Introduction
INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder characterized by chronic hyperglycemia and disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion/insulin action. DM is one of the world's oldest known diseases. In 1997, diabetes prevalence was introduced as a “basic health indicator” for member states by the World Health Organization (WHO). DM affect nearly 10% population all over the world (Burke et al., 2004). According to WHO report, the number of diabetics was 171 million in 2000 which may increase to 360 million in the year 2030 (WHO, 2000). As the number of people with DM multiply worldwide, the disease has been taking an ever increasing proportion of national and international health care budget. 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 one of 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 is thought to result from autoimmune destruction of the pancreatic \( \beta \)-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 \( \beta \)-cells are present at the time of diagnosis in 90% of individuals and include antibodies to the islet cells, to glutamic acid decarboxylase and to insulin (Zimmet et al., 1994). This form of diabetes usually
is seen in children and adolescents but it can occur at any age. Type-1 DM was formerly known as juvenile-onset diabetes and ketosis-prone diabetes and more recently it is called as insulin dependent diabetes mellitus (IDDM). 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 is characterized by IR and, at least initially, a relative deficiency of insulin secretion (Kadowaki et al., 1984; Taylor et al., 1994). In absolute terms plasma insulin concentration (both fasting and meal stimulated) usually is increased, although relative to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. IR is a common denominator of many diseases in western societies and it is a central component in the so called metabolic syndrome X and the insulin resistance syndrome (Nadig and Kotchen, 1997).

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). 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). It is reported that, the prevalence of diagnosed type-2 diabetes rose nearly by 7.6 fold from 1935 to 1996. The global figures are predicted to rise by 46 % from 150 million cases in 2000 to 221 million by 2010 (Zimmet et al., 2001). Reasons for
this rise include changes in human behavior, 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).

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 rise 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 the intestine are transported through portal circulation to the liver. Galactose and fructose are readily converted to glucose in the liver. The 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). Between meals, the 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 CO₂. 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 transporter), 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 the 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 β-cells and glucagon secretion by α-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 gluoregulatory hormones of the body are designed to maintain circulating glucose concentration within a relatively narrow range.

Insulin is produced by the β-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 decreased by dephosphorylation of glycogen synthase and glycogen phosphorylase respectively. Glycolysis is stimulated and gluconeogenesis 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 Krebs 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 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 one 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 is 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 hypoglycaemia. Other counter-regulatory 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 to be a disturbance in the metabolism of carbohydrates accompanied by alteration in the metabolism of fats and proteins. 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 raised in direct proportion to hepatic glucose output (Bogardus et al., 1984; Reverse et al., 1984; Defronzo 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 (Defronzo et al., 1982). As fasting plasma insulin and C-peptide concentrations are normal in NIDDM, the disturbances to glucose homeostasis appears 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, since 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 sorbitol. Sorbitol doesn’t readily diffuse across the cell membrane and tends to accumulate in
Fig 1: Metabolic disturbances under diabetic conditions (Harper, 2000)
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 center 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 triacyl glycerol (TAG) from adipose tissue as evidenced by high free fatty acid levels in plasma. The acetyl-CoA cannot be efficiently oxidized by Krebs 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 devasting 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). Initially β-cells compensate for insulin resistance by increasing insulin secretion and hyperinsulinemia develops. However, as time goes by the β-cell function is altered and fails to compensate for increase in insulin resistance and,
thus, blood sugar levels start to rise (Purrello and Rabuazzo, 2000) and eventually clinical diabetes is established.

Insulin resistance can be 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.

In the western world more than 80 % of patients with NIDDM are obese. Although genes are an important factor in many cases of obesity, a person's environment also plays a significant part (Manson et al., 1992; Tomás et al., 2002). In muscle insulin-stimulated transmembrane glucose uptake appears to be the major rate-limiting defect (Yki-Jarvinen, 1998). In adipose tissue insulin resistance is manifested as impaired glucose uptake and utilization but in many cases also as an impaired suppression of lipolysis and release of FFA (Boden, 1997; Golay et al., 1988) and, in addition, it can also lead to dysregulated production and secretion of adipokines and other adipose-derived biomolecules (Steppan et al., 2001; Yamauchi et al., 2001). In the 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 capacity for insulin secretion.
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. The inherited defects responsible for insulin resistance are largely unidentified. Common polymorphism in candidate genes that could potentially modulate insulin sensitivity, e.g. β-adrenergic receptors, PPARγ (peroxisome proliferator activated receptor γ), IRS-1 (insulin receptor substrate-1) and glycogen synthase, appear to be associated with human insulin resistance and type-2 diabetes (Groop, 2000). However, the quantitative importance of such polymorphism for an individual's risk to develop type-2 diabetes is limited. 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 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. In mice, various degree of insulin resistance can be created depending on the specific knockout protein and its role in the insulin-signaling cascade.

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. There are several pathways that may contribute to the development of insulin resistance and type-2 diabetes. Such pathways include 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 \textit{in vivo} and \textit{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 remain incompletely understood.

Elevated FFAs might promote accumulation of fat depots in muscle, liver and/or \( \beta \)-cells, and the accumulated triglycerides 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 triglyceride 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 all 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 arc
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).

It is generally accepted that a complex interplay between genetic and acquired factors is key in 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, but also for β-cell dysfunction and macro-and microvascular damage in diabetes (Eriksson, 2007).

HYPERGLYCEMIA INDUCED OXIDATIVE STRESS AND VASCULAR COMPLICATIONS

Oxidative stress

Oxidative stress results when the rate of oxidant production exceeds the rate of oxidant scavenging. Reactive oxygen species (ROS) can attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids. To a lesser extent carbohydrates are also the targets of ROS. 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 radicals (mainly hydroxyl radical) attack on DNA. The resulting cytotoxicity, mutations and potential for malignant change occur as a result of induced chromosomal aberrations ultimately resulting in cell death (Sinclair et al., 1991).

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 Shulman, 2002; Itani et al., 2002; Evans et al., 2003). Glucose autoxidation and non-enzymatic protein glycation may be the source of ROS that can initiate oxidative tissue damage in diabetes. 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 ($\alpha$-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 flurophore (protein browning), and the fragmentation of protein. Thus, the term autooxidative glycation was introduced to describe the process of oxidative modification of protein by high glucose concentration (Brownlee, 1996).
Introduction

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).
In addition, glycated proteins 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 $\mathrm{O}_2^*$, the release of erythronic acid and the products of oxidative cleavage of the glycated protein-carboxymethyllysine, carboxymethylhydroxylysine, and pentosidine. These compounds were termed glycoxidation products and 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 $\kappa$B (NF-$\kappa$B), 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, 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

Diabetes is a disease of complications. Sometimes a complication of disease 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).

Extensive clinical studies have shown that long-term glycemic control is an important predictor of 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). Increased oxidative stress lead to the activation of stress sensitive intracellular damage and contribute to the late complications of diabetes.

INDUCTION OF EXPERIMENTAL TYPE-1 DIABETES

Experimental DM has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. However, to induce a notable form of diabetes, at least 90-95% of the pancreas has to be removed. Otherwise, the Langerhans islets 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 (Rikieten 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. The dosage is difficult to judge to create stable NIDDM without either gradual recovery or deterioration into IDDM. Using 55 mg/kg body weight STZ dose results 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).

STZ enters the β-cell via a glucose transporter (GLUT2) 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
Fig 3: The mechanism of streptozotocin-induced toxic events in β-cells of rat pancreas (Szkudelski, 2001).
DNA damage. As a result of the STZ action, β-cells undergo the destruction by necrosis (Weiss, 1982; Szkudelski, 2001).

**INDUCTION OF INSULIN RESISTANCE BY 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, hypertriglyceridaemia and hypertension and obesity in rodents (Tobey et al., 1982; Hwang et al., 1987; Thorburn et al., 1989). The fructose-fed rat is therefore used as an animal model of IR and is considered to parallel 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 into 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 consequence is the provision of increased substrate to all metabolic pathways leading from triose phosphate. These 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 triglyceridemia (Mayes, 1993).

Another product of fructose metabolism, acetyl-CoA, is a precursor for FFA (Havel, 2005). This increase in FFA in the liver also results in the elevation of blood levels of TGs and
Figure 4: Hepatic fructose metabolism: A highly lipogenic pathway. Fructose is readily absorbed from the diet and rapidly metabolized principally in the liver. Fructose can provide carbon atoms for both the glycerol and the acyl portions of triglyceride. Fructose is thus a highly efficient inducer of de novo lipogenesis. High concentrations of fructose can serve as a relatively unregulated source of acetyl CoA. In contrast to glucose, dietary fructose does NOT stimulate insulin or leptin (which are both important regulators of energy intake and body adiposity). Stimulated triglyceride synthesis is likely to lead to hepatic accumulation of triglyceride, which has been shown to reduce hepatic insulin sensitivity, as well as increased formation of VLDL. (Basciano et al., 2005)
FFA (Bantle et al., 2000). Circulating FFA stimulates insulin release (Crespin et al., 1969). Insulin, in turn, perpetuates the buildup 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, glycosen syntahase, pyruvate dehydrogenase, Krebs cycle) (Tippett and Neet 1982; Dresner et al., 1999; Thompson and Cooney, 2000; Kruszynska et al., 2002).

Increases in FFA can cause insulin insensitivity by escalating intramyocellular lipids (Elliott et al., 2002). These include impaired glucose tolerance. Dietary fructose metabolism leads to the production of glucose. In addition, 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. Triglycerides 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.

MANAGEMENT OF DIABETES

Abnormalities in DM are insulin deficiency, insulin resistance and increased hepatic glucose output. With this in mind, therapies used to treat patients with disease are aimed at correcting one or more of these physiological abnormalities. The aim of therapy is to maintain blood glucose at normal levels. A key goal of diabetes treatment is to prevent complications because, overtime, diabetes can damage the heart, blood vessels, eyes, kidneys and nerves.

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 Ramanujam, 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).

Although 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, relatively few diabetes patients 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 insulins 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 remained the mainstay of antidibetic therapy for almost three decades. 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). Thus, these drugs could be used only in patients with type-2 DM having functional β-cells for endogenous insulin production. 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**

Repaglinide is an insulin secretagogue, the first of the meglitinide class. It is a member of the carbamoyl methyl benzoic acid family (glinides), which 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**

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**

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 thiazolidinidiones has been determined by researchers from Glaxo. Thiazolidinedione is an agonist for PPAR γ (Olefsky, 2000). This is an orphan member of nuclear hormone superfamily that mediates adipocyte differentiation and modulates insulin sensitivity through regulation of gene expression. Interestingly, triglyceride-lowering fibrates have been revealed to be PPARα agonists, another isoform of PPAR family (Kliewer et al., 1999).

Among the thiazolidinedione compounds, troglitazone of Sankyo was first to be approved in Japan and USA. But, following reports of severe liver toxicity in patients 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 edema 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.

The most bothersome side effects observed with these agents are gastrointestinal including abdominal discomfort, bloating, flatulence and diarrhoea but are reversible with discontinuation. 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

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 also used for management of diabetes mellitus but 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 also 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. Also, there are several factors that are not obvious from biological considerations alone. Therefore, therapeutic approach of several traditional medicines is rather more holistic.

There is growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely; even 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 multifactorial pathogenicity of diabetes demands multimodel 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 % respondents had used at least one form of unconventional therapy including herbal medicine (Eisenberg et al., 1993; Maclennan et al., 1996).
Therefore, it has become necessary to look for an economical as well as therapeutically effective treatment especially for usage in the developing and under-developed countries. In many developing countries, traditional medicine, in particular, the herbal medicine is sometimes the only affordable sources of healthcare. As for the developed countries, the use of herbal medicine by the sufferers of chronic diseases is encouraged by the concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer gentle means of managing such diseases (Klepser and Klepser, 1999; WHO, 2002).

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. But 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 antidiabetic plants (Gray and Flatt, 1997).

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. 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 hypoglycaemic plants showed antidiabetic 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 in traditional medicine for diabetes (Marles and Farnsworth, 1995) from which only a small number of these 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 antidiabetic 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 defense, as aldose reductase inhibitors, as modulators of intracellular second messengers and as adrenergic effects.

**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 flavanoids 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* stimulate 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 principle 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—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 *Ilex 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 Ca²⁺ efflux. Increased extracellular Ca²⁺ 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 α-adrenergic blockers which inhibit epinephrine induced hepatic glycogenolysis and hyperglycemia, but not glycogenolysis in skeletal muscle. Dihydroergotamine and yohimbine, another α-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 non-linear. 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 interlinkages 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.

The medicinal preparations in 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.

*Catharanthus roseus*

*Catharanthus roseus* belongs to the family Apocyanaceae. It is commonly known as Medagaskar periwinkle, Vinca rosea or Lanchnera rosea worldwide, in Sanskrit Nityakalyani and Sadapushpi, in Telugu Billaganneru, in Hindi Sadaphul. It is a native of Madagascar and abundantly naturalized in many regions, particularly in arid coastal locations, and grown commercially for its medicinal uses in India, Australia, Africa, and Southern Europe and cultivated as an ornamental plant almost throughout the tropical and subtropical world.

It is an evergreen subshrub or herbaceous plant growing to 90 cm tall. The leaves are oval to oblong, 2.5-9 cm long and 1-3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole and flowers are white to dark pink. The fruit is a pair of follicles 2-4 cm long and 3 mm broad. Recently, two white flowered varieties named Nirmal and Dhawal have been released by the CIMAP, Lucknow. These species has long been cultivated for herbal medicine and as an ornamental plant.

**Chemistry**

Extensive chemical studies on *Catharanthus roseus* (*C. roseus*) so far resulted in the isolation of bioactive molecules such as alkaloids, flavanoids, tannins etc. the well known anticancer drugs-vincristine and vinblastine are just 2 of 130 alkaloids that can be found in *C.*
Fig 5: *Catharanthus roseus*
Catharanthus roseus. It has ajamalicine, serpentine and antispasmodic properties. Many alkaloids have pain-relieving or anticancer properties.

Pharmacological properties

*Catharanthus roseus* is one of the few medicinal plants which have found mention in the folk medicinal literature as early as 2nd B.C. Long before modern researcher learnt of the plant's valuable and varied properties, people in faraway places were using the Madagascar periwinkle for a host of medicinal purposes. Traditionally, *C. roseus* has been used in folk medicine to treat diabetes, high blood pressure, tuberculosis etc. As an antidiabetic remedy, it was believed to promote insulin production or to increase the body's utilization of sugars from food.

Sulphates of *C. roseus* alkaloids, vincristine and vinblastine, are widely used as chemotherapeutic agents against Leukemia and Hodgkin's disease worldwide (Ozgen et al., 2003). The major alkaloid is vincamine and its closely related semi-synthetic derivative, widely used as a medicinal agent, known as ethyl-apovincaminate or vinpocetine, has vasodialating, hypoglycemic and memory enhancing actions (Chattopadhya et al., 1991). *C. roseus* is widely used as an infusion in different part of world to treat diabetes (Alexandrova et al., 2000; Heijden et al., 2004). Hot water decoction of the leaves and/or the whole plant is used for treatment of diabetes in several countries (Don, 1999). Fresh leaf juice of *C. roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits (Nammi et al., 2003). Significant antihyperglycemic activity of leaf alcoholic extract (Chottopadhya, 1999) and dichloromethanemethanol extract of leaves and twinges (Somanath et al., 2001) have been reported in laboratory animals. The extracts of *C. roseus* have been reported to have human peroxisome proliferator
receptor activating activity (Rau et al., 2006). The leaf juice of C. roseus has been reported to reduce serum total cholesterol and triglyceride in rats (Antia and Okokon, 2005). The fresh juice from the flowers of C. roseus made into a tea has been used by Ayurvedic physician in India for external use to treat skin problems, dermatitis, eczema and acne (Nayak et al., 2006).

AIM AND SCOPE

Earlier studies on antihyperglycemic and antihyperlipidemic activity of C. roseus are fragmentary and no studies are available on the efficacy of C. roseus in preventing IR. However, very little information is available on antioxidant activity of C. roseus. So, the present study was undertaken to explore possible beneficial effects of C. roseus leaf powder in prevention of diabetes and IR. Further, the biochemical basis for its antidiabetic property and protection against IR are investigated.

In the present systematic study the following aspects were undertaken to investigate the efficacy of C. roseus treatment on STZ induced type-1 diabetic and fructose fed IR 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: [Insulin (μU/ml) × Glucose (mmol/L)/ 22.5]

2. To measure the tissue damage under type-1 diabetic and IR conditions and to assess the toxicity/protective effect of C. roseus treatment, the activities of hepatic and renal
transaminases viz., glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) were measured.

3. In order to assess alterations in the peripheral utilization of glucose and glucose metabolism under insulin deficient and IR conditions and to evaluate the efficacy of *C. roseus* treatment on these changes, glycogen content of liver and muscle and activities of key enzymes of carbohydrate metabolism viz., Glycolytic enzymes: [hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) in liver and muscle], Gluconeogenic enzymes: [glucose-6-phosphatase (G6Pase) and fructose1, 6-bisphosphatase (F16BPase) in liver and kidney], Glycogenolytic enzyme: [glycogen phosphorylase in liver], HMP shunt pathway enzyme: [glucose-6-phosphate dehydrogenase (G6PDH) in liver] and Fructose metabolic enzyme: fructokinase in liver were measured.

4. The activities of intestinal disaccharidases (maltase, sucrase and lactase) were assayed in all the experimental groups.

5. To find out the alterations in lipid metabolism, hepatic and cardiac tissue lipids (total cholesterol, triglycerides, phospholipids and free fatty acids) and the activities of enzymes of lipid metabolism—fatty acid synthase (FAS) and malic enzyme in liver and lipoprotein lipase in adipose tissues were measured.

6. To assess the oxidative stress under type-1 diabetic and IR conditions and to evaluate the protective effect of *C. roseus* treatment, the extent of lipid peroxidation and protein oxidation were measured in liver, pancreas and heart.
7. Alterations in antioxidant system in liver, pancreas and heart were studied by measuring GSH levels, and by assaying the activities of GSH dependent and independent antioxidant enzymes i.e., glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD).

8. To understand the contribution of polyol pathway operation to the oxidative stress, the activities of aldose reductase and sorbitol dehydrogenase in liver, pancreas and heart were measured.