Chapter 1

Introduction
1.1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both. This is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 150 million people in 2000, expected to increase to 220 million people by 2010. In particular, the number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2025 (Sicree et al., 2006). India leads the world with largest number of diabetic subjects thus earning the dubious distinction of being termed the “Diabetes Capital of the World” (Mohan et al., 2007).

The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on its target tissues, resulting from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (Devendra, 2004). A primary metabolic effect of insulin is to stimulate the uptake of circulating glucose into muscle and adipose tissue.

1.2. TYPES OF DIABETES

Although several pathogenic processes may be involved in the development of diabetes, the vast majority of cases fall into two main categories: type 1 diabetes and type 2 diabetes.

Type 1 diabetes which is otherwise called as Insulin Dependent DM (IDDM) is a chronic autoimmune disease, characterized by an absolute insulin insufficiency due to the irreversible autoimmune destruction of the insulin secreting β-cells of the islets in the pancreas. The symptoms appear when the β-cell mass gets reduced by approximately 90% leading to severe insulin deficiency and hyperglycemia. This is mainly due to hepatic production of glucose by glycogenolysis and gluconeogenesis and decreased cellular uptake of glucose from the circulation.

Type 2 diabetes, Non Insulin Dependent DM (NIDDM) is more complex in etiology and is characterized by a relative insulin deficiency, reduced insulin action and insulin resistance of glucose transport in skeletal muscle and adipose tissue. The overall prevalence of diabetes is approximately 6% of the population, of which 90% belongs to type 2 diabetes. Despite genetic predisposition, the risk of
developing type 2 diabetes in humans increases with age and due to obesity, cardiovascular disease (hypertension, dyslipidaemia) and lack of physical activity.

Symptoms of both types of diabetic conditions include i) high levels of sugar in the blood, ii) unusual thirst, iii) frequent urination, iv) excessive hunger and loss of weight, v) blurred vision, vi) nausea and vomiting, vii) extreme weakness and tiredness and viii) irritability and mood changes.

1.3. OTHER SPECIFIC TYPES OF DM

Other specific types are currently less common causes of DM, but are those in which the underlying defect or disease process can be identified in a relatively specific manner.

1.3.1. Genetic defects of β-cell function

Several forms of the diabetic state may be associated with monogenic defects in β-cell function, frequently characterized by onset of mild hyperglycemia at an early age (generally below 25 years). They are usually inherited in an autosomal dominant pattern. Patients with these forms of diabetes, formerly referred to as maturity-onset diabetes of the young (MODY), have impaired insulin secretion with minimal or no defect in insulin action (Byrne et al., 1996; Clement et al., 1996). Mutations in the genes encoding hepatic nuclear factor 4 (HNF4), glucokinase (GK), hepatic nuclear factor 1 alpha and 1 beta (commonly known as HNF1A and HNF1B, but official symbols are TCF1 and TCF2 respectively), insulin promoter factor 1 (IPF1) and neurogenic differentiation factor 1 (NEUROD1) are the causes of the six known forms of MODY. These MODY genes encoding the transcription factors such as HNF4A, TCF1, TCF2 and IPF1 form crucial links in the cascade of transcription factors that control the appropriate expression of β-cell genes, such as insulin and glucose transporter GLUT2. Mutations of these genes may disrupt the development of β-cells in the embryo and result in dysfunctioning of β-cells in the adult (Ikegami and Ogihara, 1996; Park and Eisenbarth, 2001).

1.3.2. Genetic defects in insulin action

There are some unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to symptomatic diabetes (Taylor, 1992). The INSR gene encodes the receptor for insulin and mutations of the insulin receptor can cause diabetes and play a role in susceptibility to type 2 diabetes. The insulin receptor
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belongs to the largest family of such enzyme-linked receptors known as the receptor protein-tyrosine kinase (RTK) family (Ullrich et al., 1985). Several variants of the insulin receptor have been associated with hyperglycemia and type 2 diabetes (Caro et al., 1988; Hart et al., 1996). A heterozygous mutation changing Val-985 into methionine has been found to be more common in this type of diabetes (Hart et al., 1999). The prevalence of the mutation increased with increasing serum glucose levels, suggesting a role for this receptor variant in hyperglycemia.

1.3.3. Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes that injure the pancreas include pancreatitis, trauma, infection, pancreatic carcinoma and pancreatectomy (Larsen et al., 1987).

1.3.4. Endocrinopathies

Few hormones (eg. growth hormone, cortisol, glucagon and epinephrine) are acting antagonistic to insulin action. Diseases associated with excess secretion of these hormones can cause diabetes (eg. Acromegaly, Cushing’s syndrome and Glucagonoma) (MacFarlane, 1997). These forms of hyperglycemia typically can be resolved when the excess hormone is removed.

1.3.5. Drug or chemical induced diabetes

Many drugs can impair insulin secretion. These types of drugs may not by themselves cause diabetes but they may precipitate diabetes in persons with insulin resistance (Pandit et al., 1993). In such cases, the classification is ambiguous, as the primacy of β-cell dysfunction or insulin resistance is unknown. Certain toxins such as vacor (a rat poison) and pentamidine can permanently destroy pancreatic β-cells (Espositi et al., 1996). There are many drugs and hormones which can impair insulin action. Eg. steroids, thiazides, diuretics, phentoyin, diazoid, alloxan and streptozotocin.

1.3.6. Infections

Certain viral infections have been associated with β-cell destruction. Diabetes occurs in some patients with congenital rubella infection (Forrest et al., 1971). It is clear that congenital infection with rubella leads to a higher incidence of early-onset diabetes (Menser et al., 1978). In addition, coxsackie-B, cytomegalovirus and other viruses (eg. adenovirus and mumps) have been reported in inducing the disease (Pak et al., 1988). Coxsackie viruses have been
implicated, bolstered by perhaps coincidental presence of a peptide (PEVKEK) both in the virus and in glutamic acid dehydrogenase (GAD), one of the antigens recognized by anti-islet cell antibodies in patients with type 1 diabetes (Hyoty and Taylor, 2002).

1.3.7. Gestational DM

Gestational diabetes is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy (Doria et al., 1999).

1.4. PATHOPHYSIOLOGY OF IDDM

In IDDM, pancreatic β-cells are progressively destroyed as a consequence of autoimmune process. In the cellular immune abnormalities, antibodies to islet cell components are common features of the disease.

Cytokines produced by immune cells infiltrating pancreatic islets are important mediators of β-cell destruction in IDDM. In an activated state, T-lymphocytes and macrophages, release high levels of interleukin (IL)-1β and interferon (IFN)-γ respectively. IL-1β alone, or in combination with tumor necrosis factor (TNF)-α and IFN-γ, causes the production of nitric oxide (NO) by the inducible form of nitric oxide synthase (iNOS) in pancreatic islets (Southern et al., 1990; Eizirik et al., 1994, 1996). NO is a short-lived and highly reactive radical, which inhibits the Krebs-cycle enzyme aconitase and the electron transport chain complexes I and II leading to decreased glucose oxidation rates, ATP generation and insulin production (Corbett and McDaniel, 1994; Heitmeier et al., 1997).

There are evidences that environmental factors and genetic factors may all be involved in the pathogenesis of IDDM. Environmental risk determinants can be classified as viral infections (eg. coxsackie virus and cytomegalovirus), early infant diet (eg. breast feeding versus early introduction of cows milk components) and toxins (eg. N-nitroso derivatives) (Hyoty and Taylor, 2002). Genetic factors consist of multiple susceptibility genes with a major locus encoded by HLA region on chromosome 6p21 (Davis et al., 1994; Cox et al., 2001; Concannon et al., 2005). Class II DQ and DR genes have been consistently reported to be associated with type 1 diabetes in multiple ethnic groups (Thomson et al., 1988; Kawabata et al., 2002). In addition to HLA, several non-HLA loci have been shown to contribute to the disease susceptibility. Despite a large number of loci mapped to the genome, only a limited
number of genes have been identified as genes responsible for susceptibility conferred by these loci. Among these are the insulin gene (INS) for IDDM2 (Julier et al., 1991), CTLA4 for IDDM12 (Ueda et al., 2003), SUMO4 for IDDM5 (Guo et al., 2004) and PTPN22 encoding lymphoid tyrosine phosphatase (LYP) (Meloni et al., 2004). The factors that normally regulate the proliferation of the β-cells largely remain elusive although several factors have been identified that influence β-cells growth in vitro.

1.5. PATHOPHYSIOLOGY OF NIDDM

NIDDM is typically a polygenic disease that results from a complex interplay between genetic predisposition and environmental factors such as diet, degree of physical activity and age. Initially, in NIDDM, insulin-stimulated glucose transport in skeletal muscle is impaired. As compensation, pancreatic β-cells display augmented secretion of insulin, resulting in hyperinsulinemia. Peripheral insulin resistance, in combination with impairment in the early phase of insulin secretion, results in hyperglycemia. In the final stage, changes in insulin signaling, such as insulin’s inability to inhibit hepatic gluconeogenesis, are accompanied by a deterioration of pancreatic β-cell function. However, in addition to having hyperglycemia and insulin resistance/secretary defects, nearly 80% of diabetics are obese and have a host of other metabolic abnormalities, including dyslipidaemia [increased low density lipoprotein (LDL)-cholesterol, decreased high density lipoprotein (HDL)-cholesterol, and raised triglyceride levels], hypertension and abnormalities of coagulation and the fibrinolytic system.

Several genes have been associated with islet cell dysfunction in NIDDM, including some encoding for transcription factors (HNF-α, PPAR-γ, PDX-1, IB1 and NeuroD1), glucose metabolism (glucose transporters, glucokinase, FABP2 and UCP-2), molecules of the insulin signaling pathways (IRS-1 and IRS-2) and several others such as calpain10. On the other hand, many environmental factors can directly or indirectly affect pancreatic islet cells and possibly contribute to the development and/or progression of NIDDM.

1.6. INSULIN RESISTANCE

Insulin resistance is defined as when insulin is inefficient in causing the plasma glucose to enter the cells of a body and to be utilized by the cells for energy, even if there is enough insulin in serum i.e., the cells resist the insulin. In addition, the liver may continue to secrete glucose into the bloodstream even when the glucose is not needed. The reasons for insulin resistance occurring are still uncertain. Certain genes predispose certain people to develop insulin resistance.
Some other factors are lack of exercise, obesity and chronically high blood sugar levels that may precipitate insulin resistance in susceptible individuals.

1.7. ROLE OF APOPTOSIS IN β-CELL DEATH

It is well known that oxidative stress impairs various cellular functions and plays important roles in the pathophysiology of many diseases. In IDDM reactive oxygen species (ROS) are generated by macrophages and participate in the toxic actions that leads to necrosis or apoptosis of the insulin-producing cells (Rabinovitch et al., 1992, 1996). Under hyperglycemia, the increased blood levels of various reducing sugars promote protein glycation through the Maillard reaction, which consecutively produces Schiff's bases and advanced glycation end products. ROS are formed in this process and trigger tissue damage. Due to intrinsically very low levels of antioxidant enzyme expression and activity (Lenzen et al., 1996; Tiedge et al., 1997), islet cells are particularly at high risk for ROS-induced damage as compared with other tissues.

The macrophage cytokine IL-1β in combination with IFN-γ and TNF-α plays vital role in β-cell dysfunction and death. Signal transduction by these cytokines involves binding to specific receptors, signal transduction by cytosolic kinases (especially mitogen and stress-activated protein kinases) and/or phosphatases, mobilization of diverse transcription factors: nuclear factor-κB (NF-κB), activator protein-1 (AP-1) and signal transducer and activator of transcription-1 (STAT-1) and up-regulation and down-regulation of gene transcription.

High glucose-induced ROS generation increases the activity of NF-κB leading to apoptosis in a process that involves Bax and caspase activation. NF-κB is initially located in the cytoplasm as an inactive form through interaction with IκB, an inhibitory factor of NF-κB. Various inducers cause dissociation of this complex, presumably by phosphorylation of IκB, allowing NF-κB to be released from the complex. NF-κB then translocates to the nucleus, where it interacts with its DNA recognition sites to mediate gene transcription (Baeuerle and Henkel, 1994; Baldwin, 1996).

In rodent pancreatic β-cells, cytokines act through the generation of NO (Eizirik et al., 1996) and cytokine-induced NO triggers cell death by both apoptosis and necrosis (Mandrup-Poulsen, 1996; Saldeen, 2000). It was reported that cytokine or NO donor-induced apoptosis and necrosis were dependent on a Bcl-2-inhibitable pathway (Saldeen, 2000) and were proceed by disruption of the
mitochondrial membrane potential in insulin-producing cells (Hortelano et al., 1997; Barbu et al., 2002). Many investigators suggested that a site for NO modulation of the cell death process is the mitochondria where it has various events including disruption of mitochondrial membrane potential (Hortelano et al., 1997; Barbu et al., 2002); cytochrome-c release from mitochondria into cytosol (Brookes et al., 2000); overproduction of ROS and termination of ATP production (Green and Kroemer, 1998). Also, the release of cytochrome-c and apoptosis inducing factor from mitochondria leads to activation of caspase-9 and -3 (Zou et al., 1999) and eventually apoptosis that is characterized by cell shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation (Zhivotovsky et al., 1997), which are caused by cleavage of poly-(ADP-ribose) polymerase (PARP), inhibitors of deoxynbonuclease (such as DFF45 or ICAD) and structural proteins (Kothakota et al., 1997; Mashima et al., 1997; Kwon et al., 2007).

High glucose causes mitochondrial membrane depolarization and loss of uncoupling proteins (UCP), especially UCP3 resulting in increased oxidative stress as well as release of cytochrome-c and activation of caspases (Vincent et al., 2004). Excessive ROS may lead to defective PDX-1-binding activity and insulin mRNA expression, decreased insulin content and reduced insulin secretion in islets and consequent necrosis or apoptosis of islets (Figure 1.1) (Tanaka et al., 2002; Robertson et al., 2003).

Although β-cell death is induced by many substances or molecules, increased evidences indicate that oxidative stress plays a crucial role in β-cell death (Fridlyand and Philipson, 2004). Strategies aimed at interfering the oxidative stress induced apoptotic pathways could therefore be of potential therapeutic value. Administration of antioxidants and free radical scavengers leads to over expression of antioxidant enzymes in islets of transgenic mice and protected their β-cells from oxidative stress induced apoptosis (Lortz et al., 2003; Olcott et al., 2004). Medicinal plants such as Scoparia dulcis, Coptidis rhizome and Amomum xanthoides have also been reported for their preventive action of β-cell death in insulin secreting pancreatic cells (Park and Park, 2001).
1.8. COMPLICATIONS OF DM

The development of diabetic complications is a major cause of morbidity and mortality of individuals (Brownlee, 2001). Epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progression of diabetic complications (Jakus and Rietbrock, 2004). These complications are directly related to blood vessel disease and are generally classified into small vessel disease such as those involving the eyes, kidney and nerves (microvascular) and large vessel disease including the heart and blood vessel (Plutzky, 2003).

Diabetic complications can be classified as acute complications and chronic complications. The acute complications arise within a short time frame because of poor metabolic control, which includes Diabetic KetoAcidosis (DKA) and Hyperglycemic Hyperosmolar State (HHS). Chronic diabetic complications include
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Microvascular complications which include retinopathy, nephropathy and neuropathy, macrovascular complications which include coronary artery disease, cerebrovascular disease and peripheral vascular disease and combination of micro and macrovascular complications such as diabetic foot and diabetic dermopathy.

1.8.1. Diabetic Ketoacidosis

Diabetic ketoacidosis is characterized by accelerated rates of hepatic glycogenolysis, gluconeogenesis, ketogenesis, impaired glucose and ketoacid utilization, all of which results in elevated levels of glucose and ketoacids in blood (Befiore, 1996). These disturbances, coupled with increased muscle proteolysis, flood the blood stream with an overabundance of fuels for hepatic glucose and ketone over production (Devlin, 1997). It occurs almost only in persons with IDDM, occasionally in NIDDM.

1.8.2. Hyperglycemic Hyperosmolar State (HHS)

Hyperglycemic hyperosmolar state is characterized by severe hyperglycemia (glucose level typically greater than 600-800 mg/dl), dehydration and altered mental status in the absence of ketosis. HHS accounts for 5 -10% of hyperglycemic comas and 30 - 50% of mortality and is usually from arterial or venous thrombosis. It occurs mainly in elderly persons with NIDDM, secondary to severe stress and followed by stroke.

1.8.3. Diabetic Retinopathy

Diabetic retinopathy is a highly specific vascular complication of both IDDM and NIDDM. The basis of diabetic retinopathy is damage to the microcirculation; the major histological change is loss of pericytes and thickening of the capillary basement membrane which leads to these capillaries becoming leaky. The microcirculation also ceases to supply the retinal tissue properly leading to retinal ischemia and infarction.

1.8.4. Diabetic Neuropathy

Diabetic neuropathies are among the most common complications of diabetes. Both the prevalence and incidence of neuropathy increase with duration of diabetes. The pathogenesis of diabetic neuropathy is uncertain, metabolic factors may predominate in early disease and vascular factors at later stage. Neuropathy may develop acutely during periods of poor glycemic control and may also be precipitated by weight loss. Symptoms often tend to resolve when glycemic control is improved or weight regained.
1.8.5. Diabetic Nephropathy

Diabetic nephropathy represents a distinct clinical syndrome characterized by albuminuria, hypertension and progressive renal insufficiency. It can lead to End Stage Renal Disease (ESRD), a serious condition in which patient survival depends on either dialysis or kidney transplantation (Diabetes Control and Complications Trial, 2000).

1.8.6. Diabetic Foot

Diabetic foot may be the result of peripheral neuropathy and peripheral ischemia and often lead to ulceration and subsequent limb amputation. Diabetes is the most common cause of non-traumatic amputation of the lower limb. Gangrene develops as a result of decreased blood supply and microbial infection (Jude et al., 1999).

1.8.7. Circulatory and Cardiovascular complications of diabetes

Cardiovascular disease is the leading cause of morbidity and mortality among persons with diabetes. The risk factors include hardening of the arteries (arteriosclerosis), fatty deposits (atherosclerosis), low levels of high density lipoprotein-cholesterol (HDL-C), elevated triglycerides and high blood pressure (Jakus and Rietbrock, 2004). Among persons with diabetes several concomitant conditions may affect the etiology of atherosclerosis: obesity, hyperinsulinemia, abnormalities of platelet function and defects in blood coagulation and flow (Ginsberg, 2000).

1.8.8. Polyol pathway

The polyol pathway has been implicated in the pathogenesis of diabetic complications and the major contributor to oxidative stress in the lenses and nerves of diabetics (Rahimi et al., 1995). Sorbitol is formed from glucose under the influence of aldose reductase and is further metabolized to fructose by sorbitol dehydrogenase. Accumulation of sorbitol slowly diffuse polyol out of lens and its accumulation within the cell cause swelling and death of schwann cell with subsequent demyelination (Nishimura-Yabe, 1998). Other changes include a fall in the concentration of ATP and reduced glutathione, while the concentrating mechanism for amino acids is considerably weakened, which finally leads to cataract.
1.9. **REACTIVE OXYGEN SPECIES (ROS)**

A free radical is any atom or group of atoms that has an unpaired electron in its outer orbit. ROS refers to highly reactive oxygen containing entities which include hydrogen peroxide (H$_2$O$_2$) and lipid peroxide with no unpaired electron and superoxide (O$_2^-$), hydroxyl ("OH), peroxyl (ROO'), alkoxyl (RO') radicals, nitric oxide radicals ("NO), nitrogen dioxide ("NO$_2$), peroxynitrite ("ONOO"), ozone (O$_3$) and possibly singlet oxygen with unpaired electrons (O$^*$$^*$$^*$). Though H$_2$O$_2$ and lipid peroxide are not free radicals, they act as reservoirs for the highly reactive "OH, ROO' and RO' radicals and hence have been included under ROS (Datta et al., 2000).

The major sources of ROS in cells are mitochondrial oxidative metabolism via electron leakage from electron transport chain. A normal occurrence during cellular respiration is the formation of O$_2^*$ radicals as single electron traversing the electron transport chain bind prematurely to molecular oxygen. These O$_2^*$ radicals then readily participate in other reactions that produce other types of ROS including "OH. Enzymatic reactions involving mixed function oxidation and auto oxidation of small molecules also contribute to ROS production (Cross and James, 1991).

ROS are powerful oxidants and highly toxic to all types of biological molecules including DNA, lipid, protein and carbohydrates. ROS are shown to be involved in processes such as mutagenesis, carcinogenesis, membrane damage, lipid peroxidation as well as carbohydrate damage (Sies, 1993), most of which are mediated by "OH.

1.10. **LIPID PEROXIDATION**

The O$_2^*$ and "OH are potentially deleterious to the cell because they are able to abstract hydrogen atom from polyunsaturated fatty acids (PUFA) of cellular membranes. These free radicals react with PUFA and form new radicals (peroxyradicals) which initiates a chain reaction of lipid peroxidation in the presence of oxygen (Starner et al., 1989) leading to a variety of pathological phenomenon such as, increased permeability of the membrane, loss of integrity of the cells and inhibition of enzymes (Rao et al., 1990). Degradation of lipid peroxides usually generates a wide variety of (sometimes toxic) compounds like aldehydes, alkenals, epoxides and hydroxyenals (Esterbauer, 1982). The toxicity of these compounds is responsible for the pathological changes observed during
oxidative stress. Increased oxidative stress may contribute to the development of complications of DM.

1.10.1. Lipid peroxidation process

Lipid peroxidation is a radical mediated chain process involving 3 stages in sequence: initiation, propagation and termination. The process of lipid peroxidation is shown below (Figure 1.2):
A free radical (R*) attacks fatty acid chain of membrane lipids (LH) by abstracting hydrogen atom thus leaving an unpaired electron. This is referred to as the initiation stage of lipid peroxidation (Gutteridge, 1995). The presence of double bond in the fatty acid weakens the C-H bond on the carbon atom adjacent to the double bond and thus facilitates ‘H’ removal. This abstraction leaves an unpaired electron on the carbon -CH. The ‘C’ centered radical undergoes molecular rearrangement to form a conjugated diene (Halliwell and Gutteridge, 1990). This is followed by the addition of molecular O_2 to form lipid peroxy radicals, which in turn attacks another PUFA and abstracts hydrogen. This is the propagation stage of lipid peroxidation (Glugliano et al., 1996). The free radical initiated chain reaction propagates until two free radicals interact with each other. This is the termination stage of lipid peroxidation (Esterbauer et al., 1991). Membrane lipid peroxidation results in loss of PUFA, decreased membrane fluidity and loss of enzyme and receptor activity (Halliwell and Gutteridge, 1986). Moreover, lipid peroxidation itself has been implicated in DNA damage as many of the products from lipid peroxidation are capable of interacting with DNA to cause oxidative DNA damage (Glugliano et al., 1996).

1.11. OXIDATIVE STRESS AND DIABETES

Oxidative stress is playing a major role in the pathogenesis of both types of DM. Free radicals are formed in diabetes by glucose oxidation, protein glycation and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation. Accumulated evidence also indicates that oxidative stress-activated signaling pathways mediate insulin resistance and β-cell dysfunction. These consequences of oxidative stress can promote the development of diabetes complications. Therefore, oxidative stress, antioxidant defense, cellular redox status are regarded as the central players in diabetes and its complications.

1.12. ANTIOXIDANTS

Aerobic organisms have developed antioxidant defense system to cope up with the unwanted and toxic effects of ROS to avert damages due to oxidative stress. Antioxidants are one element of a collection of processes that retard in vivo free radical oxidation (Thomas, 2000). The antioxidants may protect against ROS either by 1) preventing the ROS formation, 2) intercepting of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance to sensitive biological targets to ROS
attack, 3) facilitating the repair of damage caused by ROS or 4) providing a favorable environment for the effective functioning of other antioxidants (Sen, 1995).

Enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and non-enzymic small molecules such as reduced glutathione (GSH), vit-C, vit-E and uric acid are directly involved in detoxification of ROS (Reunanen et al., 1998). The SOD exists in three forms namely copper-zinc SOD, manganese SOD and iron SOD. All these forms convert singlet oxygen to H₂O₂. Most H₂O₂ in tissues is removed by GPx, which uses it to oxidize GSH (Beckman et al., 1990). GSH is the primary low molecular weight thiol in the cytoplasm and is a major reserve for cysteine. GSH in conjunction with the reductant, NADPH can reduce lipid peroxides, free radicals and H₂O₂. GSH is oxidized to glutathione disulfide (GSSG) in this process, which is reconverted to GSH by glutathione reductase (GR). CAT is found in very low levels, which is localized in peroxisomes and removes H₂O₂ (Simonian and Coyle, 1996). These antioxidants work co-operatively and tend to counterbalance the effects of free radicals on the system. Deteriorative changes in the antioxidant defense system can cause accumulation of oxidative damage (Matsuo et al., 1993).

1.12.1. Antioxidants in diabetes

Under physiological conditions, a wide range of antioxidant defenses protects against the adverse effects of free radical production in vivo (Revnanen et al., 1998). Under normal conditions, potential toxic ROS generated by mitochondrial respiratory metabolism are efficiently neutralized by cellular antioxidant defense mechanisms. However, this balance can easily be broken and lead to cellular dysfunction. The balance between proxidant and antioxidant systems is very important in many disease processes including DM and is probably related to the complications associated with the disease (Ferreira et al., 1999). In addition to increased generation of free radicals in diabetes, impaired generation of naturally occurring antioxidants also result in increased oxidative injury due to failure of protective mechanisms (Bloom garden, 1997).

Several reports indicate increased lipid peroxidation and decreased SOD, CAT and GPx activity in various organs like liver, kidney, heart, lymphoid organs, lens and blood vessels etc., during diabetes (Yadav et al., 1997). Vitamin E content was decreased in diabetic rats, which lead to increased susceptibility to oxidation (Krishnamurthy et al., 1984). Reduced concentrations of ascorbic acid in diabetes
and interactions between this vitamin and biochemical mechanisms such as synthesis of structural proteins (Cunningham et al., 1994), oxidative stress (Dorchy, 1999), polyol pathway (Lindsay et al., 1998) and non-enzymic glycation of proteins suggest that disturbed ascorbic acid metabolism may be important in the pathogenesis of diabetes (Paolisso et al., 1994).

1.13. ANIMAL MODELS IN EXPERIMENTAL DIABETES

Animal models of diabetes are greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes. Inbred animal models, in which the genetic background is homogenous and environmental factors can be controlled, are therefore valuable in genetic dissection of multifactorial diseases. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations. Various types of animal models of diabetes derived either spontaneously or induced by treating with chemicals or dietary or surgical manipulations and combinations. Chemically induced models of diabetes are common in elucidating the possible role of environmental factors involved in the endocrine pancreatic destructive processes and subsequent development of diabetes. The most usual substances to induce diabetes in the rat are alloxan and streptozotocin.

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), synthesized by uric acid oxidation, exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The action of alloxan in the pancreas is lead by its rapid uptake by the β-cells (Weaver et al., 1978a; Boquist et al., 1983). The formation of ROS is followed by alloxan reduction leads to the formation of dialuric acid (Figure 1.3). The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals, superoxide radicals are able to liberate ferric ions (Fe³⁺) from ferritin and reduce them to ferrous ions (Fe²⁺). Fe³⁺ can also be reduced by alloxan radicals (Sakurai and Ogiso, 1995).

It has been proposed that one of the targets of the ROS is DNA of pancreatic islets (Li et al., 2002). Fragmentation of DNA takes place in β-cells exposed to alloxan (Takasu et al., 1991; Sakurai and Ogiso, 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the
formation of the disulfide bond and inactivation of the enzyme (Lenzen et al., 1987; Lenzen and Panten, 1988; Lenzen and Mirzaie-Petri, 1991).

It has been proposed that disturbances in intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan. Alloxan elevates cytosolic free Ca\(^{2+}\) concentration in pancreatic β-cells (Kim et al., 1994; Park et al., 1995) which leads to depolarization of pancreatic β-cells (Dean and Matthews, 1972).

![Figure 1.3. The mechanism of alloxan-induced ROS generation in β-cells of rat pancreas (Szkudelski, 2001).](image)

1.14. MODE OF ACTION OF PRESENT DRUGS FOR DM

There are various pharmacological approaches to improve glucose homeostasis. For glycemic regulation, five classes of drugs are currently available: sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolinediones and insulin, each of which has a different mode and site of action. These standard pharmacological treatments may be used individually for certain types of patients
or may be combined in a stepwise fashion to provide more ideal glycemic control in diabetic patients.

### 1.14.1. Sulphonylureas

The sulphonylureas are divided into 2 groups: first generation sulphonylureas (eg. acetohexamide, tolazamide and chlorpropamide) and second generation sulphonylureas (eg. glibenclamide and glipizide) (Proks et al., 2002). All sulphonylureas act by stimulating pancreatic β-cells to secrete insulin. They bind to β-cell receptors that are closely associated with ATP-dependent K⁺ channels; closure of these channels causes depolarisation of the cell, an influx of Ca²⁺ and stimulation of insulin secretion (Groop, 1992; Aguilar-Bryan et al., 1995). They also decrease hepatic insulin clearance, resulting in increased serum insulin concentrations (Marshall et al., 1970; Kolterman et al., 1984; Groop et al., 1988). Profound hypoglycemia is the major adverse effect associated with all sulphonylureas. Another adverse effect of the sulphonylureas is body weight gain. The anabolic effects of increased insulin levels, together with reduced urinary loss of glucose, may contribute to such weight increase. Other described effects of sulphonylureas are hepatitis and allergy.

### 1.14.2. Biguanides

The biguanides (eg. metformin, phenformin and buformin) alleviate hyperglycemia in type 2 diabetes by inhibiting hepatic glucose production and improving peripheral insulin sensitivity. Pharmacological studies indicate that these compounds act by improving peripheral sensitivity to insulin, reducing gastrointestinal absorption of glucose and decreasing hepatic glucose production, but do not stimulate insulin secretion (Klip and Leiter, 1990; Bailey, 1992). A decrease in total plasma cholesterol, LDL-C and triglyceride levels and some increase in HDL-C level were reported (Bailey, 1993; De Fronzo et al., 1995; Stumvoll et al., 1995). Lactic acidosis is the major serious adverse effect linked to the biguanides. Other adverse effects associated with biguanides are largely gastrointestinal including nausea, vomiting, diarrhoea, anorexia and abdominal discomfort.

### 1.14.3. α-Glucosidase Inhibitors

The α-glucosidase inhibitors (eg. acarbose, miglitol and voglibose) competitively and reversibly inhibit α-glucosidase, an intestinal brush border hydrolase enzyme (Balfour and McTavish, 1993). This leads to a postprandial decrease in carbohydrate absorption because complex dietary polysaccharides are
not broken down into absorbable monosaccharides. As a result, there is a decrease in hyperinsulinism and in hepatic triglyceride synthesis. Although they can be used as monotherapy, these antihyperglycemic drugs are frequently used in combination with the sulfonylureas or insulin. Clinical studies in both type 1 and type 2 diabetic patients have shown decrease in postprandial blood glucose concentration and urinary glucose excretion (Requejo et al., 1990; Chiasson et al., 1994). The major adverse effects of acarbose are on the gastrointestinal system, especially flatulence, abdominal bloating, borborygmus and sometimes diarrhoea.

1.14.4. Thiazolidinediones

Thiazolidinediones (eg. rosiglitazone, pioglitazone, darglitazone and BRL-49653) are insulin action enhancers that appear to improve glucose tolerance, decrease hepatic glucose production and increase insulin-stimulated glucose disposal (Saltiel and Horikashi, 1995). The thiazolidinediones enhance the effect of insulin in skeletal muscle, adipose and hepatic tissues without increasing pancreatic secretion of insulin. They bind to peroxisomal proliferator-activated receptors, changing insulin-dependent gene expression in the liver; the exact mechanism remains elusive. Due to its hepatotoxicity in some patients (Watkins and Whitcomb, 1998; Imura, 1998), troglitazone had been withdrawn from the market.

1.14.5. Benzoic acid derivatives

Repaglinide is the first nonsulfonylurea oral hypoglycemic agent on the market. It is indicated either as monotherapy or in combination with metformin. Repaglinide binds to the ATP-sensitive K⁺ channels on pancreatic β-cells at a receptor different from that of the sulfonylureas. However, it decreases insulin levels, whereas the sulfonylureas do not and an extrapancreatic effect leading to increased insulin sensitivity has been postulated. There have been no reports on repaglinide overdose and toxicity (Carlton, 2000).

1.15. DIABETES AND PROTEOMICS

Proteomics is a powerful tool for investigating protein expression profiles in biological systems and their modifications in response to stimuli or to particular physiological or pathophysiological conditions. It opens novel opportunities to study the mode of action, side-effects, toxicity and resistance of drug. It is also a valuable approach for the discovery of new drug targets. Although proteomics has been extensively employed to investigate cancers and other diseases, there are currently few reports concerning the proteomics study of diabetes. Using proteomic approach several protein markers for DM including glomerular transferrin (Tf),
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fibronectin and other components of glomerular extracellular matrix, tubular low-
molecular weight proteins (microglobulin, retinal-binding protein and urine protein) and other categories of proteins such as Tamm–Horsfall protein, b2-glycoprotein-1, urinary enzymes (N-acetyl-b-D-glucosaminidase, cholinesterase, glutamyl transpeptidase and alanine aminopeptidase) and tubular brush-border antigen have been identified (Hong and Chia, 1998; John et al., 2000; Larsen et al., 2001; Edvardsson et al., 2003).

Most of the proteomic studies on diabetes have been carried out in cell lines and in animal models of diabetes. Diao et al. (2006) reported that forty-three proteins were found to be either up-regulated or down-regulated in the liver, kidney and serum of the alloxan-induced diabetic mice. Sanchez et al. (2002; 2003) studied the effect of rosiglitazone on the expression of diabetes-associated proteins in pancreatic islets of type 2 diabetic mice. Liu et al. (2007) reported the differences in expression of retinal proteins between diabetic and normal rats which involved in the mechanisms and prognosis of retinal diseases caused by diabetes. The fungal polysaccharides were found to normalize the alterations in the protein profiles observed in streptozotocin-induced diabetic rat plasma (Kim et al., 2006).

The toxicity of some potential antidiabetic molecules has been evaluated by proteomic screening. Steiner et al. (1996) studied the effects of etomoxir, a potent hypoglycemic agent on the protein expression profile in rat liver by 2-D gel electrophoresis. The liver toxicity of another hypoglycemic agent, SDZ PUG 693, has also been investigated by 2-D gel electrophoresis (Arce et al., 1998).

1.16. ALTERNATIVE THERAPEUTIC APPROACHES FOR DIABETES

Although, oral hypoglycemic agents/insulin are the mainstay of treatment to diabetes and is effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1995). Therefore other agents with potent antidiabetic properties with less or no side effects are required. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. It is estimated that about one third of currently marketed drugs are related to natural products (Grabley and Thiericke, 1999; Onaga, 2001). The most commonly used drugs of modern medicine such as aspirin, anti-malarial, anticancers, digitalis, etc., have originated from plant sources. The ethnobotanical information reports about 800 plants that may possess antidiabetic potential (Alarcon-Aguilara et al., 1998).
Several medicinal plants such as *Musa sapientum* (Pari and Uma Maheswari, 2000), *Catharanthus roseus* (Singh et al., 2001), *Smallantus sonchifolius* (Aybar et al., 2001), *Momordica charantia* (Grover et al., 2001), *Helicteres isora* (Chakrabarti et al., 2002), *Coccinia indica* (Venkateswaran and Pari, 2002a), *Ficus religiosa* (Wadood et al., 2003), *Bauhinia forficata* (De Sousa et al., 2004), *Cogniauxia podoleana* Baillon (Diatewa et al., 2004) etc. were reported to posses antihyperglycemic effects.

A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes (Bailey and Day, 1989; Marles and Farnsworth, 1995). The discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using *Galega officinali*. In recent times, the safety and efficacy of these herbs have been validated by laboratory experiments and clinical trials. Although several medicinal plants have gained importance for the treatment of DM, many remain to be scientifically investigated.

1.17. GYMNEMA SPECIES

Members of Gymnema have been used in India for the treatment of diabetes for over 2,000 years (Kirtikar and Basu, 1975). The leaves were also used for stomach ailments, constipation, water retention and liver disease. Few Gymnema species have been pharmacologically evaluated by researchers.

*G. sylvestre* R. Br. belongs to the Asclepiadaceae family, is a woody, vine-like plant that climbs on bushes and trees, native to South Asia from India to the southern part of China. The leaves of this plant are also known to suppress sweet taste and intestinal glucose absorption (Chattopadhyay, 1998; Persaud et al., 1999). Anti-hyperglycemic effect of dried leaf powder of *G. sylvestre* was observed in alloxanized rabbits along with decrease in the activity of gluconeogenic enzymes and reversal of pathological changes in the liver initiated during the hyperglycemic phase (Shanmugasundaram et al., 1983). Oral administration of aqueous extracts of leaves of *G. sylvestre* normalized the blood sugar levels of STZ diabetic rats through β-cell regeneration (Shanmugasundaram et al., 1990). Various hypoglycemic principles have been isolated from this plant. The saponin fraction of the plant has been referred to contain gymnemosides and gymnemic acid (Murakami et al., 1996; Yoshikawa et al., 1997).
The triterpene glycosides isolated from this plant inhibited glucose utilization in muscles (Shimizu et al., 1996) and intestine (Shimizu et al., 1997a). In another study, water-soluble fraction of alcoholic extract of this plant significantly lowered the hepatic glycogen content of the glucose fed rats (Chattopadhyay, 1998). The presence of quercitol, lupeol, β-amyrin, stigmasterol in the leaves has also been reported. A new flavonol glycoside, kaempferol 3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside was isolated, along with four known flavonoids, kaempferol 3-O-robinobioside, rutin, quercetin 3-O-robinobioside and tamarixetin 3-O-robinobioside from this genus (Liu et al., 2004). Other species of Gymnema such as G. inodorum (Shimizu et al., 2001), G. yunnanense (Xie et al., 2003) G. foetidum have also been reported for antidiabetic activity.

1.18. GYMNEMA MONTANUM

Except a few common species, the majority of the Gymnema species shows various levels of rarity and G. montanum is one such species that has been used for this study. G. montanum H. belongs to the family Asclepiadaceae is a rare and endemic plant species of India (Hooker, 1883). It is found mainly in the Shola forests of Western Ghats, Gudalur, Nilgiri Biosphere Reserve at an altitude of 900 - 1500 MSL (Vajravelu and Bhargavan, 1983). It is a woody climber plant with terete, glabrous stem. Leaves-opposite, leathery, entire, elliptic-oblong to obovate, chartaceous, penninerved; petiole-1.0-1.5 cm; lamina-ovate-lanceolate 6-10 cm long and 4-8 cm broad. Inflorescence an umbels axillary of 3-7 flowers; peduncle-slender, about 3 cm long thinly pubescent; pedicels shorter 0.2-0.5 cm; bract-scaly, about 1.5 mm. Calyx-lobes equal, subcoriaceous, finely puberulous without, glandular. Corolla -yellow, campanulate; tube shorter than lobes; lobes recurved, 3 mm, twisted towards right in bud. Pollinia erect; pollinial bags oblong, gradually narrowed towards caudicle; Caudicle-indistinct, receptacle-brownish. Corona single, coralline of freshly process, extending lengthwise, ciliate along margin. Stigma-obconic.

G. montanum is morphologically distinguished from G. sylvestre in its woody climber, leathery leaves and size of the follicles. G. montanum is traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation and gastrointestinal ailments. There is no information about the pharmacological properties of this plant. These facts justify interest in further studies on the anti-diabetic property of this plant.
Plate 1.1. GYMNEMA MONTANUM H. - an Indian medicinal plant

A. Habitat
B, C & D - Closer view of the Climber
E - Closer view of the Pod