Introduction
INTRODUCTION

Diabetes mellitus is one of the world’s oldest known diseases. Diabetes received its name from a Greek physician Aretaeus in first century B.C. In 1750, a Latin scientist Cullen added mellitus to the term diabetes. In ancient times, Indians would call diabetes madhumeha (sweet urine disease).

As a result of extensive research done over a period of centuries, diabetes mellitus is now recognised as a metabolic disorder of multiple etiology which is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative deficiency in insulin secretion/insulin action or both.

In 1997 diabetes prevalence was introduced as a “basic health indicator” for member states by the World Health Organization (WHO). Diabetes mellitus affects nearly 10% population all over the world (Burke et al., 2004). The global population is in the midst of a diabetes epidemic with people in South East Asia and Western Pacific being mostly at risk. According to WHO report, the number of diabetics was 171 millions in 2000 which might increase to 360 millions in the year 2030 (WHO, 2000) and India tops the list. As the number of people with Diabetes mellitus (DM) multiplies worldwide, national and international health care budget too increases. Based on routine statistics, recent WHO reports estimated mortality from diabetes in the world as 987,000 deaths for the year 2002, which was 1.7% of total world mortality (WHO, 2003).

The vast majority of diabetic patients are classified into two broad categories: type-1 diabetes, which is caused by an absolute deficiency of insulin, and type-2 diabetes, which is characterized by the presence of insulin resistance (IR) with an inadequate compensatory increase in insulin secretion.

Type-1 diabetes was formerly known as juvenile onset diabetes and ketosis-prone diabetes and more recently, it is called as Insulin Dependent Diabetes Mellitus (IDDM). Type-1 diabetes is thought to result from autoimmune destruction of the pancreatic β-cells, which results in complete or almost complete loss of insulin production (Kukreja and Maclaren, 1999; Atkinson and Eisenbarth, 2001). Markers of immune destruction of β-cells are present at the time of diagnosis in 90% of individuals and include antibodies to the islet
cells, glutamic acid decarboxylase and insulin (Zimmet et al., 1994). This form of diabetes, usually seen in children and adolescents, can also occur at any age. From literature review it is revealed that 15-20% of diabetic patients suffer from type-1 diabetes (Chakrabarti and Rajagopalan, 2002). This rapid rise in the incidence of type-1 diabetes strongly suggests that the action of the environment on susceptibility genes contributes to the evolving epidemiology of type-1 diabetes (Gillespie, 2006).

Type-2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM) is usually diagnosed after 40 years of age. NIDDM is the most common form of diabetes mellitus and nearly 85% of all peoples with diabetes have NIDDM (Chakrabarti and Rajagopalan, 2002).

Insulin resistance was reported to be a characteristic feature of NIDDM in the early 1970s. Type-2 diabetes represents the final stage of a chronic and progressive syndrome representing a heterogeneous disorder caused by various combinations of insulin resistance and decreased pancreatic β-cell function, caused by both genetic and acquired abnormalities (Olefsky, 1993; Kahn, 1994; Gerich, 1998). Insulin resistance precedes the development of type-2 diabetes, sometimes by years. In individuals who will ultimately develop type-2 diabetes, it is believed that blood glucose and insulin levels are normal for many years; then at some point of time, insulin resistance develops. Initially, β-cells compensate for insulin resistance by increasing insulin secretion and hyperinsulinemia develops. However, as time goes by, the β-cell function alters and fails to compensate for increasing insulin resistance and thus blood sugar levels begin to increase (Purrello and Rabuazzo, 2000) and eventually clinical diabetes is established.

Currently, type-2 diabetes is diagnosed when the underlying metabolic abnormalities consisting of insulin resistance and decreased β-cell function cause elevation of plasma glucose above 126 mg/dl (7 mmol/liter) in the fasting state and/or above 200 mg/dl (11.1 mmol/liter) 120 min after a 75-g glucose load (Report of the Expert Committee, 1999). However, the fact that many newly diagnosed type-2 diabetic subjects already suffer from the late complications of diabetes at the time of the diagnosis (Beck Nielsen and Groop, 1994) indicates that the diagnosis may have been delayed and, in
addition, that the pre-diabetic condition is harmful to human health and requires increased awareness by physicians and the general public.

Type-2 diabetes is the most common metabolic disorder worldwide (Goldstein, 2003) and its prevalence is growing at an alarming rate in both developed and developing countries (Wild et al., 2004; Yach et al., 2006). Reasons for this increase include changes in human behaviour, increase in sedentary lifestyle, consumption of energy rich diet, stress, infections, altered immune function, altered metabolic/physiological status, drugs and hormones (Lovejoy and Digirolamo, 1992). Type-2 diabetes has a strong genetic predisposition and is more common in minority ethnic groups, i.e., Mexican-Americans, Latinos, American Indians than in individuals of European ancestry (Defronzo, 1997; Tilburg et al., 2001).

Diabetes is a disease of complications. Sometimes a complication may give a clue to the presence of the disease. IDDM and NIDDM are associated with several forms of long term complications. These include microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease and cerebrovascular disease).

GLUCOSE HOMEOSTASIS

Despite large changes in the input and utilization of glucose, the blood glucose levels are maintained at constant level. Maintenance of stable levels of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms and one in which the liver, the extra hepatic tissues, and several hormones play a part. In the post-absorptive state, the concentration of blood glucose in individual humans and many mammals is set within the range of 4.5-5.5 mM. After the ingestion of a carbohydrate meal, it may increase to 6.5-7.2 mM. During fasting, the levels fall to around 3.3-3.9 mM.

The monosaccharides, mainly glucose, galactose and fructose, arising from digestion and absorption of dietary carbohydrates in intestine, are transported through portal circulation to liver. Galactose and fructose are readily converted to glucose in liver. Liver has the primary metabolic function of regulating the blood concentration of most metabolites, particularly glucose. In the case of glucose, this is achieved by taking up excess glucose and converting it to glycogen (glycogenesis) or to fat (lipogenesis).
meals, liver can draw upon its glycogen stores to replenish glucose in the blood (glycogenolysis) or, in company with the kidney, convert non-carbohydrate metabolites such as lactate, glycerol and amino acids to glucose (gluconeogenesis). Skeletal muscle utilizes glucose as a fuel forming both lactate and CO$_2$. It stores glycogen as a fuel for its use during muscular contraction.

Liver cells appear to be freely permeable to glucose (via the GLUT-2 transport), whereas cells of extra hepatic tissues (apart from pancreatic islets) are relatively impermeable. As a result, the passage through the cell membrane is the rate-limiting step in the uptake of glucose in extra hepatic tissues, and glucose is rapidly phosphorylated by hexokinase on entry into the cells. On the other hand, it is probable that the activity of certain enzymes and the concentration of key intermediates exert a much more direct effect on the uptake or output of glucose from liver. Nevertheless, the concentration of glucose in blood is an important factor controlling the rate of uptake of glucose in both liver and extra hepatic tissues. Glucose homeostasis is maintained in normal animals by the reciprocal regulation of insulin secretion by $\beta$-cells and glucagon secretion by $\alpha$-cells of the islet of Langerhans.

**Role of hormones in glucose homeostasis**

Blood glucose concentration in the fed and post-absorptive states are regulated by the interaction between insulin and glucagon. The glucoregulatory hormones of the body are designed to maintain circulating glucose concentration within a relatively narrow range.

Insulin is produced by the $\beta$-cells of the pancreatic islets of Langerhans as a direct response to the degree of hyperglycemia. The islet cell is freely permeable to glucose via the GLUT-2 transporter, and the glucose is phosphorylated by the glucokinase. Therefore, the blood glucose concentration determines the flux through glycolysis, the citric acid cycle, and the generation of ATP. Increase in ATP concentration inhibits the ATP-sensitive K$^+$ channels causing depolarization of membrane of the $\beta$-cells, which increases Ca$^{2+}$ influx via voltage-sensitive Ca$^{2+}$ channels stimulating exocytosis of insulin. Thus, the concentration of insulin in the blood parallels that of the blood glucose (Bratanova-Tochkova et al., 2002; Soria et al., 2004)
Insulin acts at multiple steps in carbohydrate metabolism. It enhances uptake of glucose into fat and muscle cells via modulation of GLUT-4 translocation. Glycogen synthesis is increased, and glycogen breakdown is decreased by dephosphorylation of glycogen synthase and glycogen phosphorylase respectively. Glycolysis is stimulated and gluconeogenesis is inhibited by dephosphorylation of pyruvate kinase (PK) and 2, 6 biphosphate kinase. Signalling intracellular energy abundance, insulin enhances the irreversible conversion of pyruvate to acetyl Co-A by activation of the intra-mitochondrial enzyme complex pyruvate dehydrogenase. Acetyl-CoA may then be directly oxidised via the Kreb’s cycle, or used for fatty acid synthesis (Denton and Tavare, 1997).

The impaired ability of insulin to signal GLUT-4 translocation from intracellular stores is currently believed to be an important contributory factor to postprandial hyperglycemia in diabetes (Baron et al., 1988). Decreased insulin levels in diabetic animals have been shown to not only decrease transporter translocation but also diminish expression of GLUT-4 in muscle cells (Klip et al., 1990; Unger, 1991).

The importance of GLUT-4 in glucose homeostasis is best demonstrated by studies in mice in which one allele of GLUT-4 gene has been disrupted. These mice have approximately a 50 per cent reduction in GLUT-4 concentration in skeletal muscle, heart and adipocytes and they have severe insulin resistance (Shepherd and Kahn, 1999). Thus, the mechanism by which diabetes, characterized by either low insulin levels, as in type-1 diabetes, or insulin resistance, as in type-2 diabetes, could cause pathologically high plasma glucose levels via loss of regulation and expression of transmembrane glucose transporters.

Insulin is a potent inhibitor of lipolysis and even small increment in the plasma insulin concentration exerts a potent antilipolytic effect leading to a marked reduction in the plasma free fatty acid levels (FFA) (Bonadonna et al., 1990; Campbell et al., 1992). The decline in plasma free fatty acids concentration results in increased glucose uptake in muscle and contributes to the inhibition of hepatic glucose production (Kelley et al., 1993). Thus, changes in the plasma FFA concentration in response to increased plasma levels of insulin and glucose play an important role in the maintenance of normal glucose homeostasis.
Insulin, though the dominant hormone driving metabolic processes in the fed state, acts in concert with growth hormone and IGF-1. Growth hormone is secreted in response to insulin, among other stimuli preventing insulin-induced hypoglycemia. Other counterregulatory hormones include glucagon, glucocorticoids and catecholamines. These hormones drive metabolic processes in the fasting state. Glucagon promotes glycogenolysis, gluconeogenesis and ketogenesis. The ratio of insulin to glucagon determines the degree of phosphorylation or dephosphorylation of the relevant enzymes (Karam, 1997). Catecholamines promote lipolysis and glycogenolysis, while glucocorticoids promote muscle catabolism, gluconeogenesis and lipolysis.

METABOLIC DISTURBANCES

Diabetes has always been considered, a disturbance in the metabolism of carbohydrates accompanied by alteration in the metabolism of fats and proteins. Fig 1 shows the pathophysiology of type 1 and type 2 diabetes. The changes are mainly the result of a low insulin/glucagon ratio. Hepatic glucose output is controlled by basal levels of insulin and glucagon. In NIDDM, fasting blood glucose is increased in direct proportion to hepatic glucose output (Bogardus et al., 1984; DeFranzo et al., 1985), and appears unlikely to be a result of decreased insulin action at the periphery as it has not been shown to correlate closely with insulin-stimulated glucose disposal (DeFranzo et al., 1982). As fasting plasma insulin and C-peptide concentrations are normal in NIDDM, the disturbances to glucose homeostasis appear to result from insulin insensitivity.

Hyperglycemic conditions arise due to (1) high rates of glycogenolysis and gluconeogenesis, (2) decreased utilization of glucose by the peripheral tissues due to the decreased peripheral uptake of glucose from blood.

The action of hyperglycemic hormones becomes more prominent due to lack of insulin. As carbohydrates cannot be used as fuel in diabetes, fat is used as fuel. High glucagon level decreases the hepatic fructose 2, 6-biphosphate level, thereby decreasing the utilization of glucose. The insulin dependent enzymes are also less active. Net effect is inhibition of glycolysis and stimulation of gluconeogenesis leading to hyperglycemia.

One of the consequences of hyperglycemia in human DM is increased metabolism of glucose by sorbitol (polyol) pathway. Aldose reductase catalyses the reduction of glucose to
Fig. 1 The pathophysiology of Type 1 and Type 2 Diabetes mellitus
sorbitol. Sorbitol doesn’t readily diffuse across the cell membranes and tends to accumulate in the cell. Under hyperglycemic condition, high glucose flux through the sorbitol pathway accounts for one-third of glucose metabolism. This has important implications in terms of redox changes of NADP⁺ and NAD⁺ couples and metabolism of glucose by alternative pathways (Jeffrey and Jornvall, 1983). Conversion of glucose to sorbitol by aldose reductase requires NADPH and forms NADP⁺ and thereby competes with other NADPH requiring reactions. Conversion of sorbitol to fructose by sorbitol dehydrogenase is coupled to reduction of NAD⁺ to NADH and this inhibits glycolysis at the glyceraldehyde dehydrogenase step for NAD⁺ (Gonzalez et al., 1986). Increased flux of glucose via polyol pathway has also consequences for the overall antioxidant status leading to depletion of glutathione (GSH) as a result of competition between aldose reductase and glutathione reductase for NADPH.

When blood glucose level exceeds the renal threshold, glucose is excreted in urine. Due to osmotic effect, more water accompanies the glucose (polyuria). To compensate for this loss of water, thirst centre is activated and more water is taken (polydipsia). The loss and ineffective utilization of glucose leads to breakdown of fat and protein. This leads to loss of weight. To compensate the loss of glucose and protein, patient will take more food (polyphagia). The need for fatty acid breakdown to meet the energy requirements would lead to production of more acetyl CoA. The enzyme carnitine-acyl transferase is activated by a low insulin/glucagon ratio since the malonyl CoA level is low. There is increased mobilization of triacylglycerol (TAG) from adipose tissue as evidenced by high free fatty acid levels in plasma. The acetyl-CoA cannot be efficiently oxidized by Kreb’s cycle since the availability of oxaloacetate is limited. The stimulation of gluconeogenesis is mainly responsible for the depletion of oxaloacetate. The excess of mitochondrial acetyl CoA therefore is diverted to ketone bodies leading to enhanced ketogenesis. The net effect is the increased mobilization and utilization of fat for meeting energy requirements. Increased breakdown of proteins for providing substrate for gluconeogenesis and the absence of anabolic effect of insulin are responsible for muscle wasting.

Acute metabolic complications in DM include diabetic ketoacidosis, hyperosmolar non-ketotic coma and lactic acidosis. Ketosis is a more common complication of uncontrolled IDDM. The excessive production of ketone bodies by the liver exceeds the
capacity of peripheral tissues to utilize the ketone bodies leading to ketonemia and ketonuria. The accumulation of the acidic ketone bodies lowers the blood pH leading to diabetic ketoacidosis. In addition to acidosis, ketosis also leads to dehydration. The hyperglycemia and glycosuria produce osmotic diuresis. If not treated promptly and properly the condition may be fatal. Patient may become unconscious, comatose and die.

Both types of DM are equally devastating with respect to their later complications i.e. nephropathy, neuropathy and aggravated atherosclerosis which lead to cardiovascular disorders.

INSULIN RESISTANCE

Insulin resistance was first described in the 1930s when Himsworth reported diabetes patients who did not respond to insulin treatment (Himsworth, 1936). Insulin resistance has a strong predictive value with respect to development of type-2 diabetes and together with decreased insulin production from the β-cells of the pancreas it provides the pathophysiological background for the disease (Reaven and Banting, 1988).

Insulin resistance is defined as an impaired effect of a certain amount of insulin in target tissues, i.e. mainly muscle, fat and liver. Insulin resistance can manifest itself as either unresponsiveness or insensitivity to insulin. Unresponsiveness implies that there is an impaired maximal effect of insulin. Insensitivity, on the other hand, means that a higher insulin concentration than normal is necessary to produce a certain effect, i.e. the dose-response curve for insulin is shifted to the right (Kahn, 1978). In most conditions of insulin resistance, there is a combination of unresponsiveness and insensitivity.

Although genes are an important factor in many cases of obesity, a person's environment too plays a significant part (Manson et al., 1992; Tomás et al., 2002). In adipose tissue, insulin resistance results in an impaired antilipolytic action of insulin, which results in increased non-esterified fatty acid (NEFA) release. Adipose tissue lipolysis has been shown to be the pathway most sensitive to insulin action compared with other insulin regulated pathways (Kovacs and Stumvoll, 2005). In addition, it can also lead to deregulated production and secretion of adipokines and other adipose-derived biomolecules (Steppan et al., 2001; Yamauchi et al., 2001).
When the amount of NEFA is greater than needed, as after a meal, NEFAs are esterified and stored as TAGs. Large amounts of fat in this way can be stored as lipid droplets in adipocytes. Other cells can store only limited amounts of fat. If concentrations of NEFAs are high in the plasma, transport of fat in to cells increases throughout the body, including cells with low capacity for fat storage, such as hepatocytes, myocytes, pancreatic and kidney cells (Schaffer, 2003). Fatty acid overload in tissues other than adipose tissue can lead to cell and tissue death, a phenomenon called lipotoxicity (Schaffer, 2003). Lipotoxicity is involved in the pathogenesis of insulin resistance, type-2 diabetes and cardiovascular diseases.

In muscle, insulin-stimulated transmembrane glucose uptake appears to be the major rate-limiting factor (Yki-Jarvinen, 1998). Increased intra myoecellular fat accumulation has been associated with insulin resistance (Shulman, 2000). Defects in mitochondrial oxidative phophorylation were found in insulin resistant and type-2 diabetics (Kelley et al., 2002) who display increased muscle fat content. Like adipose tissue, skeletal muscle can also produce and release cytokines (Lang et al., 2003), but the concentrations of these have not been associated with cardiovascular risk factors (Yki-Jarvinen, 2005).

Hepatic insulin resistance is characterized by an impaired ability of insulin to suppress hepatic glucose production. Fat accumulation in the liver is associated with hepatic insulin resistance (Kotronen and Yki-Jarvinen, 2008). In liver, there is attenuated insulin action with respect to glucose uptake and storage as well as suppression of glucose and VLDL production (Del Prato et al., 1997; Mevorach et al., 1998). Interestingly, there can also be insulin resistance in the insulin-secreting β-cells of the endocrine pancreas and this can be of importance in type-2 diabetes leading to an attenuation of proinsulin synthesis and hence the capacity for insulin secretion. Liver specific insulin receptor knockout (LIRKO) mice develop severe glucose intolerance due to failure of insulin to induce hepatic glucose uptake and suppress hepatic glucose production (Michael et al., 2000). LIRKO-mice also develop a pro-atherogenic lipoprotein profile (Biddingger et al., 2008) showing that hepatic insulin resistance alone is sufficient to induce dyslipidemia and atherosclerosis. Moreover, in humans, fatty liver is associated with a failure of insulin to reduce gluconeogenesis and serum NEFAs, independently of obesity (Seppa-Lindroos et al., 2002). Environmental factors like physical inactivity, a high energy and high fat diet,
smoking and stress strongly interact with a genetic predisposition to promote development of the insulin resistance.

Although immense research efforts have been made in order to elucidate the mechanisms underlying insulin resistance, there is still no consensus on the exact defects at the cellular and molecular levels. Several pathways may contribute to the development of insulin resistance and type-2 diabetes. Such pathways, including metabolic factors, e.g., glucose and fatty acids in elevated concentrations, can exert detrimental effects.

Experimental hyperglycemia has been shown to cause insulin resistance both in vitro and in vivo (Garvey et al., 1986; Bonadonna et al., 1993; Iozzo et al., 2001). Hyperglycemia alone exerts detrimental effects on insulin secretion and insulin action (Unger and Grundy, 1985), a phenomenon commonly referred to as glucose toxicity (Rossetti et al., 1990). One of the consequences of hyperglycemia induced IR is increased metabolism of glucose by hexosamine pathway. Several studies in rats suggested that increased hexosamine biosynthesis leads to skeletal muscle insulin resistance in vivo and in vitro which may be a mechanism involved in glucotoxicity (Rossetti et al., 1987; Hawkins et al., 1996). Moreover, glucose-induced activation of different protein kinase C (PKC) isoforms has been shown to interfere with insulin receptor signalling and produce insulin resistance (Berti et al., 1994; Kawano et al., 1999). However, the mechanism by which hyperglycemia causes insulin resistance, still remains incompletely understood.

Elevated FFAs might promote accumulation of fat deposits in muscle, liver and/or β-cells, and the accumulated triacylglycerols might provide an environment that could interfere with metabolic signalling and thus action in these different tissues (Nyholm et al., 1999). A link between insulin resistance and triacylglycerol content in muscle biopsies has been established (Phillips et al., 1996; Pan et al., 1997). Moreover, it was shown that elevation in plasma FFA concentrations can lead to an attenuated effect of insulin to stimulate IRS-1-associated PI-3 kinase activity in muscle (Dresner et al., 1999).

Neurohormonal mechanisms clearly can be involved, and glucocorticoids (Rooney et al., 1993; Lambillotte et al., 1997), growth hormones (Fowelin et al., 1993), sex steroids and catecholamines (Rizza et al., 1980) as well as insulin itself have marked effects on insulin sensitivity in various tissues. As visceral adiposity appears to be a very important
component of the development of type-2 diabetes and cardiovascular disease, adipose-related mechanisms are of interest. In adipose dysfunction, inflammatory mediators such as cytokines and chemokines as well as inflammatory cells, i.e., lymphocytes, neutrophils and macrophages may play important roles (Devaraj et al., 2004).

A complex interplay between genetic and acquired factors is responsible for the pathobiology of IR. It is likely that stressors in various forms hit the organism at many different levels, which adds up to a pathogenic process moving towards insulin resistance and diabetes. Such stressors, i.e., social, psychological, neural, endocrine, metabolic, inflammatory factors, may merge into a final common pathway namely oxidative stress at the cellular level. It is proposed that this is a critical pathway for the development of insulin resistance in insulin’s target tissues, and for β-cell dysfunction and macro- and microvascular damage in diabetes (Eriksson, 2007).

HYPERGLYCEMIA INDUCED OXIDATIVE STRESS AND VASCULAR COMPLICATIONS

Oxidative stress

When the rate of oxidant production exceeds the rate of oxidant scavenging, the situation leads to oxidative stress. Reactive Oxygen Species (ROS) can attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids and to a lesser extent, carbohydrates. Increased free oxygen radical activity can initiate peroxidation of lipids. The increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound proteins and receptors (Baynes, 1991). Oxidation of protein molecules not only inactivates them but also introduces a tag for in vivo protein degradation by proteosome system (Tsu Chung et al., 2000). DNA is probably the most biologically significant target of oxidative attack. Strand scission, destruction and fragmentation of bases and deoxyribose sugars have all been reported to occur following free radical (mainly hydroxyl radical) attack on DNA. The resulting cytotoxicity, mutations and potential for malignant change occur due to induced chromosomal aberrations ultimately resulting in cell death (Sinclair et al., 1991).

Extensive studies revealed that, increase in oxidant production, observed in diabetes and insulin-resistant states, are the products of altered metabolism of glucose, FFA, and other metabolites, which are the result of insulin deficiency and resistance (Boden and
Glucose autoxidation and non-enzymatic protein glycation may be the source of ROS that can initiate oxidative tissue damage in diabetes (Fig 2). Glucose is prone to transition metal-catalyzed autoxidation with the formation of superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^-$) (Jiang et al., 1990). Monosaccharides, such as glucose, can enolize and therefore reduce transition metal and molecular oxygen, yielding ($O_2^-$), whose dismutation forms $H_2O_2$ spontaneously. The latter can be further decomposed into $OH^-$, a process catalyzed by transition metal ions such as copper and iron. Experiments in vitro revealed that glucose incubated with protein undergoes a similar process, with the formation of $O_2^-$, $H_2O_2$, $OH^-$, and dicarbonyls (α-ketoaldehyde). The latter is more reactive to protein, with the formation of ketoamine adducts. The on-site-formed $OH^-$ can attack protein attached to glucose and induce site-specific damage, including the oxidation of amino acids, the generation of fluorphore (protein browning), and the fragmentation of protein. Thus, the term autoxidative glycation was introduced to describe the process of oxidative modification of protein by high glucose concentration (Brownlee, 1996).

Glycated proteins also may serve as a source of oxygen radicals. Amadori adducts of protein glycation may undergo oxidation in the presence of oxygen and transition metals leading to the formation of $O_2^-$, the release of erythronic acid and the products of oxidative cleavage of the glycated protein carboxymethyllysine, carboxymethyl hydroxylysine, and pentosidine. These compounds, termed glycoxidation products, were used as the biomarkers of glucose-dependent protein damage (Kyselova et al., 2004). Besides affecting the functions of these molecules, oxidative stress also triggers a series of cellular responses including the activation of protein kinase C (PKC), the transcription nuclear factor κB (NF-κB), and JNK stress associated kinases (Koya et al., 1998; Mohamed et al., 1999; Ho et al., 2000). Inappropriate activation of these important regulatory molecules can have deleterious effects on cellular functions and is thought to contribute to the pathogenesis of various diabetic vascular complications. Through subsequent ROS-induced DNA damage and poly-ADP ribose polymerase activation, ADP-ribose polymers attach to and inhibit the cytosolic glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. This inhibition in turn mediates the activation of four proposed mechanisms of hyperglycemia-associated tissue damage- the polyol pathway, the hexosamine pathway, protein kinase C activation,
Fig. 2. Biochemical pathways along which glucose metabolism can form ROS. Under physiological conditions, glucose primarily undergoes glycolysis and oxidative phosphorylation. Under Pathologic conditions of hyperglycemia, excessive glucose shunted to other pathways: autooxidation; enolization and ketoaldehyde formation; PKC activation; dicarbonyl formation and glycation; sorbitol metabolism (Paul Robertson, 2004).
and formation of advanced glycation end products (Nishikawa et al., 2000; Ceriello, 2003).

Although there is controversy about the antioxidant status in diabetes, several studies report decreased plasma or tissue concentration of superoxide dismutase, CAT, GSH and ascorbic acid in both diabetic animals and patients (Hink et al., 2001). Thus enhanced oxidant production with decreased antioxidant potential of diabetes intensifies the oxidative stress.

Vascular complications

Both IDDM and NIDDM are associated with several forms of long term complications. These include microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease and cerebrovascular disease). Many clinical studies have shown that long-term exposure to hyperglycemia causes diabetic vascular complications. Oxidative stress has been postulated to be a major contributor to the pathogenesis of these events (Ellis et al., 2000; Bonetti et al., 2003; Etoh et al., 2003).

INDUCTION OF EXPERIMENTAL TYPE-1 DIABETES

Several methods are employed to induce experimental diabetes in laboratory animals. Understanding diabetic pathology would require a lifetime of serial studies. The use of animal models largely circumvents these problems. Surgical removal of pancreas is an effective method. However, to induce a notable form of diabetes, at least 90-95% of the pancreas has to be removed (Akbarzadeh et al., 2007). Otherwise, the islets of Langerhans in the remaining pancreas may undergo hypertrophy and secrete sufficient amount of insulin for fulfilling the normal metabolic needs. The second method of inducing diabetes in animals is by injecting diabetic drugs such as alloxan or streptozotocin (STZ).

Streptozotocin (STZ) induced diabetes

Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is an antibiotic derived from Streptomyces achromogenes (Lewis and Barbiers, 1960). It was developed as an anticancer agent. However, since the discovery of its diabetogenic effects following systemic application (Rakieten et al., 1963), STZ is now used mainly to induce diabetes in experimental animals. When injected intravenously or subcutaneously, it
induces diabetes resembling human type-1 or type-2 diabetes, depending on particulars (Ito et al., 1999). The final symptoms of insulin deficiency are clearly seen in rats afflicted with diabetes chemically by STZ. High dosages of the β-cell toxin streptozotocin induce severe insulin deficiency and IDDM with ketosis. Lower dosages, calculated to cause a partial reduction of β-cell mass, could be used to produce a mildly insulin-deficient state of NIDDM without a tendency to ketosis. Injection of STZ at 55mg/kg body weight resulted in the toxicity of β-cells with emergence of clinical type-1 diabetes within 2-4 days (Ekrem et al., 2005; Baskar et al., 2006).

Fig.3 shows the mechanism of streptozotocin induced toxic events in β-cells of rat pancreas. STZ enters the β-cell via a glucose transporter (GLUT-2) and causes alkylation of DNA (Elsner et al., 2000). DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of STZ than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD⁺ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, H₂O₂, and OH⁻ are also generated. Furthermore, STZ liberates toxic amounts of NO (nitric oxide) that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, β-cells undergo destruction by necrosis (Weiss, 1982; Szkudelski, 2001).

INDUCTION OF INSULIN RESISTANCE

Several insulin resistant animal models are available which include genetic, drug induced and nutritionally or diet induced animal model.

Genetic model of insulin resistance

The inherited defects responsible for insulin resistance are largely unidentified. Common polymorphisms in candidate genes that could potentially modulate insulin sensitivity. For example, α-adrenergic receptors, PPARy (peroxisome proliferator activated receptor), IRS-1 and glycogen synthase, appear to be associated with human insulin resistance and NIDDM (Groop, 2000). Mutations in candidate genes involved in insulin-stimulated glucose transport, (e.g., the insulin receptor, glucose transporters and signalling proteins) can lead to marked insulin resistance, but these are rare (Fujimoto, 2000). For
Fig. 3. The mechanism of streptozotocin-induced toxic events in β-cells of rat pancreas (Szkudelski, 2001).
example, defects in the insulin receptor gene are too rare to account for the common forms of insulin resistance (Krook and O'Rahilly, 1996). In recent years, monogenic and polygenic knockout mouse models as well as tissue-specific knockout models have been created (Mauvais-jarvis et al., 2002).

**Drug/chemical induced insulin resistance**

β-Adrenergic agonists especially parenteral (used in pre-mature labour, eg., Ritodrine) cause insulin resistance and aggravate hyperglycemia (Krentz, 2002). Pharmacological doses of glucocorticoids induce *ob* gene expression in rat adipocyte tissue within 24 h, which is followed by complex metabolic changes resulting in decrease in food consumption, reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and TAG levels (Shalam et al., 2006). Glucocorticoids are reported to activate adipose tissue lipolysis, which is probably also an important factor in promoting insulin resistance, since insulin sensitivity was normalised when lipolysis (Ekstrand et al., 1992) or lipid oxidation (Guillaume-Gentil et al., 1993) was inhibited. The effects of glucocorticoids in vivo appear to include both an impairment of insulin-dependent glucose uptake in peripheral tissues and a stimulation of gluconeogenesis in the liver (Rizza et al., 1982; Rooney et al., 1993). This appears to be mediated primarily by an impairment of glucose transport, and dexamethasone-induced insulin resistance in 3T3-L1 adipocytes probably involving the GLUT4 translocation machinery (Sakoda et al., 2000)

**Diet/Nutritional induced insulin resistance**

Changes in diet have been studied as contributing factors to the development of insulin resistance. Along with an increase in total energy consumption over the past few decades, there has been a shift in the types of nutrients consumed in the diet. The most widely used animal model of insulin resistance is the high fat fed rat (Storlien et al., 1986; Ramirez et al., 1990; Han et al., 1997). Storlien et al. (1986) demonstrated that feeding rats with a high fat diet (59 % of energy) for 24 d induced whole body insulin resistance and inhibited insulin-stimulated glucose utilization in liver, skeletal muscle and brown and white adipose tissue.

Similar to the genetic models, a sucrose-rich diet evokes glucose intolerance
associated with hyperinsulinemia, increased plasma FFAs, and hypertriglyceridemia (Lombardo et al., 1983). High-sucrose diets impaired insulin action in rats at both the hepatic and peripheral levels (Storlien et al., 1988; Pagliassotti and Prach, 1995; Podolin et al., 1998).

**High-fructose diet**

High dosage of fructose in the diet has been shown to induce IR accompanied by deleterious metabolic consequences including hyperinsulinaemia, hyperglycemia, glucose intolerance, hypertriacylglycerolemia and hypertension and obesity in rodents (Hwang et al., 1987; Thorburn et al., 1989). The fructose-fed rat is therefore used as an animal model of IR and is considered parallel to multiple metabolic syndrome (syndrome X) observed in humans (Reaven and Banting, 1988).

Most of the metabolic effects of fructose are due to its rapid utilization by the liver and its entry in to the pathway of glycolysis or gluconeogenesis at the triose phosphate levels after by passing the phosphofructokinase regulatory step (Underwood and Newsholme, 1965) leading to a far reaching consequences to carbohydrate and lipid metabolism. The metabolic consequences include immediate hepatic increase in pyruvate and lactate production, activation of pyruvate dehydrogenase, and a shift in balance from oxidation to esterification of non-esterified fatty acids resulting in increased secretion of VLDL. These effects are augmented by long-term absorption of fructose, which causes enzyme adaptations that increase lipogenesis and VLDL secretion leading to triacylglycerolemia (Mayes, 1993). A schematic representation of hepatic fructose metabolism is given in Fig 4.

Acetyl-CoA is another product of fructose metabolism and it is a precursor for FFA (Havel, 2005). This increase in FFA in the liver also results in the elevation of blood levels of TAGs and FFA (Bantle et al., 2000). Circulating FFA stimulates insulin release (Crespin et al., 1969). Insulin, in turn, perpetuates the build up of FFA, as insulin reduces oxidation (lipolysis) of FFA (Cave et al., 2007). Elevated plasma FFA concentration can induce insulin resistance in muscle via multiple mechanisms involving alterations in a variety of intracellular lipid signalling molecules, which exert their inhibitory effects on multiple steps (insulin signal transduction system, glucose transport, glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, Krebs cycle) (Tippett and Neet 1982; Dresner et al.,
Fig. 4. Hepatic fructose metabolism: A highly lipogenic pathway
Increases in FFA can cause insulin insensitivity by escalating intramyocellular lipids (Elliott et al., 2002).

The increased concentration of FFA in the liver increases hepatic glucose production. Fructose consumption, however, does not directly promote insulin secretion from pancreatic cells, which is necessary for glucose metabolism (Teff et al., 2004). Glucose, produced as a result of gluconeogenic precursors from fructose metabolism, stimulates insulin release, but the fructose-induced insulin resistance prevents the insulin from effectively metabolizing glucose. As a result, increased amounts of glucose circulate throughout the body. Insulin resistance can also lead to compensatory hyperinsulinemia where the body attempts to balance the reduced effects of insulin by producing and releasing more insulin (Suga et al., 2000). Insulin also is important for leptin gene expression and leptin secretion (Havel, 2002). Leptin is one of a number of hormones that signals the brain that enough food has been consumed (Rohner and Jeanrenaud, 1996). Plasma leptin levels in fructose-fed rats are increased 2-fold compared to control rats in response to oral glucose loads (Lee et al., 2006) suggesting that leptin resistance may be present in these animals. Triacylglycerols promote leptin resistance by preventing leptin from crossing the blood brain barrier (Banks et al., 2004). Consequences of leptin resistance include an increase in caloric intake due to decreased satiety signals.

**CLINICAL PRESENTATION and MANAGEMENT OF DIABETES**

The clinical presentation of diabetes is heterogeneous and it depends on the type of diabetes and the duration. The clinical presentation of type 1 diabetes is usually acute with classical osmotic symptoms like polydipsia, polyuria, nocturia, weight loss, fatigue with associated symptoms of muscular cramps, blurred vision and persistent fungal or bacterial infections. Approximately, 5-10% of type 1 diabetics present in diabetic keto acidosis featured by marked polyuria, polydipsia , nausea and vomiting, dehydration and reduced level of consciousness and acidic respiration whereas, the presenting clinical features of type 2 diabetes range from none at all to those associated with life threatening hyperglycemia emergency of the hyperosmolar non-ketotic syndrome which includes micro and macrovascular complications.(WHO, 1999).
The management of diabetes aims to maintain blood glucose at normal levels and a key goal of diabetes treatment is to prevent complications of diabetes as long time exposure to hyperglycemia can damage the heart, blood vessels, eyes, kidneys and nerves. Abnormalities in DM are insulin deficiency, insulin resistance and increased hepatic glucose output. With this in mind, therapies used to treat patients with this disease are aimed at correcting one or more of these physiological abnormalities.

**Current approaches to diabetes management**

1. **Therapeutic lifestyle changes**

   Current recommendations of the American Diabetes Association include a trial of diet and exercise as first line therapy for the treatment of NIDDM (Chakrabarti and Rajagopalan, 2002). Caloric restriction leads to decrease in body weight, reduction in total, abdominal subcutaneous and visceral fat (Janssen *et al.*, 2002) and blood pressure (Itoh *et al.*, 2001) associated with reduction in insulin levels, and improvements in the lipid profile (Krotkiewski *et al.*, 1979; Weinstock *et al.*, 1998). Significant improvement in insulin resistance with diet alone (Torjesen *et al.*, 1997), combined diet and exercise regimens and regular physical activity without caloric restriction (Ross *et al.*, 2000) has been reported.

   Regular physical exercise improves insulin sensitivity and glucose tolerance due to upregulation of muscle GLUT-4. Beneficial effects of meditation and yoga have been reported in patients with coronary heart disease (CHD) (Manchanda *et al.*, 2000). However, its role in the management of IRS without CHD is not known. Progression from impaired glucose tolerance to diabetes can also be effectively prevented by lifestyle interventions (Pan *et al.*, 1997).

   The diabetes control and complications study (Okubo *et al.*, 1995) and United Kingdom Prospective Diabetes Study demonstrated that good metabolic control through intensive drug therapy and strict lifestyle management could reduce the risk of developing diabetes complications, but relatively few diabetics have adopted this strict regimen. Some patients with NIDDM are satisfactorily treated with diet alone. Others require a combination of diet and oral hypoglycemic drugs.
2. Pharmacotherapy

Currently, six different classes of hypoglycemic agents are being used—insulin, sulfonylureas, meglitinides, biguanides, alpha-glucosidase inhibitors and thiazolidiones for the treatment of diabetes and insulin resistance associated disorders (Chakrabarti and Ramanujam, 2002).

Insulin

With the introduction of several new insulin preparations since 1996, and more on the way, insulin therapy options for type-1 and type-2 diabetes have expanded. Insulin therapies are now able to more closely mimic physiologic insulin secretion and thus achieve better glycemic control in patients with diabetes. If the desired level of glycemic control cannot be achieved with diet and exercise within three-month period, pharmacological intervention is recommended (American Diabetes Association, 1995). Generally, initiation of therapy in most cases starts with insulin. Insulin therapy affords effective glycemic control, yet its several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment (Piedrola et al., 2001), and in the event of excess dosage ‘fatal hypoglycemia’ limits its usage.

Sulfonylureas

Sulfonylureas have been extensively employed in the treatment of type 2 diabetes since their introduction in the 1950s. This is the choice of drug for type 2 diabetics, having functional β-cells for endogenous insulin production. Sulfonylureas can increase insulin secretion by enhancing pancreatic β-cell responsiveness to glycemic stimuli (Efendic et al., 1979). They attach to β-cell surface receptors (ATP-dependent potassium channels) causing depolarization, calcium influx, and stimulation of insulin release (Aguilar-Bryan et al., 1995). All sulfonylureas have been associated with weight gain and thus may not be optimal first choice for obese patients (Turner et al., 1996).

Drug-induced hypoglycemia is a potential effect of first (chlorpropamide) and second generation sulfonylureas (glyburide, glipizide, glimepiride) (Harrower, 1996). A high mortality rate has been shown when glyburide is used in combination with metformin.
Adverse effects may include skin sensitivity, yellowing of skin or eyes, dark urine, unusual bleeding or bruising, fever, sore throat, jaundice, hematologic complications, hyponatremia and fluid retention. (Krentz and Bailey, 2005).

**Meglitinides**

The carbamolymethyl benzoic acid derivative repaglinide is an insulin secretagogue, and is the first of the meglitinide class. This is structurally different from the traditional Sulfonylureas, but shows chemical resemblance to the non-sulfonylurea moiety of the glibenclamide molecule. Nateglinide, the newest member of the class has recently become available (Pratley et al., 2001).

The meglitinides stimulate the release of insulin from the pancreatic β-cells. However, this action is mediated through a different binding site on the ‘sulfonylurea receptor’ of the β-cells and the drugs have somewhat different characteristics when compared with sulfonylureas (Fuhlendorff et al., 1998). In contrast to glibenclamide, meglitinides do not stimulate calcium dependent exocytosis. Glibenclamide, not meglitinide, can stimulate insulin secretion *in vitro* even in the complete absence of glucose, whereas in presence of 5 or 10 mmol/L of glucose, meglitinides are 5 times more potent than glibenclamide in insulin secretion (Hatorp et al., 1999).

Unlike commonly used sulfonylureas, the meglitinides have a very quick onset of action and a short half-life (Weaver et al., 2001). Repaglinide and nateglinide may cause hypoglycemia as well as headache, nasal congestion, joint aches, back pain, constipation, and diarrhoea.

**Biguanides**

The mode of action and pharmacokinetics of biguanides are distinct from those of sulfonylureas. Biguanides, such as phenformin and metformin, decrease hepatic glucose output through inhibition of gluconeogenesis and to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues (Stumvoll et al., 1995; Bailey and Turner, 1996; Cusi and Defronzo, 1998). They also stimulate weight loss and improve lipid profile (Zhou et al., 2001). Situations, in which metformin therapy should be avoided, include cardiogenic
or septic shock, congestive heart failure, severe liver disease, and pulmonary insufficiency with hypoxemia or severe tissue hypoperfusion (Chakrabarti and Ramanujam, 2002).

Adverse effects of metformin can include hypoglycemia as well as gastrointestinal upset. Malabsorption of vitamin B12 and anemia are less common adverse effects (Dunn and Peters, 1995).

Thiazolidinediones

Thiazolidinedione is an agonist for Peroxisome Proliferator-activated Receptor gamma (PPARγ) (Olefsky, 2000). Activation of this receptor increases the transcription of certain insulin sensitive genes influencing adipocyte differentiation and function. Reduction of insulin resistance is necessary to improve the blood glucose level in type-2 diabetic patients with obesity and insulin resistance (Greene, 1999). A thiazolidinedione-based compound, ciglitazone, was derived from fibrate lipid lowering agents by Takeda and was reported to be a novel oral hypoglycemic agent that potentiated the peripheral actions of insulin. Subsequently, many attempts to synthesize new analogues have been made and the molecular target of thiazolidinediones has been determined by researchers from Glaxo. Interestingly, triacylglycerol-lowering fibrates have been revealed to be PPARα agonists, another isoform of PPAR family (Kliewer et al., 1999).

Troglitazone is the most studied drug among the thiazolidinedione group. However, following reports of severe liver toxicity in patients after taking this drug, the product was withdrawn from the market. Rosiglitazone and pioglitazone are the two thiazolidinedione analogues now in the market. Because these agents do not increase insulin secretion, hypoglycemia does not pose a risk when thiazolidinediones are taken as monotherapy. Drug-induced hypoglycemia may occur when thiazolidinediones are combined with sulfonylureas (Mudaliar and Henry, 2001).

Significant weight gain has been reported with all thiazolidinediones which is a matter of concern as most of the type-2 diabetic patients are already obese. The thiazolidinediones are relatively safe in patients with impaired renal function, but caution should be used in patients with hepatic dysfunction (Krentz and Bailey, 2005). The manufacturers recommend these agents not to be prescribed for patients with serum
transaminase levels that exceed 2.5 times the upper limit of normal. Adverse effects of these medications include risk of fracture and may also include fluid retention and peripheral oedema as well as upper respiratory tract infection, sinusitis, and muscle or tooth pain.

**Alpha-glucosidase inhibitors**

Alpha-glucosidase inhibitors act by inhibiting the enzyme alpha-glucosidase found in the brush border cells that line the small intestine, which cleaves more complex carbohydrates into sugars. Because these drugs inhibit the breakdown and subsequent absorption of carbohydrates from the gut following meals, the largest impact of these drugs is on postprandial hyperglycemia (Rodger *et al.*, 1995). Acarbose and miglitol are the two agents available in the market in this class.

Since the action of this group of drug is reduction of glucose absorption in the small intestine, increased carbohydrate is delivered to the large bowel. Fermentation produces, abdominal discomfort, bloating, flatulence and diarrhoea but these symptoms are reversible with the discontinuation of the drug. Therapy with acarbose has been linked to elevations in serum transaminase levels and use of this agent is contra indicated in patients with liver cirrhosis (Champbell *et al.*, 1996).

**NEED FOR ALTERNATIVE MEDICINE**

Diabetes mellitus is one of the refractory diseases identified by Indian Council of Medical Research (ICMR) for which an alternative medicine is a need.

Different types of oral hypoglycemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents has been successful in maintaining euglycemia and controlling long-term microvascular and macrovascular complications. Though, insulin therapy is used for management of diabetes mellitus, there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment (Piedrola *et al.*, 2001). Further problems with conventional therapy in developing countries include insulin supply, storage, injection, dietary control and complications from malnutrition, lack of trained health care workers and
lack of education for the patients (Gill, 1988). In such situations, the incidence of diabetes-related mortality is far greater than in well served urban areas.

Alternative strategies to the current modern pharmacotherapies of DM are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorders, as well as the enormous cost and poor availability of modern therapies for many rural populations in developing countries. Furthermore, arguments are being forwarded not to isolate a single drug to target the reversal of major aspects of the disease (Bailey, 2000), since biological systems are too complex to be fully understood through conventional and isolated experimentations as they are not always linear. In addition to this, there are many other factors, which are not obvious from biological considerations alone. Therefore, therapeutic approach of traditional medicines is more holistic.

The growing interest in herbal remedies can be accounted for their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown (Valiathan, 1998). In many developing countries, traditional medicine, in particular, the herbal medicine is sometimes the only affordable source of health care (Hamdan, 2004). Even the WHO approves the use of plant drugs for different diseases including diabetes mellitus (WHO, 2002).

The multi factorial pathogenicity of diabetes demands multi model therapeutic approach. Thus, further therapeutic strategies require the combination of various types of multiple agents. The power of self-preservation or adjustment has been the motto of traditional medicinal practice, which prescribes polyherbal formulations. The polyherbal formulations have the synergistic, potentiative agonistic/antagonistic pharmacological agents within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. The multiple activities of plant based medicinal preparations meant for diabetes offer enormous scope for combating the threat of the diabetic epidemic. Surveys conducted in Australia and US indicate that almost 48.5 and 34.0% respondents had used at least one form of
unconventional therapy including herbal medicine (Eisenberg et al., 1993; Maclennan et al., 1996). In developed countries, the use of herbal medicines is encouraged to eliminate the adverse effects of chemical drugs. Treatment using medicines of natural origin appears to offer gentle means of managing chronic diseases like diabetes (Klepser and Klepser, 1999; WHO, 2002). In many developing countries, traditional medicine, in particular, the herbal medicine is sometimes the only affordable sources of healthcare.

With the increasing incidence of diabetes in rural population throughout the world, the inability of current therapies to control all the metabolic defects of the disease and their pathological consequences, and the great expense of modern therapy, there is a clear and urgent need for the development of alternative strategies for diabetes therapy.

**Herbs in the treatment of diabetes**

Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on dietary measures, which included the use of traditional plant therapies. However, after the advent of insulin and other hypoglycemic drugs (synthetic) this field of work largely remained unexplored. The yawning gap for additional agents to combat hyperglycemia and its accompanying complications presents an opening to revisit traditional anti-diabetic plants (Gray and Flatt, 1997). World Health Organization (1980) has also recommended the evaluation of the plants effective in conditions where we lack safe modern drugs. Biguanides developed from prototypic plant molecule is an excellent example of anti-diabetic drug development from plants. Thus, it is prudent in the current context to look for new and if possible more efficacious hits from the vast reserves of phytotherapy.

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meagre. Many studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. Moreover, during the past few years some of the bioactive drugs isolated from hypoglycemic plants showed anti-diabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy (Bnouham et al., 2006).
Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential, which can play a major role in protection against molecular damage induced by ROS (Cao et al., 1997; Vaya et al., 1997). Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of the medicinal plant treatments for diabetes received scientific scrutiny for which WHO has also recommended attention (WHO, 1980).

**Herbal hypoglycemic constituents and mechanism of action**

World wide over 1200 species of plants have been recorded as traditional medicine for diabetes (Marles and Farnsworth, 1995) from which only a small number have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of Type-2 diabetes.

The study of traditional remedies for diabetes mellitus yields an excellent return in potential for new sources of anti diabetic drugs. There are more than 200 pure compounds from plant sources reported to show blood glucose lowering activity. The wide variety of chemical classes indicates that a variety of mechanisms must be involved in the lowering of the blood glucose level. Some of these compounds may have therapeutic potential, while others may produce hypoglycemia as a side effect of their toxicity especially hepatotoxicity.

Numerous mechanisms of actions have been proposed for these plant extracts. Some hypotheses relate to their effects on the activity of pancreatic β-cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. Other mechanisms may involve improved glucose homeostasis by increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis, by decreasing gluconeogenesis, inhibition of intestinal glucose absorption, and reduction of glycemic index of carbohydrates, as antioxidant defence, as aldose reductase inhibitors, as modulators of intracellular second messengers and as adrenergic effectors.
Plant hypoglycemics stimulating β-cells or insulinomimetic activity

In recent years there have been several comprehensive reviews covering plant hypoglycemics. Barbarine, an alkaloid from the leaves of *Zizyphus jujuba*, stimulated the β-cells of pancreas (Aydin *et al*., 1995). Water soluble alcoholic extracts of *Gymnema sylvestre* leaves potentiate insulin release from pancreatic β-cells (Chakravarty *et al*., 1996) Ganoderan B, a glycan from *Ganoderma lucidum* (Hikino *et al*., 1989), the active principle as (-) epicatechin and flavonoids from the bark of the tree *Pterocarpus marsupium* were shown to posses preventive as well as restorative properties of β-cells (Chakravarthy *et al*., 1980). The active constituent of *Momordica charantia* i.e., polypeptide-P (Plant insulin) was identified as insulinomimetic (Baldwa *et al*., 1977; Khanna *et al*., 1981).

Plant hypoglycemics modulating the carbohydrate metabolism

Quinoline derivatives inhibit hepatic gluconeogenesis from lactate and alanine. Hypoglycin from *Blighia sapida* stimulates hepatic glycolysis. (Feng and Patrick, 1958). Galegine, a guanidine from *Galega officinalis*, blocks succinic dehydrogenase and cytochrome oxidase and thus increasing anaerobic glycolysis and decreasing gluconeogenesis resulting in enhanced glucose uptake and hypoglycemia (Oliver-Bever and Zahnd, 1979). Charantin, a steroid glycoside from *Momordica charantia*, showed enhancement of glucose uptake in muscle tissue and of glycogen accumulation in muscle and hepatic tissue but with no effect on glucose uptake (Marles and Fransworth, 1995).

Plant hypoglycemics inhibiting intestinal glucose absorption

Seeds of *Trigonella foenum* have insulinomimetic activity or inhibition of intestinal glucosidase (Petit *et al*., 1993). Tea polyphenolics, apart from their much-cited antioxidant activities, also have been reported to inhibit α-amylase and sucrase, and have been shown to be the principal substance for suppressing postprandial hyperglycemia (Hara and Honda 1990; Matsumato *et al*., 1993; Valsa *et al*., 1997). Furthermore, these polyphenolics also inhibit glucose transport across the intestine by inhibiting sodium-glucose co-transporter-1 (S-GLUT-1) (Kobayashi *et al*., 2000a). Catechin (+), epicatechin (-), epigallocatechin (-) and epicatechin gallate (Kobayashi *et al*., 2000b), isoflavones from soybeans (Vadavanam *et al*., 1999), polyphenolics compounds, tannic acid, chlorogenic acid (Welsh *et al*., 1989), crude
saponins fractions from *Gymnema sylvestre* (Murakami *et al*., 1996; Yoshikawa *et al*., 1997) and other saponins from several plant extracts (Yoshikawa *et al*., 1996; Yoshikawa *et al*., 1997) have been shown to possess potent inhibitor of Na\(^+\)-GLUT-1 mediated transporter of glucose and antihyperglycemic activity. The water soluble dietary fibres of guar, gum, pectin (Johnson and Gee, 1981), polysaccharides (Yuan *et al*., 1998), saponins (Matsuda *et al*., 1998) have been reported to increase the viscosity of gastrointestinal content thereby decreasing the gastric emptying rate and suppressing delaying the digestion and absorption of carbohydrates. The manipulation of Na\(^+\)-GLUT-1 mediated transport along with α-amylase and α-glucosidase inhibitory activity by plant phenolics make them very exciting candidates in the control and management of hyperglycemia.

**Plant hypoglycemics acting as antioxidants**

Free oxygen radicals are important mediators of β-cell destructors in IDDM. Nicotinamides antioxidant activity has some effect in preventing IDDM. Trigonelline, from *Trigonella foenum*, an inhibitor of the enzyme poly ADP-ribose synthetase, causes depletion of NAD\(^+\) from pancreatic β-cells and is also a potent hydroxyl-radical scavenger. Nicotinamide can prevent the β-cell toxicity of streptozotocin (STZ) and alloxan (Ledoux *et al*., 1988). Several phytochemicals were reported to act against the deleterious effects of oxidative stress such as anthraquinones of aloe vegetable (Malterud *et al*., 1993), saponins from *Pinax ginseng* (Huong *et al*., 1998), polyphenols (Tiwari, 2001) and flavonoids from *Sideritis raeseri* (Gabrieli *et al*., 2005). The active tannoid principle isolated from *Emblica officinalis* has antioxidant activity (Bhattacharya *et al*., 1999).

**Plant hypoglycemics acting by modulating intracellular second messengers**

The most famous plant product for the stimulation of intracellular cAMP is forskolin, a diterpene from *Coleus forskohlii*. It is an adenylate cyclase activator which increases intracellular cAMP by stimulating its biosynthesis. Theophylline and other methyl xanthenes from *Camellia sinensis* and *Illyz guayusa* and papaverine from *Papaver somniferum* are phosphodiesterase inhibitors which increase intracellular cAMP by preventing its breakdown (Gearien and Mede, 1981; Hill *et al*., 1987; Zawalich, 1988). Theophylline is orally hypoglycemic when administered chronically to normal rats, but this *in vivo* effect was not attributed to its phosphodiesterase inhibition, but rather to its
induction of intracellular $\text{Ca}^{2+}$ efflux. Increased extracellular $\text{Ca}^{2+}$ might enhance calcium-stimulated ATPases, which would result in decreased cellular ATP levels, enhanced lipolysis, and reduced glycogenolysis. This effect is also seen with administration of caffeine (Tobin et al., 1976).

**Plant hypoglycemics acting by adrenergic effects**

Ergot alkaloids, occurring in fungi such as *Claviceps purpurea*, and at least one group of higher plants, *Rivea corymbosa* and closely related *Ipomoea* and *Argyreita* species are $\alpha$-adrenergic blockers which inhibit epinephrine induced hepatic glycogenolysis and hyperglycemia, but not glycogenolysis in skeletal muscle. Dihydroergotamine and yohimbine, another $\alpha$-adrenergic blocking alkaloid from *Pausinystalia yohimbe*, and *Pierre* prevented epinephrine-induced inhibition of insulin release (Henquin et al., 1982).

Multiple defects in the pathophysiology of diabetes are mostly understood imprecisely, and therefore warrant not isolating a single drug target to the reversal of all or majority of aspects of the disease (Bailey, 2000), as biological systems are too complex to be fully understood through conventional experimentation and also because they are nonlinear. Therefore, the unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem.

The concept of synergy is central to the holistic approach. The trend of the modern concept to isolate pure compounds may not achieve the desired results as observed in the natural version. Once an active principle is isolated from the natural product without its synergical colleagues to support and/or balance its action, it may lose its character as present in its natural form. However, the natural/holistic approach attempts to solve problems by taking these in their entirety, with all their inter linkages and their complexity. This may be the reason why Ayurvedic preparations have different permutations according to the disease conditions.

Synergistic interactions are documented for constituents within a total extract of a single herb, as well as between different herbs in a formulation. Many of the most effective phytomedicines are on the drug market as whole extracts of plants, and practitioners always believe that synergistic interactions between the components of individual or mixtures of herbs are a vital part of their therapeutic efficacy. Preparation of traditional medicines
contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms.

The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β-cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer an exciting opportunity to develop them into novel therapeutics.

**Moringa oleifera**

*Moringa oleifera* Lam (syn Pterigosperma Geartn) (Fig 5a and b) belongs to the monogeneric family Moringaceae and it is one of the best known, most widely distributed and naturalized species (Nadkarni, 1976). It is popularly known as drumstick or horseradish in English, Munaga in Telugu, Shobhanjana in Sanskrit, Murungai in Tamil, Soanjna in Hindi, Sajna in Bengali, and Nugge in Kannada. *Moringa oleifera* is a native of western and sub Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia (Mughal et al., 1999) and is now distributed in Philippines, Cambodia, Central America, North and South America (Morton 1991). The tree ranges from a height of 5-10 m. The relative ease with which it propagates through both sexual and vegetative means and its low demand for soil nutrients and water, makes its production and management easy. *Moringa oleifera* was well known to the ancient world, but only recently has it been ‘rediscovered’ as a multipurpose tree with a tremendous variety of potential uses. *Moringa* trees have been used to combat malnutrition especially among infants and nursing mothers. Three non-governmental organizations in particular- Trees for Life, Church World Service and Educational Concerns for Hunger Organizations have advocated *Moringa* as a natural nutrition for the tropics.

Fuglie Lowell (1999 and 2000) reported and has extensively documented as video, throughout West Africa, countless instances of life saving nutritional rescue which are attributed to *Moringa*. It is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. Many parts of this plant i.e., leaves, immature pods, flowers and fruits are edible and are used as a highly nutritive vegetable in many countries (Anwar and Banger 2003). *Moringa* leaves have been reported to be a rich source of β-carotene, protein, Vitamin C, calcium and potassium and act as a good source of natural antioxidant due to the presence of
ascorbic acid, flavonoids, phenolics and carotenoids. In Philippines, the *Moringa oleifera* tree is considered as ‘mother’s best friend’ because the utilization of leaves increase women’s milk production and is sometimes prescribed for anemia (Siddhuraju and Becker, 2003). The leaves are also used as fodder with a high nutritive value. Besides *M. oleifera* is a highly valued plant to treat a variety of ailments.

**Phytochemistry**

*Moringa oleifera* is rich in compounds containing the simple sugar, rhamnose and a fairly unique group of compounds called glucosinolates and isothiocyanates (Fahay *et al.*, 2001; Bennett *et al.*, 2003). The stem bark has been reported to contain two alkaloids i.e., Moringine and Moringinine (Karharo, 1969). Purified whole gum exudate from *M. oleifera* has been found to contain L-arabinose, galactose, glucuronic acid. Flowers reported to contain some flavanoid pigments such as alkaloids, kaempherol, isoquercitin (Faizi *et al.*, 1994a; Siddhuraju and Becker, 2003). Antihypertensive compounds, thiocarbamate and isothiocyanate glycosides have been isolated from *M. oleifera* pods (Faizi *et al.*, 1998). The leaves of *M. oleifera* are a rich source of natural antioxidants due to the presence of various types of antioxidants like ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al.*, 2005; Makker and Becker, 1996).

The high concentration of ascorbic acid, β-carotene, β-cytosterol, calcium, iron, essential amino acids like methionine, tryptophan, lysine, vitamins A, B, C, α-tocopherol, nicotinic acid, folic acid, makes the leaves and pods of *M. oleifera* a virtually ideal dietary supplement (Makker and Becker, 1996). The PIXE analysis of aqueous extract of *M. oleifera* leaves revealed the presence of appreciable quantities of Ca, Zn, K, Mn, Na, Sr (Gowrishanker *et al.*, 2010). Recently interest has been generated in isolating hormones, growth promoters from the leaves of *M. oleifera*.

**Medicinal Uses**

*Moringa oleifera* has numerous medicinal uses which have long been recognized in Ayurvedic and Unani systems of medicines (Mughal *et al.*, 1999). *M. oleifera* contains nitrile mustard oil glycosides and thiocarbamate glycosides which are antihypertensive (Faizi *et al.*, 1994a) and are very rare in nature (Faizi *et al.*, 1995). Bioassay guided fractionation of ethanol extract of *M. oleifera* leaves led to the isolation of pure compounds
(Niazinin, Niazimicin and Niazimin A+B) which are found to control blood pressure (Gilani et al., 1994a). *Moringa* roots, leaves, gum and seeds have been found to possess diuretic activity (Caceres et al., 1992). *M. oleifera* leaves were found to contain lipid lowering activity in the serum of high fat diet fed rats which may be attributed to the presence β-sitosterol (Ghasi et al., 2000). The seeds (Caceres et al., 1992) and the leaves (Gilani et al., 1992) of *M. oleifera* have been reported to have antispasmodic activity. The hepatoprotective activity of *M. oleifera* leaves (Fakurazi, 2008), roots (Ruckmani et al., 1998) has been reported which may be due to the presence of quercitin. *M. oleifera* leaves were found to possess strong antioxidants and radical scavenging activities and the leaves were found to preserve and enhance the process of spermatogenesis in mice (Lilibeth et al., 2010). The aqueous extract of leaves of *M. oleifera* has shown to lower the blood sugar (Ndong, 2009; Jaiswal, 2009).

The roots of *M. oleifera* are reported to be rich in antimicrobial agents and are reported to contain a powerful antibacterial and fungicidal, Pterigospermin (Kurup et al., 1954). *M. oleifera* root (Eilert et al., 1981), Flowers (Das et al., 1957) and bark (Bhatnagar et al., 1961) have been found to possess anti bacterial and antifungal activity. The fresh leaf juice was found to inhibit the growth of microorganisms, *staphylococcus aureus* and *Pseudomonas aeruginosa*, which are pathogenic to humans (Caceres et al., 1991). Niazimicin has been proposed to be a potent chemoprotective agent in chemical carcinogenesis (Guevara et al., 1999) and Niaziminic (9+10), a thiocarbamate from the leaves of *M. oleifera*, exhibits inhibition of tumor promoter induced Epstein-Barr virus activation (Muranakami et al., 1998). Aqueous leaf extract of *M. oleifera* was reported to be useful in treating hyperthyroidism (Thahiliani and Kar, 2000). Besides, the leaves showed significant radiation protection to the bone marrow chromosomes in mice (Rao et al., 2001). The flowers and leaves are considered to be of high medicinal value with antihelminthic activity (Bhattacharya et al., 1982).

As a result of scientific evidence, *M. oleifera* is coming to the forefront as an important source of naturally occurring phytochemicals. Different parts of *M. oleifera* are incorporated in various marketed health formulations such as Rumalaya and Septilin (the Himalaya Drug Company, Banglore, India), Orthoherb (Walter Bushnell Ltd, Mumbai, India), Kupid Forte (Pharma Products Pvt Ltd, Thayavur. India), and Livospin (Herbs APS
Pvt Ltd, Patna, India) which are reputed as remedies available for a variety of human health disorders. *Moringa* seeds have specific protein fractions for skin and hair care. Purisoft, a cosmetic product, consists of peptides of *Moringa* seeds which protects the human skin from environmental influences and *M. oleifera* seed extract is a globally acceptable innovative solution for hair care (Stussi et al., 2002)

In addition to the pharmacological properties, powder from seed kernels works as a natural anticoagulant. *Moringa* seeds are one of the best natural coagulants discovered so far (Ndabigengesere and Narasiah, 1998) which can reduce turbidity of water up to 99%. (Muyubi and Evison, 1995b). The pleasant tasting edible oil from the seeds was highly valued by the ancient Roman, Greek and Egyptian civilizations for use in making perfume and in protecting skin.

**AIM AND SCOPE**

Earlier studies on antihyperglycemic and antihyperlipidemic activity of *M. oleifera* are fragmentary and no studies are available on the efficacy of *M. oleifera* in preventing IR. However, very little information is available on antioxidant activity of *M. oleifera* in both insulin resistant and insulin deficient conditions. So the present study was undertaken to explore possible beneficial effects of aqueous extract of *M. oleifera* leaf (AEMO) in prevention of IR and diabetes. Further, the biochemical basis for its protection against IR and anti diabetic property were investigated.

In the present systematic study, the following aspects were undertaken to investigate the efficacy of AEMO treatment on fructose fed IR and STZ induced type-1 diabetic animal models.

1. The body weight, plasma glucose, insulin and lipid profile were measured at 15 day intervals during the experimental period. Homeostasis Model Assessment (HOMA), used as an index to measure the degree of IR, was calculated using the formula: \[\text{HOMA} = \frac{\text{Insulin (}\mu\text{U/ml}) \times \text{Glucose (mmol/L))}}{22.5}\].
2. At the end of the experimental period (60 days), 12 h fasted animals were subjected to oral glucose tolerance test. In six experimental groups, the plasma glucose levels were measured at 0, 30, 60 and 120- min intervals after glucose load. Except in STZ diabetic group (D and D+MO), plasma insulin was assayed in
remaining four groups (C, C+MO, F and F+MO) at 0, 30, 60 and 120 min intervals. Action of insulin on glucose disposal was measured using glucose insulin index, which is the product of the areas under the curve of glucose and insulin during OGTT.

3. To assess the tissue damage under IR and type 1 diabetic conditions and to assess the toxicity/protective effect of AEMO administration, the activities of hepatic and renal transaminases (GPT and GOT) and plasma transaminases, urea and creatinine were measured.

4. Immediately after sacrifice of experimental animals by cervical dislocation individual organ weights (liver, kidney, pancreas, heart and testes) were recorded and the relative organ weights were calculated.

5. In order to assess the alterations in peripheral utilization of glucose and glucose metabolism under IR and insulin deficient conditions and to evaluate the efficacy of AEMO administration on these changes, glycogen content of liver and muscle and activities of key enzymes of carbohydrate metabolism were assessed. The activities of glycolytic enzymes; Hexokinase (HK), Phosphofructokinase (PFK) and Pyruvate kinase (PK) in liver and muscle, gluconeogenic enzymes: Glucose 6 phosphatase (G6Pase) and Fructose 1, 6 bisphosphatase (F1,6BPase) in liver and kidney, glycogenolytic enzymes: Glycogen phosphorylase in liver, HMP pathway enzyme: Glucose 6 phosphate dehydrogenase(G6PDH) in liver and fructose metabolic enzyme: Fructokinase (FK) in liver were measured.

6. The activities of intestinal disaccharidases (maltase, sucrase and lactase) were assayed in all experimental rats.

7. To find out the alterations in lipid metabolism, hepatic and cardiac tissue lipids (total cholesterol, phospho lipids, triacylglycerols and free fatty acids ) and the activities of lipid metabolic enzymes: Fatty acid synthase (FAS) and Malic enzyme (ME) in liver and Lipoprotein lipase (LPL) in adipose tissue were assayed.

8. To assess the oxidative stress in IR and type 1 diabetic conditions and to evaluate the protective effect of AEMO, extent of lipid peroxidation was measured in liver, pancreas, heart and testes. Alterations in antioxidant status in liver, pancreas, heart, kidney and testes were studied by measuring GSH levels and by assaying
the activities of GSH dependent (glutathione reductase, glutathione S transferase, glutathione peroxidase) and independent (superoxide dismutase and catalase) antioxidant enzymes.

9. To understand the contribution of polyol pathway to the oxidative stress in IR and insulin deficient conditions, sorbitol dehydrogenase activity was measured in liver, pancreas, kidney, heart and testes.

10. Histological examinations of liver, pancreas, kidney and testes were done to find out the pathological changes if any, due to IR and type 1 diabetic conditions and to assess the protective /toxic effect of AEMO on the microscopic structure of the tissues.

11. Preliminary qualitative phytochemical analysis of aqueous extract of *M. oleifera* leaf was performed by following standard methods. Trace elements in the aqueous extract of *M. oleifera* leaf were estimated using PIXE technique.