glucose oxidation leads to generation of oxygen free radicals during diabetes (Santini et al. 1997). In a transition metal dependent reaction enediol form of glucose is oxidized to an enediol radical anion. It is believed that transition metals such as iron (Fe) and copper (Cu) play a crucial role in this and catalyzes auto oxidation of glucose. An enediol radical anion then converts into reactive ketoaldehyde and to superoxide anion radical. Further superoxide radical converts into H₂O₂ by superoxide dismutase enzyme. H₂O₂ can yield highly reactive •OH radical in the presence of transition metals Fe or Cu (Jiang et al. 1990). Superoxide radical also reacts with nitric oxide to generate another highly reactive peroxynitrite radical (Hogg et al. 1993).
Fig. 1. Generation of major free radicals in diabetes.

Oxygen is converted to \( \cdot O_2^- \) via the activation of enzymatic and nonenzymatic pathways, which is then, dismutated to \( \cdot H_2O_2 \) by superoxide dismutase (SOD). \( \cdot H_2O_2 \) can be converted to water (\( H_2O \)) by catalase or glutathione peroxidase (GSH-Px) or to \( \cdot OH \) by Fenton reaction. Glutathione reductase regenerates glutathione (GSH). In addition, \( \cdot O_2^- \) reacts rapidly with nitric oxide (\( \cdot NO \)) to form peroxynitrite radical (\( ONOO^- \)).
**Mitochondria and oxidative stress**

An imperfectly coupled electron transport in mitochondria leads to production of superoxide radicals, a major source of free radical insult in diabetes. In the normal process of mitochondrial respiration, oxygen is essential for the complete metabolism of glucose and other substrates during the production of ATP. In normal physiological conditions electrons derived from oxidation of substrates are funneled through the redox carriers of the respiratory chain (complexes I, III, and IV) to the final electron acceptor, molecular oxygen. Oxygen entered in the respiratory chain, between 0.4 and 4% converts into the free radical superoxide. This free radical is quickly eliminated by the action of antioxidant enzyme, superoxide dismutase. The mitochondrial Mn-SOD degrades this oxygen free radical to \( \text{H}_2\text{O}_2 \). Then, \( \text{H}_2\text{O}_2 \) is detoxified to water and molecular oxygen within the mitochondria by the action of glutathione peroxidase (Fig. 2). \( \text{H}_2\text{O}_2 \) diffused into the cytosol will be detoxified by catalase in peroxisomes. \( \text{H}_2\text{O}_2 \) can also generate highly reactive \( \cdot\text{OH} \) radical in the presence of reduced transition metals such as Cu or Fe.

Under hyperglycemic condition, enhanced glycolysis provides excess pyruvate into tricarboxylic acid cycle, leading to increased generation of electron donor, NADH and flavin adenine dinucleotide (FADH\(_2\)). These electron donors increase electron flux through the mitochondrial electron transport chain. Consequently, there is an increase of the ATP/ADP ratio and hyper-polarization of the mitochondrial membrane potential. This high electrochemical potential difference generated by the proton gradient leads to partial inhibition of the electron transport in complex III, resulting in an accumulation of electrons in coenzyme Q. In turn, this drives partial reduction of \( \text{O}_2 \) to generate the free radical anion superoxide. Therefore, the excessive production of ROS by mitochondrial dysfunction is believed to play a critical role in the pathogenesis of diabetic complications.

**Other sources of free radicals in diabetes**

Advanced glycation end products (AGEs) derived from hyperglycemia also results in production of free radicals. Hyperglycemic condition favors nonenzymatic formation of
Fig. 2. Superoxide radical generation by mitochondria.

CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; CV, ATP synthase; TCA, tricarboxylic acid cycle; UCP, uncoupling protein; Q, coenzyme Q.
Fig. 3. Pathways involved in AGEs formation in diabetes.
Amadori products. This results with the formation of AGEs by several modulations (Hori et al. 1996). Auto oxidation, lipoxidation and elevated levels of fructose-3-phosphate also involved in the formation of AGEs under hyperglycemic condition (Fig.3). These AGEs are reported to inactivate enzymes, alter the structures and function of enzymes and elevate the generation of free radicals (Baynes and Thorpe, 1999). The major enzymatic sources involved in the generation of free radicals in diabetes are NADPH oxidase, xanthine oxidase and lipoxygenase. NADPH oxidase enzyme destroys invading bacteria by releasing ROS. It was initially thought that NADPH oxidase expresses only in phagocytic cells. Presently, several forms of this enzyme have been discovered and it is understood that it is expressed in many tissues. Over-expression of NADPH oxidases and enhanced generation of ROS has been implicated in several diseases including renal, diabetes, atherosclerosis, and hypertension. These enzymes are one of the important sources of hyperglycemia induced oxidative stress in kidney and arterial cells and implicated in diabetic vasculopathy and nephropathy (Cerillo, 2008; Paravicini and Touyz, 2008; Spinetti et al. 2008). Xanthine oxidase converts hypoxanthine to xanthine and xanthine to uric acid. Both the reactions involve production of superoxide radicals. Studies have reported that over-expression of xanthine oxidase is one of the free radical contributors in diabetes.

**Antioxidants**

The primary defense against oxidative stress in the cell includes antioxidant molecules such as GSH, vitamin C and vitamin E and enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase and glutathione-S-transferase. Antioxidants counter free radicals by preventing the formation of ROS, by eliminating already generated ROS by scavenging, trapping and quenching them or by binding metal ions into inactive chelates. Many studies reported endogenous antioxidant defense system is altered in diabetes. Antioxidants such as vitamin C, vitamin E, and α-lipoic acid, etc. can prevent, and even reverse many early changes in the vascular and neurological tissues in diabetic animals. Vitamins C and E and α-lipoic acid (a superoxide scavenger) are required to regenerate glutathione and oxidized vitamins C and E. They have been shown
to be effective in ameliorating diabetic complications by improvement of nerve conduction velocity and blood flow to the peripheral nerves, leukocyte adhesion in the retina, cataract formation, and mesangial expansion (Abiko et al. 2003; Gaede et al. 2001). The beneficial influence of α-lipoic acid may due to its hypoglycemic and antioxidant strength. It has also potential to prevent protein glycation (Suzuki et al. 1992) and inhibit ALR activity in cultured lens under hyperglycemic conditions (Altabore et al. 1997). α-Lipoic acid counters oxidative damage by direct free radical scavenging and metal chelation, interaction with other antioxidants and increasing intracellular GSH (Obrosova et al. 1998).

The effectiveness of the vitamins C and E are established in diabetic animal models. Administration of vitamins C and E alone or in combination to diabetic animals, normalized levels of lipid peroxidation, isoprostanones, plasma malondialdehyde, and other cellular markers of oxidative stress such as nuclear factor-κβ (Gaede et al. 2001; Abiko et al. 2003). They are capable of improving early functional markers of complications including diabetic retinopathy, nephropathy, and neuropathy. These vitamins can ameliorate or reverse cardiovascular abnormalities such as forearm blood flow, nerve conduction velocity, vascular permeability and contractility, endothelial dysfunction, and albumin-uria. Some studies have even reported that in diabetic animal models, late pathological changes in the retina and the peripheral nerves can be prevented by vitamins C and E (Kowluru and Kennedy, 2001). In other studies, high doses of vitamin E normalized parameters of oxidative stress and inhibited vascular abnormalities caused by hyperglycemia-induced production of DAG and PKC activation in the retina and glomerulus (Koya et al. 1997; Kunisaki et al. 1995). In clinical studies, antioxidant treatments such as vitamins C and E and α-lipoic acid provided positive results to show that they can prevent or stop only early markers of diabetic retinopathy, nephropathy, neuropathy, and cardiovascular disease. Other antioxidants such as trolox, analog of vitamin E (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), reported to prevent sugar induced lens opacification in cultured lenses (Ansari et al. 1994) and significantly improved motor nerve conduction velocity, nerve blood flow and inhibited
thermal hyperalgesia. Trolox also countered lipid peroxidation and activity of anti-oxidant enzymes in sciatic nerves of diabetic rats (Sharma and Sayyed, 2006).

2.0. Aldose reductase

Polyol pathway is the earliest proposed mechanism to explain pathophysiology of diabetic complications. The polyol pathway constitutes two enzymatic reactions: the first is the NADPH–dependent reduction of glucose to sorbitol by the action of ALR and the second step is the oxidation of sorbitol to fructose by the action of sorbitol dehydrogenase using NAD as cofactor (Fig.4). ALR was first identified to provide energy to sperm cells by converting glucose to fructose in the seminal vesicle. Accumulation of high amounts of galactitol and sorbitol in rat lens during sugar-induced cataractogenesis has been reported. These findings suggest a pathological role of ALR in the development of diabetic complications. It is postulated that accumulation of polyols in the lens would lead to an increase in intracellular osmotic pressure, excessive hydration, gain of Na\(^+\), and loss of K\(^+\) leading to cataractogenesis (Kinoshita et al. 1981).

ALR (EC 1.1.1.21) has 316 amino acid residues composing a monomeric protein with a molecular mass of 36 kDa. The primary structure was first determined for rat lens ALR (Carper et al. 1989). This enzyme shares structural similarities with another NADPH-dependant oxidoreductase, human liver aldehyde reductase (Wermuth et al. 1987) and to p-crystallin, a major structural component of the lens of frog. These structures are composed of \(\alpha/\beta\)-barrel motif with a core of eight parallel \(\beta\)-strands connected by peripheral \(\alpha\)-helices with a large hydrophobic active site. The NADP\(^+\) coenzyme molecule is bound to the carboxyl terminus of the \(\beta\)-strands in an extended conformation. The nicotinamide ring is centered in the active site cavity. This highly hydrophobic active site pocket is formed by aromatic residues (Trp20, Tyr48, Trp79, Trp111, Phe121, Phe122 and Trp219); apolar residues (Val47, Pro218, Leu300 and Leu301); polar residues (Gln49, Cys298 and His110); and three possible proton donors, which were Tyr48, His110 and Cys298, were also identified. Fig.5 shows the three dimensional structure and active site residues of ALR solved by the X-ray crystallography studies.
Fig. 4. Polyol pathway.
Fig. 5. Structure of ALR (β/α barrel structure).

The representative frame shows eight α-helices (in red) surrounding a core of eight β-strands (in yellow) all in parallel orientation. The arrow on the β-strand points from the N- to the C-terminus. The active site is located on the C-terminal side of the β-strands and is associated with three loops (in white and blue).

**Active site residues.** The crystal structure suggests the active site is a large, deep, elliptical hydrophobic pocket with the involvement of Tyr-48 and His-110 that participates in acid-base catalysis. This pocket emerges as the binary complex of enzyme and undergoes a conformational change upon NADPH binding at the C-terminal end of the barrel.
2.1. Physiological role of ALR

Osmoregulatory influence of ALR in kidney: ALR is highly localized in the medullary portion of the kidney and involved in the synthesis of sorbitol. Sorbitol is one of the organic osmolyte that balance the osmotic pressure of extracellular sodium chloride. Cells in the renal inner medulla are exposed to large alterations of extracellular tonicity and respond to hypertonic stress by accumulating non-perturbing organic osmolytes such as sorbitol, myo-inositol and the methyl amines betaine and glycerophosphorylcholine (Yancey et al. 1982). It is reported that line of renal papillary epithelial cells exposed to increased extracellular osmolarity stimulates an increase in intracellular sorbitol (Bagnasco et al. 1987). These findings support osmoregulatory role of ALR in the renal homeostasis. Further, the defect in kidney function, unable to concentrate urine to normal level is reported with ablation of ALR gene in mice (Aida et al. 2000). In addition, some of the non-renal cells such as Chinese hamster ovary cells (Kaneko et al. 1990), cultured human retinal pigment epithelial cells (Henry et al. 1993), and human embryonic epithelial cells (Ferraretto et al. 1993) under hyper-osmotic stress results in increased expression of ALR which may provide an insight into the osmoregulatory role of ALR.

ALR in glucose toxicity: ALR has been implicated in the development of diabetic complications. The enzyme hexokinase phosphorylates glucose into glucose-6-phosphate under normal glycemic condition. Under normoglycemia, approximately 3% of nonphosphorylated glucose enters the polyol pathway, the alternate route of glucose metabolism (Morrison et al. 1970), whereas more than 30% of glucose is metabolized through this pathway under hyperglycemia (Gonzalez et al. 1984). Since in the tissues like lens, nerve, kidney, etc. undergo insulin independent uptake of glucose, ALR is the only enzyme that catalyzes the reduction of aldoses to polyols. This enzyme was identified as the major protein accountable of hyperglycemic injury. The elevated polyol path activity under hyperglycemic conditions leads to pathogenesis of complications by imparting osmotic stress, oxidative stress, reductive stress, glycative stress and PKC stress.
Under diabetic conditions, sorbitol is formed more rapidly than it is converted to fructose, resulting in a net accumulation of sorbitol. Sorbitol accumulation is also enhanced by the polarity of the polyol, which hinders facile penetration through membranes and subsequent removal from tissues through diffusion. The intracellular accumulation of polyols can thus produce a hyperosmotic effect that results in an influx of fluid. Increased fluid influx, in turn is associated with membrane permeability changes and subsequent onset of cellular pathology (Kinoshita, 1974).

Accelerated polyol pathway induces oxidative stress by causing depletion of NADPH. The increased flux of glucose into polyol pathway leads to over utilization of cofactors NADPH and NAD⁺ by polyol pathway enzymes ALR and sorbitol dehydrogenase respectively. Depletion of these cofactors or redox state change leads to imbalances of cascade of interrelated metabolic paths. Lack of availability of NADPH strongly alters the activities of glutathione reductase and nitric oxide synthase. Glutathione reductase is an antioxidative enzyme that maintains the level of tissue glutathione. Glutathione is one of the major molecules of detoxifying system. Depletion of glutathione increases harmful effects of free radicals under diabetic conditions (Chung et al. 2003). It is reported that reduced level of glutathione increases susceptibility to H₂O₂ in the endothelial cells culture in high glucose medium (Kashiwagi et al. 1994). Accumulation of sorbitol and the relative osmotic stress also could generate oxidative stress (Vincent et al. 2004). Similarly, production of nitric oxide from L-arginine by nitric oxide synthase is suppressed resulting from the depletion of NADPH, thereby reducing the release of nitric oxide to elicit microvascular derangement and slowing of nerve conduction (Cameron et al. 1993; Stevens et al. 1994).

The pseudohypoxia or reductive stress theory focuses on the NAD⁺ consumption via the polyol pathway during the oxidation of sorbitol to fructose by sorbitol dehydrogenase. This may result in a shift in the cytosolic redox state (increased NADH/NAD⁺ ratio), recapitulating a metabolic phenotype of hypoxia (Demopoulos et al. 2005; Srivastava et
al. 2005). The resulted pseudohypoxia triggers a cascade of biochemical changes contributing to the development of chronic complications in affected diabetic tissues.

Increased amount of fructose via polyol pathway leads to glycative stress during hyperglycemia. The enzyme, 3-phosphokinase converts fructose to fructose-3-phosphate. Increased level of fructose-3-phosphate leads to the generation of 3-deoxyglucosone, a central precursor in the formation of AGEs. Additionally, fructose is believed to be more reactive that glucose because it has the ability to adopt the open chain form (Roscic and Horvat, 2006) and therefore reacts with the free amino groups of proteins, leading to non-enzymatic glycation via the formation of Schiff bases and further rearrangements and conformational changes by the Maillard reactions, leading to the formation of AGEs (Khalifah et al. 1999). Generation of AGEs by ALR dependent and independent mechanisms and their interaction with the receptor of AGEs (RAGE) initiates signal transduction events (Vincent et al. 2004) due to activation of pleiotropic transcription factors, such as nuclear factor-κβ, resulting in the production of ROS and causing pathological changes in gene expression (Tripathi and Srivastava, 2006).

Diacylglycerol (DAG) level elevates chronically in the hyperglycemic condition. Because elevated NADH:NAD\(^+\) via polyol pathway stimulates the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate, a precursor of diacylglycerol, subsequently increases de novo synthesis of DAG (Xia et al. 1994). Studies indicate that during diabetes, amount of DAG is increased in vascular tissues such as the retina (Shiba et al. 1993), aorta, heart (Inoguchi et al. 1992) and renal glomeruli (Ishii et al. 1996). Also a nonvascular tissue, such as liver and skeletal muscle shows elevated levels of DAG (van Herpen and Schrauwen-Hinderling, 2008). Increased DAG activates PKC isoforms. PKC has been associated with vascular alterations such as increases in permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation and inhibition. These perturbations in vascular cell homeostasis caused by different PKC isoforms are linked to
the development of pathologies affecting large vessel (athero-sclerosis, cardiomyopathy) and small vessel (retinopathy, nephropathy and neuropathy).

2.2. ALR inhibitors

ALR enzyme of the polyol metabolic pathway has been implicated in the etiology of the long-term complications of diabetes, as well as many other pathological conditions such as ischemia, abnormal vascular smooth cells proliferation, cancers, and mood disorders. Hence ALR is one of the potential target for the prevention of diabetic complications and inhibition of the enzyme is considered to be a promising approach to prevent diabetic complications. The highly plastic and flexible nature of active site of ALR enzyme allows inhibitors with diverse chemical structure to interact at its active site. A wide range of ARIs has been discovered and showed to inhibit this enzyme in vitro and in preclinical studies. Although many new molecules have progressed to the clinical trials, only one molecule, epalrestat is in the market. The presence of acidic group is the most common characteristic of current ARIs, which provides hydrogen bond interaction with the active site of the enzyme that contains the Tyr48, His110 and Trp111 residues and the nicotinamide moiety of the NADP+. Another common feature of several ARIs bears one or more aromatic groups. The presence of aromatic groups helps inhibitors to bind in the hydrophobic pocket of ALR, Trp-111, Phe-122 and Leu-300 residues (Bruno et al. 2002).

Presently available ARIs can be divided into mainly three groups: Carboxylic acid ARIs, Hydantoin ARIs, and Structurally diverse ARIs.

A. Carboxylic Acid ARIs: Arlestatin, tolrestat, epalrestat and zopolrestat are few major carboxylic acid ARIs. Epalrestat is the only ARI commercially available as a drug (ONO Pharmaceutical Co., Japan). These ARIs show low in vivo activity due to their poor tissue penetration, which has been attributed to their low pKa values, thus causing ionization at physiological blood pH (Mylari et al. 2005).
**B. Hydantoin ARIs:** Sorbinil and fidentalstat are two most thoroughly studied compounds of this class of chemotype. The development of hydantoin derivatives is a solution to the inadequate pharmacokinetic profile of carboxylic acids. They present higher pKa values in comparison to carboxylic acids and are partially ionized at physiological pH, thus able to pass through cell membranes. This group of compounds exhibits better pharmacokinetic properties but unacceptable side effects related to toxicity such as skin rash and hypersensitivity or liver toxicity has rendered them undesirable as drugs (Pau et al. 2004). Sorbinil shows high selectivity against ALR compared to carboxylic acid ARIs (Ramana et al. 2004). Sorbinil significantly improved the conduction velocity in all three nerves, the peroneal motor nerve, the median motor nerve and the median sensory nerve in diabetic patients without any symptomatic neuropathy (Gupta and Dubois, 2001). Many clinical studies were carried out to establish its beneficial influence in humans, due to its hypersensitivity reaction in the early weeks of therapy limits its use as drug. Similar type of adverse reaction was noticed with other hydantoin derivatives.

**C. Structurally diverse ARIs:** Some of the molecules without carboxylic acid and hydantoin moieties also show potent inhibition of ALR enzyme. Spiroimide derivatives have been developed such as minalrestat and ranirestat exhibits potent inhibition of ALR. Few of other most characteristic chemotypes refer to tetrahydropyrrolo [1,2-a] pyrazine-1,3-dione derivatives (Negoro et al. 1998), 5-arylidene-2,4-thiazolidinedione derivatives (Bruno et al. 2002), N-nitromethyl sulfonanilide derivatives (Inoue et al. 2000), sulfonylpyridazinone derivatives (Mylari et al. 2005), N-(3,5-difluorophenol-4-hydroxyphenyl) derivatives (Alexiou et al. 2008) and carboxymethylated pyridoindole derivatives (Stefek et al. 2008). The flavonoids (2-phenyl-4H-1-benzopyran-4-one) and phenyl sulfonyl nitromethanes also exhibited potent activity against ALR.

Several ARIs have been shown to inhibit the enzyme; the clinical efficacy of these molecules is not satisfactory. The reasons lined to withdraw ARIs in clinical trials include i) polyol pathway may act synergistically with other pathways to cause diabetic complications, (ii) partly be related to post-translational modification of the enzyme, (iii)
the specificity and selectivity of inhibitors, (iv) severe toxic profiles and poor pharmacokinetic and pharmacodynamic properties. Thus, there is great necessity to develop new potent, safe ARIs with improved pharmacodynamic and pharmacokinetic properties.

2.3. Aldose reductase inhibitors from fungal source

Microbial metabolites are attracting researchers by its bioactive nature and more uses in modern medicine, veterinary medicine and agricultural applications. The new microbial metabolites discovered have been a fascinating and exciting adventure. Several studies established fungi to be excellent sources of novel bioactive secondary metabolites and greater structural and molecular diversity. It has always been an attractive field of drug research because fungi construct unique complex molecules using established metabolic pathways. Secondary metabolites formed along with the metabolic pathway may be biologically active.

Secondary metabolites are the products of metabolism not essential for normal growth, development or reproduction of an organism. These compounds serve to meet the secondary requirements of the producing organisms. They empower them to survive interspecies competition, provide defensive mechanisms and facilitate reproductive processes. Some of these secondary metabolites possess therapeutic qualities that improve the quality of life for millions of people. Many secondary metabolites have proved invaluable as antibacterial or antifungal agents, anticancer drugs, cholesterol-lowering agents, immune suppressants, antiparasitic agents, herbicides, diagnostics, and tools for research. Microbial metabolites may be utilized as fermentation product directly in the medicine, agriculture, or in other fields, using as starting material for subsequent chemical or microbiological modification and as lead compounds for chemical synthesis of new analogs or as templates in the rational drug design studies.

Metabolites usually not produced during the phase of rapid cell growth, trophophase. Stationary phase of cell growth, idiophase favors the production of secondary metabolites. Exhaustion of one of nutrient source, carbon, nitrogen or phosphate, etc.
initiates the production of secondary metabolites. One of the best examples includes biosynthesis of penicillin by *Penicillium Chrysogenum* starts when glucose is exhausted in the culture medium and starts utilizing lactose. Liquid and solid state fermentation techniques are most common methods used to produce secondary metabolites. In liquid fermentation method fungi are inoculated into a growth medium in flask and then transferred into a fermenter. The important parameters like medium composition, pH, agitation, temperature, aeration are controlled. In the case of solid state fermentation fungal culture develops on the surface of and at the interior of a solid support and in the absence of free water. It is reported solid state fermentation holds an important potential for the fungal metabolite production (Robinson et al. 2001). At the end of fermentation suitable down stream processing is used to extract and purify metabolites.

Mycophenolic acid is one of the first crystalline secondary metabolite obtained from *Penicillium glaucoma*. Penicillins and cephalosporins are the most successful classes of metabolites originated from fungus. Cephalosporins isolated from *Cephalosporium acremonium* fulfills the treatment of patients allergic to penicillins. Currently, fourth generation cephalosporins are most successful and widely prescribed class of antimicrobial agents due to their broad spectrum of activity and excellent safety profiles. Other than antibiotics some of wonder drug likes lovastatin and cyclosporin A are obtained from fungi. Cyclosporin A is one of commonly using immunosuppressant during and after bone marrow and organ transplants in humans also obtained from fungi. This primary metabolite produced by several fungi, including *Trichoderma polysporum* and *Cylindrocarpon lucidum*. Lovastatin is the secondary metabolite of *Aspergillus terreus*. This statin has been used to reduce or remove low density lipoproteins from blood vessels in humans, acts by inhibiting HMG-CoA reductase, enzyme involved in *de novo* synthesis of cholesterol in the liver. Hence, metabolites from fungi may serve an important source of new biologically active molecules (Pescitelli et al. 2009; Siddiqui et al. 2011). Challenges to discover new and exciting molecules form fungi remain the core activity of many research groups.
Several chemically diverse ARIs have been isolated from fungal sources. Sclerotiorin, a chlorine-containing fungal metabolite belongs to a member of azaphilone class, initially isolated from \textit{Penicillium sclerotiorum}. The compound isolated from \textit{Penicillium frequentan} is ported as a potent inhibitor of ALR (Chidananda et al. 2006). WF-2421 (Alpha-formamido-5'-(2-formamido-1-hydroxyethyl)-beta, 2',6-trihydroxy-3-biphenyl propanoic acid) is a novel ARI produced by \textit{Humicola grisea} which exhibited inhibitory potency against partially purified ALR of rabbit lens (Nishikawa et al. 1991). Aldostatin is a fungal metabolite isolated from a culture filtrate of \textit{Pseudeurotium zonatum} which has shown bovine lens ALR inhibitory activity. Some of the other ARIs obtained from fugal origin include salfredins from \textit{Crucibulum} sp. (Matsumoto et al. 1995), WF-3681 from \textit{Chaetomella raphigera}, citrinin (4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid) from \textit{Penicillium citrinum}, DHMI (3,4-dihydro-6-methoxy-3,7-dimethyl-1H-2-benzopyran-8-ol) from \textit{Penicillium corylophilum} (Deruiter et al. 1992), asperaldin from \textit{Aspergillus niger} CFR-1046 (Rao et al. 2003), and moniliformin isolated from \textit{Fusarium proliferatum}.

\textbf{2.4. Nigerloxin-A novel aldose reductase inhibitor from \textit{Aspergillus niger}}

Nigerloxin is a secondary metabolite derived from solid state fermentation of \textit{Aspergillus niger} CFR-W-10. Nigerloxin, chemically is 2-amido-3-hydroxy-6-methoxy-5-methyl-4-(prop-1’-enyl) benzoic acid and has a molecular weight of 265 and a molecular formula $C_{13}H_{15}NO_{5}$ (Fig.6). This compound has been understood to be a bioactive molecule with significant potential to inhibit \textit{in vitro} ALR and lipoxygenase enzyme activities. The IC$_{50}$ values of nigerloxin against rat lens ALR and lipoxygenase enzyme were found to be 69 $\mu$M and 79 $\mu$M respectively. Nigerloxin also shows a free radial scavenging property using 1,1-diphenyl-2-picryl hydrazyl (DPPH) with an ED$_{50}$ value of 66 $\mu$M.
3. Scope of present investigation

Aldose reductase (EC 1.1.1.21) catalyzes the conversion of glucose to sorbitol and causes the accumulation of sorbitol in various tissues under the condition of hyperglycemia. The accumulated intracellular sorbitol causes development of diabetic complications such as cataract, neuropathy, and nephropathy. It has been reported that inhibitors of aldose reductase reduce the tissue sorbitol content in diabetic animals and are useful as therapeutic agents for diabetic complications. Aldose reductase inhibitors based therapy suffers from limitations due to pharmacokinetic problems, reversibility of diabetic neuropathy, adverse reactions and poor reproducibility of clinical measurement. A strong demand has therefore developed for treatment of secondary complications of diabetes mellitus including cataract, diabetic neuropathy and diabetic gastrointestinal disturbances, etc.

Nigerloxin is a bioactive molecule obtained from Aspergillus niger by solid state fermentation; It is chemically 2-amido-3-hydroxy-6-methoxy-5-methyl-4-(prop-1’-enyl) benzoic acid. In vitro studies have previously showed that nigerloxin produces inhibition of soybean lipoxygenase and rat lens aldose reductase. There are no extensive studies which support the aldose reductase inhibition and free radical scavenging activity of nigerloxin. The present study was intended to establish the aldose reductase inhibitory
potential and antioxidant activity in vivo of this fungal metabolite nigerloxin in diabetic animal model with the following objectives:

1) Assessing the antioxidant property of novel fungal metabolite nigerloxin in vivo, especially in the condition of diabetes induced oxidant stress.

2) Evaluation of the aldose reductase inhibitory potential of nigerloxin in vivo in rat eye lens in induced diabetic animals; Further studies on anti-cataractogenic potential of nigerloxin.

3) Evaluation of the aldose reductase inhibitory potential of nigerloxin in vivo in the kidney of induced diabetic animals; Further studies on renal protective potential of nigerloxin.