CHAPTER 1

INTRODUCTION

&

REVIEW OF LITERATURE
1.1. Introduction

Diabetes mellitus (DM) is a chronic metabolic syndrome caused by insulin deficiency or insulin resistance leading to disturbances in carbohydrate, protein and lipid metabolism. The worldwide prevalence of diabetes among adults (aged 20-79) was about 285 million in 2010 and is predicted to be 439 million by 2030. China has the highest number of diabetics (92.4 million) and India with 62.0 million diabetics stood at number two in 2011 (IDF 2011, Yang et al. 2010).

Diabetes mellitus (DM) is categorized into two major types: insulin dependent diabetes mellitus (IDDM; type 1 DM) and non-insulin dependent diabetes mellitus (NIDDM; type 2 DM). Type 1 DM is characterized by absolute deficiency of insulin and type 2 DM results from insulin resistance and/or impaired secretion.

Many pharmaceutical preparations such as sulfonylureas, metformin, α-glycosidase inhibitors, biguanides etc. are used for the treatment of DM (Larner 1985, Lebovitz 2004). Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. Even insulin therapy does not reinstate a permanent normal pattern of glucose homeostasis and carries an increased risk of hypoglycemia.

Alternatively, there are several medicinal plants used in the treatment of DM all over the world and provide useful source for the development of drugs (Shukla et al. 2000). The antihyperglycemic/anti-diabetic activities of various plants i.e. Ficus bengalensis, Trigonella foenum graecum, Brassica nigra, Eugenia jambolana, Cassia auriculata, Musa sapientum etc have already being investigated in Department of Biochemistry, UCMS, Delhi (Shukla et al. 1994, Murthy et al. 1989, Sharma et al. 2006, Gupta et al. 2010, Dikshit et al. 2012).

W. coagulans (family Solanaceae) commonly known as Doda paneer locally, is a rigid grey shrub 60-120 cm in height occurring in drier parts of India. Hot aqueous extract of W. coagulans at a dose of 1.0 g/kg has been shown to possess glucose lowering effect in streptozotocin induced DM in rats (Hemalatha et al. 2004). Jaiswal et al. 2010 have also reported the antidiabetic effect of aqueous and ethanolic extracts of W. coagulans at a dose of 750 mg/kg bw/day in STZ induced diabetic rats. Hoda et
al. 2010 reported the antihyperglycemic and antihyperlipidemic effects of aqueous and chloroform extracts of *W. coagulans*, given orally at a dose of 1.0 g/kg for 14 days in experimental DM in rats. Some steroid like compounds (withanolides) isolated from the roots and other parts of this plant have been shown to possess hormone like activity (Budhiraja et al. 1987). Withanolides isolated from aqueous extract of fruit of *W. coagulans* have anti-hyperglycemic, cardio-protective, hepatoprotective and anti-inflammatory activities (Budhiraja et al. 1983 & 1986, Maurya et al. 2008). In above mentioned reports, the doses of aqueous extract of *W. coagulans* fruit used were very high i.e. 750-1,000 mg/kg bw/day which roughly comes to about 200 mg/rat/day and approximately 10,000 mg/human being/day, which is very high in terms of physiological and nutritional ranges. Therefore, it is important to study the beneficial effects of lower doses of *W. coagulans* on glycemic control which will be easier to administer and should be relatively free of side effects. Moreover, none of the previous studies have attempted to elucidate the mechanism of action of antihyperglycemic effects of active principle isolated from *W. coagulans* fruit in nicotinamide-STZ induced DM model, which is considered similar to type 2 DM in humans (Masiello et al. 1998).

In view of the above mentioned reports and lacunae in the knowledge, further studies were planned to elucidate the antihyperglycemic effects and mechanism of action of active component isolated from water extract of fruit of *W. coagulans*.

**1.2. Historical perspective and global burden of diabetes**

Diabetes mellitus is a non-communicable disease (NCD) and a major cause of mortality worldwide (Devendra et al. 2004). The earliest mention of diabetes like illness characterized by polyuria can be traced to Egyptian Papyrus dating back to around 1550 BC. In Ayurvedic System of Medicine of India, Charak and Sushruta supplemented the earlier information and presented a comprehensive picture of diabetes (Madhumeha), its possible predisposing factors, clinical features, course, complications along with principles of medical health care (Nadkarni 1954).

Today, DM is the fourth leading cause of death and one of the most challenging health problems in the 21st century. The total world population in year 2011 was 7.0
billion and the prevalence of diabetes was about 8.3% of total world population. According to IDF 2011 report, about 366 million people in 2011 have been affected with DM and this figure is likely to reach 552 million by 2030. More than 80% of diabetes has been observed in low and middle-income countries. China with 90 million people has the highest number of diabetics in 2011 and this number will increase upto 129.7 million by 2030, whereas India stands at number two with 62.0 million diabetics in 2011 and this number will increase upto 101.2 million by 2030 (IDF 2011). Population based studies on diabetes also show that a substantial proportion of those found to have diabetes had not been previously diagnosed. It is expected that about 183 million people remained undiagnosed by year 2011. The highest number of people with diabetes are between 40 to 59 years of age which caused 4.6 million deaths in 2011. (IDF 2011, Hu 2011).

1.3. Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disease, characterized by hyperglycemia, caused by insulin deficiency or resistance leading to disturbances in carbohydrate, protein and lipid metabolism (Scheen 1997). Aretaeus, a Cappadocian physician of the second century wrote: "The epithet diabetes has been assigned to the disorder, being something like passing of water by a siphon". He perceptively characterized ‘Diabetes’ as "being a melting-down of the flesh and limbs into urine." From Latin, meaning "sweetened with honey". ‘Mellitus’ distinguishes this disease from diabetes insipidus, which is caused by impaired renal reabsorption of water (Berg et al. 2002).

Diabetes mellitus is heterogeneous group of metabolic diseases characterized by elevation in fasting plasma glucose due to relative or absolute deficiency of insulin. It has been divided into two major categories i.e. Type 1 DM (Insulin dependent diabetes mellitus, IDDM) and Type 2 DM (Non insulin dependent diabetes mellitus, NIDDM). Type 1 DM is an inborn error in β-cell function caused by unavailability or paucity of insulin secretion and comprises 5-10% of total DM, whereas, 90% burden of diabetes is due to type 2 DM (insulin resistance), the occurrence of which is linked to urbanization and lifestyle changes (Berg et al. 2002). Indians are also more prone to diabetes as a race. There have been significant changes in the dietary pattern in the country with the advent of processed ‘fast’ foods becoming increasingly available and
physical activity has declined due to the changing nature of jobs. This shift in dietary patterns and physical activity has led to rising prevalence of overweight and obesity. Diabetes is also a recognized major risk factor for the development of macrovascular and microvascular complications. The rising burden of diabetes, the numerous chronic complications and the considerable economic burden on patients, families and the country need appropriate solutions (ADA 2010).

1.3.1. Type 1 Diabetes mellitus

It is characterized by absolute deficiency of insulin caused by an autoimmune destruction of insulin producing pancreatic β-cells and accounts for only 5–10% of patients with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 DM, or juvenile-onset diabetes. Type1 DM is induced by a combination of genetic susceptibility, a diabetogenic trigger and exposure to the driving antigen. It is a poly genetic disease i.e. many genes contribute to its onset. However, some forms of type 1 DM have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 DM fall into this category, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not associated with HLA (ADA 2010).

1.3.2. Type 2 Diabetes mellitus

This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non-insulin dependent diabetes (NIDDM), type 2 DM, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. It comprises an array of dysfunctions resulting from the combination of inadequate insulin secretion and/or resistance to insulin action, characterized by hyperglycemia and is associated with macrovascular (i.e. coronary, peripheral vascular) and microvascular (i.e. retinal, renal, possibly neuropathic) complications (ADA 2010). Unlike patients with type 1 DM, patients with type 2 DM are not absolutely dependent upon insulin for life. This distinction was the basis for the older terms i.e. insulin dependent (type 1 DM) and non-insulin
dependent diabetes (type 2 DM) respectively. However, many patients with type 2 DM are ultimately treated with insulin, as they retain the ability to secrete some endogenous insulin, they require insulin but do not depend on insulin.

Insulin resistance, which has been attributed to elevated levels of free fatty acids in plasma leading to decreased glucose transport into muscle cells and elevated hepatic glucose production as well as increased breakdown of fat. Most of the overweight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Thus, their insulin concentrations may be high, yet inappropriately low for the normal glycemic status. Beta-cell dysfunction is a major factor across the spectrum of pre-diabetes to diabetes. A study of obese adolescents by Bacha et al. (2010) confirms what is increasingly being stressed in adults as well, β cell dysfunction happens early in the pathological process and does not necessarily follow stage of insulin resistance.

1.3.2.1. Insulin resistance

Insulin resistance means that the ability of insulin to dispose of glucose in the liver, skeletal muscle and other peripheral tissues is compromised. It is usually characterized by higher fasting and post-glucose loading insulin levels and a decreased responsiveness of tissues to the insulin driven clearance of glucose from the bloodstream.

A cluster of metabolic problems associated with insulin resistance including elevated plasma glucose, lipid regulation abnormalities (elevated triglycerides and small low-density lipoproteins as well as decreased high-density lipoproteins), high blood pressure, a prothrombic state and obesity (especially central obesity) occur commonly together (Kelly, 2000, Wilcox 2005). This combination is referred to as either “The Metabolic Syndrome” or “Syndrome X.” Research suggests that this cluster of metabolic disorders seems to interact to promote the development of type 2 DM, atherosclerosis and cardiovascular disease. While insulin resistance might lie at the heart of the problem, all of these metabolic disorders appear to be closely related and can together or independently contribute to health problems. Obesity appears to be predictably accompanied by insulin resistance with the degree of insulin resistance often in direct proportion to the amount of visceral body fat (Kelly 2000).
Lipolysis generates excessive non-esterified fatty acids (NEFA) and perhaps increased expression of tumor necrosis factor-α (TNF-α) by adipocytes and skeletal muscles, which can induce insulin resistance by inhibiting the tyrosine kinase activity of insulin receptors. Normally insulin receptors phosphorylate insulin receptor substrate (IRS) proteins which are linked to the activation of phosphatidylinositol-3-kinase (PI3K)/protein kinase-B and Akt pathway. In DM, reduced expression of PI3K, consequent reduced phosphorylation and activation of Akt arrests the insulin action (Marshall et al. 1991). Several other lifestyle factors also contribute towards insulin resistance e.g. environment, diet, exercise, smoking and stress etc.

1.4. Diagnosis of diabetes mellitus

American Diabetic Association and International Diabetic Federation (2011) have modified the diagnostic criteria for DM. They have also introduced the new theory of diagnosis for the early detection of diabetes mellitus called ‘Prediabetes’ characterized by slight increase in plasma glucose, impaired fasting plasma glucose (IFG) and impaired glucose tolerance (IGT), indicating the relatively high risk for the future development of DM.

1.4.1. Criteria for the diagnosis of DM

\[ \text{HbA}_1^C \geq 6.0\% \]

OR

\[ \text{FPG} \geq 126 \text{ mg/dl} \] (Fasting is defined as no caloric intake for at least 8 h)

OR

Two-hour Plasma Glucose (PG) ≥ 200 mg/dl during an Oral Glucose Tolerance Test (OGTT)

[After glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water]

1.4.2. Categories of increased risk for diabetes

\[ \text{FPG} = 100 – 125 \text{ mg/dl} \]

OR

\[ 2\text{-h PG on the 75g OGGT} = 140 – 199 \text{ mg/dl} \]

OR

\[ \text{HbA}_1^C \ 5.7 – 6.4\% \]
1.4.3. Criteria for testing for diabetes in asymptomatic adult individuals

1. Overweight (BMI ≥ 25 kg/m²) and have additional risk factors:
   - Physical inactivity
   - First-degree relative with diabetes
   - Members of a high-risk ethnic population (e.g. African American, Latino, native American, Asian American, Pacific Islander)
   - Hypertension (≥ 140/90 mmHg or on therapy for hypertension)
   - HDL cholesterol < 35 mg/dL (0.90 mmol/L) and/or a triglyceride level > 250 mg/dL (2.82 mmol/L)
   - HbA1c ≥ 5.7%
   - History of CVD

2. In the absence of the above criteria, testing for diabetes should begin at the age of 45 years.

3. If results are normal, testing should be repeated at 3-year intervals, with consideration of more frequent testing depending on initial results and risk status.

1.5. Insulin

1.5.1. Insulin discovery, structure and biosynthesis

Insulin was the first peptide hormone discovered. Before Abel (1926) crystallized insulin, Murnaghan (1967) and Jensen and Evans (1935) hydrolyzed the N-terminal phenylalanine of the B-chain, proving that insulin was indeed a protein, till then all hormones were believed to be small molecules. Insulin is an anabolic peptide-hormone with 51 amino acids that is secreted by the β-cells in the Islets of pancreas (Harfenist and Craig 1952) (Fig. 1). With the elucidation of the primary sequence of insulin, it became known that insulin is a two chain heterodimer consisting of a 21 amino acid A-chain linked to a 30 residue B-chain by two disulfide bonds derived from cysteine residues (Sanger 1959, Ryle et al. 1955) (Fig. 1.2a). An intra chain disulfide bond also exists in the A-chain. Proinsulin, a 9 KD protein contains both the A and B-chains of insulin in a continuous single chain joined through an intervening region called the C-
peptide (Steiner 1969 & 1967). The C-peptide is a variable length peptide segment consisting of 26-31 residues depending on the species, which links the carboxy terminus of the B-chain to the amino terminus of the A-chain via two dibasic residue links (Arg-Arg and Lys-Arg) (Fig. 1.2b). Proinsulin is cleaved at these dibasic links by a trypsin-like enzyme to release two chains of insulin and free C-peptide.

Von Mering and Minkowski (1890) first noted that removal of the pancreas led to the development of diabetes mellitus in dogs. Schafer (1916) first speculated that the antidiabetic hormone, which he decided to call “insuline” was from the pancreatic islets. Barron (1920) noted that ligation of the pancreatic duct with destruction of the exocrine pancreas, only resulted in diabetes if the islets, named by Dr. Langerhans in 1869, were also destroyed. Subsequently the work of Banting and Best (1922) in the early 1920's resulted in the identification of a substance in extracts of pancreas that had the remarkable ability to reduce blood glucose levels in diabetic animals (Bliss, 1982), and by 1923 these pancreas extracts were being used to successfully treat diabetic patients. Insulin regulates a host of other cellular processes, such as protein and fat synthesis, RNA and DNA synthesis, as well as cell growth and differentiation.

Chan et al. (1976) subsequently discovered that there was an additional precursor of insulin, preproinsulin. Preproinsulin is a 12 KD single chain polypeptide which consists of proinsulin extended at the amino terminus by a 24 amino acid signal peptide region of hydrophobic residues (Lomedico et al. 1977, Steiner et al. 1980). Fig. 1.3 illustrates the steps involved in the conversion of the information encoded within the 1500 bases of the human insulin gene sequenced by Bell et al. (1980) into preproinsulin and its proteolytic conversion to insulin.

Proinsulin, the direct precursor of insulin has many of the physical properties of insulin despite the larger size of this molecule. Proinsulin has been shown to aggregate, forming dimers and Zn$^{2+}$ coordinated hexamers in a manner similar to insulin (Frank and Veros, 1968), have a comparable isoelectric point (Chou 1987, Rosen 1987) and solubility (Steiner et al. 1972) also reacts with insulin antisera (Steiner and Oyer 1967, Rubenstein et al. 1969). This prompted the suspicion that the structure of insulin and proinsulin is similar, if not identical to that of the native
**Fig. 1.1.** Endocrine pancreas

**Fig. 1.2.** Primary structures of porcine insulin and porcine proinsulin. The primary sequence of porcine insulin (a) and proinsulin (b).
**Fig. 13.** Diagramatic illustration of the processing of insulin

**Fig. 14.** Glucose induced insulin secretion
insulin. It was also found that proinsulin is an agonist of insulin and displays 3-5% biological activity (Gliemann and Sorenson 1970, Freychet 1974), therefore the binding regions of insulin are accessible even when the relatively large C-peptide is linked to insulin. The full 3-D structure of proinsulin has not been determined despite its successful crystallization (Fullerton et al. 1970, Blundell and Wood 1982), possibly due to the lack of a fixed orientation of the C-peptide, however its insulin moiety has appeared to be very similar to that of crystalline insulin (Blundell et al. 1972).

1.5.2. Insulin secretion from pancreatic islets

Insulin affects a wide range of physiological processes, although it is known for its important regulatory role in glucose homeostasis. In response to elevation in plasma glucose, insulin secretion is increased and it stimulates glucose uptake, glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycemia.

Glucose is a key regulator of insulin secretion. It increases the ATP : ADP ratio and triggers closure of ATP-sensitive K⁺ channels. This in turn causes membrane depolarization and stimulates the opening of voltage-dependent Ca²⁺ channels. The resultant Ca²⁺ influx leads to increased cytosolic Ca²⁺ concentrations and promotes exocytosis – an effect mediated through protein kinase C or through direct stimulation of insulin granules (Fig. 1.4). Although glucose provides the primary stimulus several other molecules such as FFA, amino acids and keto acids also influence stimulus-secretion coupling (Prentki 1997, Deeney et al. 2000). In addition, a number of hormones and neuro-modulators stimulate insulin secretion, including glucagon-like polypeptide-1 (GLP-1) that increases cAMP levels and activates protein kinase C through specific G-protein-coupled receptors (Thorens 1995). Another pathway operates via binding of cholinergic agents to muscarinic receptors, which stimulates the production of inositol trisphosphate (IP3) and diacylglycerol (DAG) and thus increases intracellular calcium concentrations and promotes protein kinase C activity (Wollheim and Biden 1986, Hughes et al. 1990, Persaud et al. 1989). The exact mechanism by which Ca²⁺ and its associated protein kinases induce transport of secretory granules to the plasma membrane and subsequently stimulate granule exocytosis is currently unclear.
1.5.3. Insulin receptor signaling pathway

Insulin regulates both metabolism as well as gene expression: the insulin signal passes from the plasma membrane receptor to insulin-sensitive metabolic enzymes and to the nucleus, where it stimulates the transcription of specific genes. Insulin receptors are large transmembrane glycoprotein complex (400 KD) consisting of two identical $\alpha$-chains (135 KD) protruding from the outer surface of the plasma membrane and two transmembrane $\beta$-chains (95 KD) with their carboxyl termini protruding into the cytosol (Fig. 1.5). Insulin receptor acts as membrane-bound allosteric enzyme and its two subunits perform distinct functions required for transmission of the signal to the cell interior (Rosen 1987, Wilcox 2005). The $\alpha$-chains contain the insulin binding domain and $\beta$ chains contain the protein kinase activity that transfer a phosphoryl group from ATP to the hydroxyl group of tyrosine residues in specific target proteins. Signaling through the insulin receptor begins when binding of insulin to $\alpha$ chain activates the tyrosine kinase activity of the $\beta$-chains and each $\alpha\beta$ monomer phosphorylates three critical tyrosine residues near the carboxyl terminus of the $\beta$-chain of its partner in the dimer (Kwok et al. 1986). This autophosphorylation opens up the active site so that the enzyme can phosphorylate tyrosine residues of other target proteins (White et al. 1985).

Insulin action occurs at three distinct stages (Fig. 1.6). Stage 1, insulin binds to its receptors and activates tyrosine kinase of insulin receptors with subsequent phosphorylation and interaction of intracellular substrates with downstream signaling molecules. Stage 2, complex set of distinct phosphorylation and dephosphorylation cascades [phosphotidylinositol 3 kinase (PI3K) / protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) pathway] leading to activation of several key regulatory enzymes involved in cell growth and metabolism (Goldfine 1987). The final biological effects of insulin make up stage 3 actions involving the movement of insulin regulated GLUT4 from intracellular pool to the plasma membrane and activation of the enzymes involved in glucose, lipid and protein metabolism and transcriptional regulation of several genes (Eleftheria et al. 1997) (Fig. 1.6).
Fig. 1.5. Schematic diagram of insulin receptor homodimer

Fig. 1.6 Schematic presentation of insulin signaling pathways
1.5.4. Glucose transporter 4 (GLUT4)

The intracellular uptake of glucose is mediated through trans-membrane glucose transporter (GLUT) protein. Till date, 12 isoforms of facilitative glucose transporters are known i.e. GLUT1 – GLUT12, which are characterized in terms of structure, function and tissue distribution (Joost and Thorens 2001, Joost et al. 2002). While GLUT1 is the major isoform found in most cells including immortalized and transformed cell lines, other GLUT isoforms show tissue-specific distribution (Czech et al. 1992). Normally, GLUT4 is exclusively expressed in insulin-responsive tissues i.e. skeletal and adipose tissues (James et al. 1989), where it mediates the uptake of glucose. Insulin stimulates the translocation of specific GLUT4 containing vesicles from an intracellular pool to plasma membrane resulting in an immediate 10-20 fold increase in glucose transporter (Shepherd and Kahn 1999, Bryant et al. 2002). GLUT4 is involved in the first step of the glucose utilization cascade which is highly regulated, level of regulation include gene transcription, protein synthesis and degradation. In diabetes, the expression of GLUT4 is affected both at transcription and translation levels. Several studies reported in animals models of diabetes have shown decreased mRNA and protein expression of GLUT4 and its impaired trafficking to the plasma membrane (Mohammad et al. 2002 & 2006, Furuta et al. 2002). The deficiency of insulin in the diabetic state leads to reduced expression of GLUT4 and its translocation to the plasma membrane (Olson and Pessin 1995, Karnieli and Armoni 2008), which results in decreased uptake of glucose by the cells and contributes to the significantly elevated plasma glucose levels.

1.5.5. Insulin: mechanism of action and regulation

Insulin exerts number of important metabolic effects mediated via change in the expression of more than 100 genes (O’Brien et al. 1996). For example, insulin regulates the expression of genes involved in amino acid uptake, lipid metabolism in muscle and adipose tissue, cell growth, development and survival (Yenush et al. 1997, Kimball et al. 1994, Sell et al. 1994). In type 2 DM, loss of glycemic control generally involves impairment of both insulin action (i.e. peripheral insulin resistance) and insulin secretion (i.e. β-cell dysfunction) (Tripathy et al. 2000). However, a common underlying molecular mechanism has not yet been identified for the majority of cases of type 2 DM.
The central importance of insulin in regulating glucose metabolism and the prevention of diabetes has stimulated research over last 80 years in attempt to understand the mechanism of action of this peptide hormone. It is now known that specific membrane transporters (GLUT4) facilitate the movement of glucose into cells to reduce plasma glucose concentrations in response to insulin stimulation (Fig. 1.7). Two major types of glucose transporters are known: Na⁺ dependent and Na⁺-independent transporters, only the Na⁺ independent transporters possess an insulin responsive isoform (Ullrich 1979, Hopfer 1987, Haase and Koepsell 1989). These transporters are located on the luminal side of intestine and kidney cells and absorb glucose against its concentration gradient by coupling the movement of glucose into these cells with the concomitant movement of Na⁺ into the cell. Although twelve isoforms have been identified (GLUT 1-12) (Joost et al. 2002, Rosholt and King 1995), however GLUT4 is the transporter that is in highest concentration in insulin-sensitive tissues such as skeletal muscle and adipose tissue (Fukumoto et al.1989, Charron and Kahn 1990). GLUT 4 and to a lesser extent GLUT1 enable these cells to increase their glucose uptake thereby lowering the circulating glucose levels. Since the intracellular concentration of glucose is low due to the rapid phosphorylation of glucose to glucose-6-phosphate and its dissimilation to other metabolic products, the presence of active transporters in the plasma membrane favor the movement of glucose into cells.

The impaired ability of insulin to signal GLUT4 translocation from intracellular stores is currently believed to be an important contributory factor to postprandial hyperglycemia in DM (Baron et al. 1988). Animal studies have also demonstrated that insulin resistance is associated with decrease translocation of glucose transporters to the plasma membrane in muscle cells (Bourey et al. 1990). In fact, decreased insulin levels in diabetic animals have been shown not only to decrease transporter translocation but also diminish expression of GLUT4 in muscle cells (Klip et al. 1990, Unger 1991). Thus, it appears that insulin serves not only to acutely increase glucose transporter translocation but also to maintain a basal level of expression of transporters in cells. Therefore, one mechanism by which diabetes characterized by either low insulin levels as in type 1 DM or insulin resistance as in type 2 DM could cause pathologically high plasma glucose levels via loss of regulation and expression of transmembrane glucose transporters.
Fig. 1.7. Effect of insulin on GLUT4 translocation
1.6. Effect of insulin on metabolism

1.6.1. Carbohydrate metabolism

Insulin stimulates glucose uptake from the systemic circulation as well as suppresses hepatic gluconeogenesis thereby serving a primary role in glucose homeostasis and preventing the metabolic disorder of diabetes mellitus (table 1.1.) (Scott 1912, Schafer 1916, Sanger 1959, Blis 1982, Jonsson 1994, Duville 1997). Insulin stimulates the transport of glucose across the cell membranes mainly in muscle and adipose tissue. In essence, the diabetics are in biochemical starvation mode despite a high concentration of blood glucose because insulin is deficient; the entry of glucose into cells is impaired. The liver sticks in a gluconeogenic and ketogenic state. The excessive level of glucagon relative to insulin leads to decrease in the amount of glucose-6-phosphate and fructose-2,6-bisphosphate in the liver. Hence, glycolysis is inhibited and gluconeogenesis is stimulated because of the opposite effects of Glucose-6 phosphate, F-2,6-BP on phosphofructokinase and fructose-1,6-bisphosphatase. The high glucagon / insulin ratio in diabetes also promotes glycogen breakdown (Berg et al. 2002). Insulin enhances glycogen synthesis by increasing the activity of glycogen synthase and inhibits glycogenolysis by bringing down cAMP level as a result of inhibition of adenyl cyclase and/or stimulation of a cyclic nucleotide phosphodiesterase and inhibition of cAMP dependant protein kinase (Larner et al. 1979). Therefore, during insulinopenia, decreased glucose utilization and increased glycogen breakdown lead to aggravation of hyperglycemia (Berg et al. 2002).

1.6.2. Lipid metabolism

The metabolic pathways of carbohydrate and lipid are intricately interwined. Insulin has profound effect on lipid metabolism i.e. it stimulates fatty acid biosynthesis as well as incorporation of fatty acids into triacylglycerol (TAG). It stimulates the enzyme ATP citrate lyase, which provides NADP⁺ for the fatty acid biosynthesis. These fatty acids are exported from the liver as lipoproteins. Insulin activates lipoprotein lipase (LPL), which break lipoproteins to provide free fatty acids to tissues for the TAG biosynthesis (Murray et al. 2012). On the other hand, Insulin inhibits hormone sensitive lipase by reducing cAMP level in adipocytes (Towler and Hardie 2007). Insulin also facilitates the entry of glucose in adipocytes and synthesizes glycerol. This glycerol is used to synthesize TAG within adipocytes. During diabetes
mellitus, significant elevation in lipid concentration may be due to increased mobilization of free fatty acids from adipose tissue due to the activation of hormone sensitive lipase during insulin deficiency. Dysfunction of lipoprotein lipase in insulin deficiency also contributes to hypertriglyceridemia due to impaired catabolism of triacylglycerol rich particles. Insulin also increases the receptor mediated removal of LDL-C and hence insulin deficiency may cause dyslipidemia with increased level of LDL-C. Reduced formation of HDL-C is another characteristic feature of diabetic dyslipidemia, which is due to impaired metabolism of TAG rich lipoproteins which provides a significant portion of HDL-C metabolism.

When carbohydrate utilization is impaired, lack of insulin leads to the uncontrolled breakdown of lipids and proteins. Large amounts of acetyl-CoA are then produced by \( \beta \)-oxidation, however much of the acetyl CoA cannot enter the citric acid cycle, because there is insufficient oxaloacetate for the condensation step, therefore they are diverted towards ketogenesis (Berg et al. 2002, Murray et al. 2012). Thus a striking feature of diabetes is the shift in fuel usage from carbohydrates to fats and glucose, which is abundant remains un-metabolized.

Table 1.1. Effect of insulin on metabolism

<table>
<thead>
<tr>
<th>Metabolic effects</th>
<th>Target enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose uptake (muscle, adipose)</td>
<td>Glucose transporter (GLUT4)</td>
</tr>
<tr>
<td>Glucose uptake (liver)</td>
<td>Glucokinase expression</td>
</tr>
<tr>
<td>Glycogen synthesis (liver, muscle)</td>
<td>Glycogen synthase</td>
</tr>
<tr>
<td>Glycogenolysis (liver, muscle)</td>
<td>Glycogen phosphorylase</td>
</tr>
<tr>
<td>Glycogen, Acetyl-CoA production (liver, muscle)</td>
<td>Phosphofructokinase-1</td>
</tr>
<tr>
<td>Fatty acid synthesis (liver)</td>
<td>Acetyl-CoA carboxylase</td>
</tr>
<tr>
<td>Triacylglycerol synthesis (adipose)</td>
<td>Lipoprotein lipase</td>
</tr>
</tbody>
</table>
1.6.3. Protein metabolism

Insulin plays a significant role in protein metabolism. It enhances the uptake of amino acids by various tissues and facilitates the transportation across cell membrane (Wool et al. 1972). Insulin increases the formation of aminoacyl t-RNA (Manchester 1970) and has been shown to increase the number of ribosomes and their translational efficiency in muscles. Several in vitro studies have shown that insulin suppresses proteolysis in skeletal & cardiac muscles and liver. In diabetes mellitus, insulin deficiency leads to decrease in protein synthesis, increased proteolysis and insulin therapy diminishes proteolysis in insulin dependant diabetes (Rannels et al. 1975).

1.7. Diabetes associated complications

Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia. It is well established that hyperglycemia leads to dyslipidemia, oxidative stress and endothelial dysfunction, which are further associated with micro and macrovascular complications (Bierman et al. 1966, Ceriello 2000, Garber 2002).

1.7.1. Dyslipidemia

Metabolism of lipids is impaired in DM and typical dyslipidemia is characterized by increased concentration of triacylglyceride rich lipoproteins i.e. very low density lipoprotein (VLDL) accompanied by a low concentration of high density lipoprotein cholesterol (HDL-C) (Taylor et al. 1981, Garber 2002). Insulin increases lipogenesis under normal conditions, it stimulates fatty acid biosynthesis as well as incorporation of fatty acids into TAG. It also stimulates the enzyme ATP citrate lyase (Ramakrishna and Benjamin. 1981), which provides NADPH for fatty acid synthesis. Insulin inhibits hormone sensitive lipase by reducing cAMP levels in adipocytes (Towler and Hardie 2007). In DM, there is increased mobilization of free fatty acids from adipose tissue into the circulation (Hales et al. 1965, Best and Taylor 1989), leading to increased influx into the liver, where they are catabolized to acetyl CoA. Increased production of acetyl CoA leads to increased formation of ketone bodies (McGarry et al. 1975). There is also increased synthesis of TAG in liver, which is converted mainly to VLDL, the levels of which is increased in plasma (Balasse et al. 1972). Hypercholesterolemia, hypertriglyceridemia and increased LDL levels have also been reported in chemically induced diabetes in experimental animal models (Bar-On et al. 1976, Subbiah et al. 2006) (Fig. 1.8).
1.7.2. Oxidative Stress

Oxidative stress, defined as imbalance between production of reactive oxygen species (ROS) and antioxidant defenses, leads to several biochemical effects and is an important causative factor in several chronic diseases (Halliwell 1994, Frei 1999). Oxygen and its activated intermediates may react with cellular components with resultant degradation of essential molecules, which causes various degree of toxicity and leads to either transient or irreversible damage. The term ROS includes superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (OH), lipid hydroperoxide (lipid-O$_2$H) or related species.

1.7.3. Free radicals

Molecular species capable of independent existence that contain an unpaired electron in atomic orbital are called free radicals (Halliwell 1994). Many of these free radicals are highly reactive and can either donate an electron or extract an electron from other molecules.

1.7.3.1. Superoxide anion (O$_2^-$)

Superoxide anions are formed by the addition of one electron to oxygen molecule. Glucose and other molecules like adrenaline, thiol and flavin nucleotides produce superoxide anions and these reactions are accelerated by the presence of transition metals such as iron or copper. The electron transport chain in the inner mitochondrial membrane reduces oxygen to water and electrons are leaked into the mitochondrial matrix and results in the production of superoxide anion (Brownlee 2005).

Superoxide anion is produced by reduction of oxygen by transfer of single electron

\[
O_2 + e^- \rightarrow O_2^-
\]

and by auto-oxidation of reduced transition metals

\[
Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^-
\]

\[
Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^-
\]
Fig. 1.8. Schematic diagram of effect of insulin deficiency/resistance on lipid metabolism
1.7.3.2. *Hydrogen peroxide (H₂O₂)*

H₂O₂ is produced as a result of a spontaneous dismutation of superoxide anion

\[
2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

and two electron reduction of oxygen

\[
2O_2^- + 2e^- + 2H^+ \rightarrow H_2O_2
\]

In addition, several enzymatic reactions including those catalyzed by glycolate oxidase and D-amino acid oxidase produce H₂O₂ directly.

1.7.3.3. *Hydroxyl radical (*OH*)*

Hydroxyl radical is probably the final mediator of most free radical induced tissue damage and react with sugar, amino acids, lipids and nucleotides. The most important reaction in vivo for hydroxyl radical formation is likely to be the transition metal catalyzed decomposition of superoxide anion and hydrogen peroxide (Stoh and Bagchi 1995).

Hydrogen peroxide can react with ferrous or cuprous ions to generate the hydroxyl radical.

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-
\]

Superoxide anion and hydrogen peroxide can react together directly to produce the hydroxyl radical in presence of transition metal ions at a rapid rate:

\[
Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2
\]

The net result of the reaction is known as Haber Weiss reaction:

\[
O_2 + H_2O_2 \rightarrow ^\cdot OH + OH^- + O_2
\]

1.7.3.4. *Nitric oxide (NO)*

In 1987, it was shown that endothelial-derived relaxing factor was in fact the free radical nitric oxide (Palmer et al, 1987). NO is generated from the guanidino nitrogen of L-arginine by an N-hydroxyl-L-argeinine intermediate yielding citrulline. This reaction is catalyzed by two major types of nitric oxide synthases: inducible (iNOS) and the two isoforms of constitutive nitric oxide (eNOS) (Kiechle and Malinski 1993). Both enzymes are flavoprotein and require NADPH as a cofactor. Following radical-radical interaction with superoxide, nitric oxide produces a potent oxidant peroxynitrite (ONOO⁻).

\[
NO^- + O_2^- \rightarrow ONOO^-
\]
1.7.3.5. Harmful effects of free radicals

Free radicals are highly reactive and react with various biomolecules and biomembranes leading to the formation of oxidized products or harmful intermediates. These radicals rapidly react with oxygen to form corresponding peroxyl radical (ROO\(^{\cdot}\)) and alkoxyl radical (RO\(^{\cdot}\)).

1.7.4. Lipid peroxidation

Lipid peroxidation occurs when free radicals are generated adjacent to polyunsaturated fatty acids (PUFA) such as arachidonic acid and linolenic acid in membrane lipids. The reactive radical extracts a hydrogen atom from one of the \(-\text{CH}_2-\) groups in fatty acids and generate a carbon centered radical (R\(^{\cdot}\)), which binds with molecular oxygen and form peroxyl radical (ROO\(^{\cdot}\)). Peroxyl radicals attach with membrane proteins such as receptors and enzymes and extract hydrogen atoms from adjacent fatty acid side chains. Thus extraction of a hydrogen atom can set off a free radical chain reaction that leads to conversion of lipids into lipid hydroperoxides (ROOH). The existences of lipid peroxides within a membrane severely disrupts its functioning by altering fluidity and allow ions such as Ca\(^{2+}\) to pass across the membrane (Halliwell, 1994). The whole process is as follows:

1. **Initiation**
   
   \[
   \text{ROOH} + \text{metal}^{(n^+)} \xrightarrow{} \text{ROO}^{\cdot} + \text{metal}^{(n-1)} + \text{H}^+ \\
   X^- + RH \xrightarrow{} R^{\cdot} - XH
   \]

2. **Propagation**
   
   \[
   R^{\cdot} + \text{O}_2 \xrightarrow{} \text{ROO}^{\cdot} \\
   \text{ROO}^{\cdot} + RH \xrightarrow{} \text{ROOH} + R^{\cdot}
   \]

3. **Termination**
   
   \[
   R^{\cdot} + R^{\cdot} \xrightarrow{} RR \\
   \text{ROO}^{\cdot} + R^{\cdot} \xrightarrow{} \text{ROOR} \\
   \text{ROO}^{\cdot} + \text{ROO}^{\cdot} \xrightarrow{} \text{ROOR} + \text{O}_2
   \]
Lipid hydroperoxides decompose in the presence of iron and copper ions to form a wide range of cytotoxic aldehydes as malondialdehyde and hydroxynonenal which themselves are capable of chemically modifying proteins and DNA (Slatter et al. 2000).

1.8. **Mechanism of induction of oxidative stress in DM**
Oxidative stress and resultant damage are hallmarks of chronic disease and cell death; diabetes is no exception. A casual relationship between chronic hyperglycemia and diabetic microvascular disease, long inferred from various animal and chronic studies, has now been definitely established by data from Diabetic Complications and Control Trial (DCCT). Hyperglycemia may cause tissue damage through 5 major mechanisms: (1) increased flux of glucose and other sugars through the polyol pathway, (2) increased intracellular formation of AGEs (advanced glycation end products), (3) increased expression of the receptor for AGEs and its activating ligands, (4) activation of protein kinase (PK) C isoforms, and (5) overactivity of the hexosamine pathway (Fig. 1.9). Several lines of evidence indicate that all 5 mechanisms are activated by a single upstream event i.e. mitochondrial overproduction of reactive oxygen species (ROS).

1.8.1. **Polyol pathway flux**
The polyol pathway is based on a family of aldo-keto reductase enzymes that can use a wide variety of carbonyl compounds as substrates and reduce these by NADPH to their respective sugar alcohols (polyols). Several mechanisms have been proposed to explain how hyperglycemia-induced increase in polyol pathway flux could damage the tissues involved (Chung et al. 2003). The most cited is an increase in redox stress caused by the consumption of NADPH. Since NADPH is a cofactor required to regenerate reduced glutathione (GSH) and GSH is an important scavenger of ROS, this could induce or exacerbate intracellular oxidative stress. Overexpression of human aldose reductase reduces the expression of genes that regulate regeneration of glutathione (Vikramadithyan et al. 2005). It has also been demonstrated that decreased glutathiolation of cellular proteins is related to decreased NO availability in diabetics, which would decrease GSNO (Lee and Chung 1999). Restoration of NO levels, increases glutathiolation of cellular proteins which inhibit aldose reductase activity and prevent sorbitol accumulation.
1.8.2. Intracellular AGE formation

AGEs are formed by the nonenzymatic reaction of glucose and other glycerating compounds derived from glucose and fatty acid oxidation (Wautier et al. 2004, Candido et al. 2003). In DM, AGEs are found in increased amounts in extracellular matrix (Niwa et al. 1997). Intracellular production of AGE precursors can damage cells by three general mechanisms. Firstly, intracellular proteins modified by AGEs have altered function. Secondly, extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with matrix receptors (integrins) that are expressed on the surface of cells. Finally, plasma proteins modified by AGE precursors bind to AGE receptors and induce the production of ROS, which in turn activates the pleiotropic transcription factor nuclear factor (NF)-κB, causing multiple pathological changes in gene expression (Goldin et al. 2006).

1.8.3. Protein kinase C activation

PKCs are a family of at least 11 isoforms that are widely distributed in mammalian tissues. This enzyme phosphorylates various target proteins. In DM, De novo synthesis of DAG from glucose-derived precursors via triose phosphate enhanced and increases PKC translocation and activity. (Shiba et al. 1993, Scivittaro et al. 2000). In addition, activation of PKC is sensitively regulated through redox changes in sulphhydryl groups of cysteine rich region of PKC (Gschendt et al. 1991). PKC signaling system enhanced phospholipase A₂ activity. The free arachidonic acid released by phospholipase A₂ can fuel enzymatic production of prostaglandins (PG). Since PG synthesis is a well recognized source of free radicals and the increased PG synthesis may also contribute to concomitant occurrence of lipid peroxidation.

1.8.4. Hexosamine pathway flux

Several lines of evidence have established that the excessive flux of glucose or FFA into cell activates hexosamine biosynthetic pathway. It has been proposed that the activation of this pathway leads to insulin resistance and the development of late complications of DM (Hawkins et al. 1997, Schleicher and Weigert 2000, Tang et al. 2000, Hebert et al. 1996). The hexosamine pathway also functions as a cellular
Fig. 19. Various mechanism of hyperglycemia induced oxidative stress
“sensor” of energy availability and mediates the effects of glucose on the expression of several gene products including leptins. (Rossetti 2000, McClain 2002).

1.9. Antioxidant defense systems

Antioxidants prevent free radical-induced tissue damage by preventing the formation of free radicals, scavenging them or by promoting their decomposition (Fig. 1.10). These can be dividing into three main groups:

1.9.1. Antioxidant enzymes

1.9.1.1. Superoxide dismutase

Superoxide dismutase (SOD) marks the first line of defense against oxidative damage. SOD catalyzes the dismutation of superoxide anion of H₂O₂.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

There are three isozymes of SOD in the mammalian body, copper zinc-superoxide dismutase in the cytoplasm of cells, manganese-superoxide dismutase in the mitochondrial matrix and extracellular superoxide dismutase I the extracellular space (Marklund and Marklund 1974).

1.9.1.2. Chain breaking antioxidants

In this group, the antioxidants receive electron from a radical or donate an electron to a radical with the formation of stable byproducts (Halliwell, 1995). They are divided into aqueous and lipid phase antioxidants.

1.9.2. Aqueous phase chain breaking antioxidants:

1.9.2.1. Reduced glutathione (GSH)

GSH is ubiquitous tripeptide (γ-Glu-Cys-Gly), present in high concentration in tissue. It is an important naturally occurring antioxidant, which detoxify ROS of exogeneous and endogeneous origins. GSH and its oxidized form i.e. glutathione disulphide constitute the major thiol redox system. Altered thiol disulphide status coupled with resultant oxidation of proteins and sulphhydryl has profound effects on metabolic processes. These include altered cell cycle responses, impaired functions of a variety of enzymes and protein and activity of transcription factors such as NF-kB (Aw 1999).
1.9.2.2. **Vitamin C**

Vitamin C acts as a free radical scavenging agent for superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radical and singlet oxygen.

1.9.2.3. **Lipid phase chain breaking antioxidants**

Vitamin E is an important antioxidant and plays a crucial role in limiting lipid peroxidation. Vitamin E is naturally present in mammalian cell membranes and is known as a micromembrane stabilizer. It protects against cell membrane damage form lipid peroxidation.

1.9.3. **Transition metal binding proteins**

Transition metal binding protein (ferritin, transferin, lactoferin etc) acts as a crucial component of the antioxidant defense system by sequestering iron and copper. Therefore they are not available to derive the formation of hydroxyl radicals. The main copper binding protein, ceruloplasmin, might also function as a antioxidant enzyme that may catalyze the oxidation divalent iron.

\[
4 \text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O}
\]

Fe\(^{2+}\) is the form of iron that derives the Fenton reaction and the rapid oxidation of Fe\(^{2+}\) to the less reactive Fe\(^{3+}\) form is therefore an antioxidant effect (Young and Woodside 2001).

1.9.4. **Decreased antioxidant enzymes**

There is enough evidence that hyperglycemia may decrease the activity of a number of enzymes. Decrease in activity of antioxidant enzymes in diabetes is linked to the progressive glycation of these enzymes (West 2000), which could alter their structure and function so that they are unable to scavenge free radicals, exacerbating oxidative stress in DM. About 50% of SOD in RBCs of diabetic patients is glycated resulting in low activity (Arai et al. 1987).

1.10. **Experimental diabetes mellitus**

Experimental diabetes can be produced either by surgery i.e. partial pancreatectomy and genetic manipulation by selective inbreeding or by destruction of pancreatic islets by chemical agents.
Fig. 1.10. Association of various free radical defense mechanisms generated during oxidative stress.
1.10.1. Spontaneous diabetes mellitus

Spontaneous diabetes mellitus is introduced into animals by two methods i.e. transgenic or gene knockout techniques. In the transgenic technique, the foreign DNA is introduced into fertilized eggs of the animals by microinjection. In contrast, the gene knockout procedure, involves inactivating or “knocking out” gene in the animals. In gene knockout, genes are isolated, inactivated and implanted into embryonic stem cells (ES). Transgenic and gene knockout animal models can be used as experimental animal models for human disease. Zucker diabetic fatty rats, Otsuka Long-Eveans Tokushima fatty (OLETF) rats, Sur 1 knockout mouse etc. are also good models of spontaneous DM (Seghers et al. 2000, Sugimoto et al. 2001, Wen et al. 2002).

1.10.2. Induction of diabetes mellitus by chemicals

Alloxan and streptozotocin are the most prominent diabetogenic chemical compounds in experimental diabetes research. Both compounds are β-cytotoxic glucose analogues, while their mechanisms of cytotoxic action are different. (Szkudelski 2001) (Fig. 1.11).

1.10.2.1. Alloxan

The name “Alloxan” emerged from amalgamation of the words “Allantoin” and “Oxalsaura” (oxaluric acid) (Lenzen and Panten, 1988). Alloxan (2,4,5,6-tetraoxypyrimidine, 5,6-dioxyuracil) was is an oxygenated pyrimidine derivative, which was first described by Brugnatelli in 1818 (Fig. 1.12). Wöhler and Liebig used the name “alloxan” and described its synthesis by uric acid oxidation (Lenzen and Panten 1988). Alloxan is a hydrophilic, unstable substance with a half-life, at neutral pH and 37 °C of about 1.5 min which is longer at lower temperatures (Lenzen and Munday 1991). Alloxan exerts its diabetogenic action when it is administered parenterally, intravenously, intraperitoneally or subcutaneously.

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent DM (called "Alloxan diabetes") in these animals, with characteristics similar to type 1 DM in humans. Alloxan is selectively
toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction (Lenzen 2008). Alloxan also induces fragmentation of DNA, which leads to stimulation of DNA repair by ADP-ribose poly-synthase, an enzyme which requires NAD$^+$ (Yamamoto et al. 1981). Increased activity of this enzyme leads to depletion of intracellular NAD$^+$ and this in turn could impair the ability of $\beta$-cells to synthesize insulin (Okamoto 1981).

1.10.2.2. Streptozotocin
Streptozotocin (STZ, Zanosar) (Fig. 1.13) is a naturally occurring nitosourea derivative synthesized by *Streptomyces achromogenes* and is used to induce both insulin-dependent and non-insulin-dependent DM (IDDM and NIDDM, respectively) in experimental animals (Rakieten et al. 1963). Chemically, STZ is 1-methyl-1-nitrosourea linked to D-glucose and molecular formula is 2-deoxy-2-([methyl(nitroso)amino] carbonyl]amino)-$\beta$-D-glucopyranose (Fig. 1.13). The nitrosourea moiety provides its cytotoxic effect and deoxyglucose moiety facilities its entry into $\beta$ cells (Like and Rossini 1976). Initially hyperglycemia is observed by one hour after injection followed by hypoglycemia and again hyperglycemia state is developed within 72 hours (Junod et al. 1969).

STZ is cytotoxic agent to the insulin-producing beta cells by generation of reactive oxygen species. STZ is taken up by pancreatic $\beta$-cells via glucose transporter GLUT2 and alkylate DNA (Schnedl et al. 1994). Therefore, intracellular antioxidants or NO scavengers substantially attenuate STZ toxicity. Fragmentation of DNA induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenic action of STZ than DNA damage. Poly ADP-riboylation lead 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 (Nukatsuka et al. 1998). Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, STZ liberates toxic amounts of NO that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, $\beta$-cells undergo the destruction by necrosis (Yamamoto et al. 1981, Szkudelski 2001).
Fig. 1.11. Schematic diagram of alloxan and streptozotocin induced β-cell death.

Fig. 1.12. Alloxan

Fig. 1.13. Streptozotocin
**Fig. 1.14.** Nicotinamide

**Fig. 1.15.** Schematic diagram of protective effect of nicotinamide against streptozotocin induced β-cell damage.
1.10.2.3. Nicotinamide (NAD)

Nicotinamide (vitamin B3) (Fig. 1.14) a water-soluble vitamin, is a weak PARP inhibitor, antioxidant (Melo et al. 2000), improves energy status and inhibits apoptosis and cell death in ischemic tissues (Ibrahim and Sherine 2008). It is a biochemical precursor of β-nicotinamide adenine dinucleotide (NAD+) and considered to be necessary for cellular function and metabolism. Nicotinamide may prevent islet cell nitric oxide production by inhibiting the expression of iNOS in β-cells. The role of oxygen radicals, generated during STZ-induced islet cell damage, as possible mediators of the expression of iNOS has already been suggested. Nicotinamide is involved in NAD biosynthesis, thus supply of nicotinamide can restore the islet cell content of NAD towards normality and can also inhibit the DNA repair enzyme PARP, thereby preventing cellular NAD depletion and inhibiting apoptosis (Fig. 1.15) (Masiello et al. 1998, Szkudelsk 2012).

1.11. Treatment of Diabetes Mellitus

1.11.1. Insulin

The discovery of insulin by Banting and Best was a boon to cure the diabetes mellitus. Insulin is the absolute requirement for survival of patient in type 1 DM, whereas type 2 DM may require insulin in later stage. Most insulin preparations are manufactured enzymatically or by recombinant DNA technology. Based on the pharmacodynamic properties, the types of insulin currently available are rapid acting, short acting, intermediate and long acting. The three sources of insulin that are clinically used at present are bovine, procrine and human (Skyler 1998).

Insulin regulates blood glucose homeostasis by reducing the release of glucose from liver through inhibition of hepatic gluconeogenesis and increase in glucose transport into peripheral tissues viz. muscle and adipose tissue. Insulin induces the translocation of GLUT4 protein from cellular storage vesicles to the cell membrane. As the daily necessity of insulin injections can be painful along with hypoglycemia seen with insulin therapy.
1.11.2. Oral anti-diabetic agents

These act by different mechanisms as follows:

1.11.2.1. Insulin secretagogues

The functional defect in insulin secretion can be ameliorated by pharmacological agents, which act on ATP-dependent K⁺ channels and augment their closure by glucose. These agents have insulinotropic effect (Lebovitz 2004). There are 2 different groups of these agents, which are clinically used: (i) Sulphonylureas (ii) Non-sulphonylureas.

1.11.2.1.1. Sulphonylureas

The sulphonylureas were the first widely used oral anti-hyperglycemic medications: First generation (e.g. tolbutamide, chlorpropamide, tolazamide etc.) and second generation (e.g. glibenclamide, glyburide, gliclazide etc.) sulfonylureas (Fig. 1.16). They are insulin secretagogues, triggering insulin release by direct action on the K_ATP channel of the pancreatic β cells. It inhibits the sulphonylurease receptors 1, the regulatory subunit of the ATP-sensitive potassium channels (K_ATP) in pancreatic β cells. This inhibition causes cell membrane depolarization opening voltage-dependent channel. These result in an increase in intracellular calcium in the β cells and subsequently stimulation of insulin release (Lebovitz 2004). It also increases the production of diacylglycerol and activates protein kinase C. Simultaneously, it enhances the expression of glucose transporter isoforms and activates insulin receptor substrate/phosphatidylinositol 3-kinase pathway (Muller 2000). However, the significant side effect of sulphonylureas drugs is hypoglycemia and weight gain, which has been implicated as a cause of secondary drug failure (Holman and Turnover 1991, van Staa et al. 1997).

1.11.2.1.2. Non-sulphonylureas

This relatively new class of medications is currently represented by meglitinide i.e. available in the market as repaglinide. Repaglinide is a benzoic acid derivative and nateglinide is a phenylalanine derivative (Fig. 1.17). The mechanism of action of these drugs is similar to that of the sulfonylureas (closure of the potassium adenosine triphosphate channel, leading to calcium-dependent insulin secretion). However, they
bind to the sulfonylurea receptor at a different site and with different kinetics than the sulfonylureas (Owens et al. 2000, Damsbo et al. 1999).

1.11.2.2. Insulin sensitizers

1.11.2.2.1. PPAR-γ agonist

Common to this group of drugs is thiazolidine 2,4-dione nuclear structure and changing the side chain has generated several agents viz englitazone, roglitazone and pioglitazone etc (Fig. 1.18). Troglitazone, an earlier thiazolidinedione was introduced first in 1997 in the United States. These agents enhance certain action of insulin via peroxisome proliferator-activated receptor gamma (PPARγ). Thiazolidinediones improve insulin sensitivity, particularly in the peripheral tissues (Eckland and Tz 1997). The major side effects of rosiglitazone and pioglitazone are weight gain, edema, anemia, pulmonary edema and congestive heart failure.

1.11.2.2.2. Biguanides

Over 30 years ago various biguanides (e.g. metformin, phenformin, buformin) were used (Fig. 1.19). Its major action in patients with DM is to decrease hepatic glucose output, primarily by decreasing gluconeogenesis, but it may also to a lesser extent, increase glucose uptake by skeletal muscles. Metformin might produce high risk of lactic acidosis and weight gain (Beckmann 1971).

1.11.2.3. α-Glucosidase inhibitors

Acarbose and miglitol are the α-glucosidase inhibitors (Fig. 1.20), which competitively inhibit enzymes in the small intestinal brush border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. α-Glucosidase inhibitors act locally at the intestinal brush border, are not absorbed and are excreted in feces (Lebovitz 1998).

1.11.2.4. Incretin

Incretin is also insulin secretagogues. The two molecules i.e. glucagon-like-peptide-1 (GLP1) and gastric inhibitory peptide (GIP) are fulfilling the criteria of incretin, which is rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP
agonists bind to membrane GLP receptors resulting in increase in insulin release from pancreatic β-cells. Endogenous GLP has a half life of only few minutes. DPP-4 inhibitors increase blood concentration of incretin GLP-1 by inhibiting its degradation.

1.11.3. Mineral supplementation
Several trace elements and other metal ions influence the insulin signaling and glucose metabolism, which serve as cofactors and/or components of enzymes and transcription factors e.g. chromium, magnesium and vanadium (Gurson CT, Saner 1971, Pugazhenthithi and Khandelwal 1993). Initially, these were considered to be the membrane-bound insulin receptor (Tamura et al. 1984) but later findings pointed out post receptor functions in the cytosol in addition to insulin receptor substarte 1 (IRS-1). Under conditions where good antilipolytic action occurred, vanadate shows marginal activation of phosphorylation of insulin receptor (Mooney et al. 1989).

However, treatment of IDDM with insulin injection is physically painfully and causes obesity as well as hypoglycemia. Similarly, prolong use of above mentioned chemically synthesized pharmacological drugs formulation are also associated with serious effects, which are depicted in table 2.

Table 2. Limitations of hypoglycemic medications

<table>
<thead>
<tr>
<th>Anti-Diabetic Drugs</th>
<th>Limitations/Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas</td>
<td>Hypoglycemia, weight gain</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Gastrointestinal disturbances</td>
</tr>
<tr>
<td>Alpha-glucosidase Inhibitors</td>
<td>Gastrointestinal disturbances</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Liver toxicity, weight gain, high LDL cholesterol, high cost</td>
</tr>
<tr>
<td>Meglitinides</td>
<td>Hypoglycemia, weight gain</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hypoglycemia, weight gain</td>
</tr>
</tbody>
</table>
Fig. 1.16. Sulphonylureas.

Fig. 1.17. Non-sulphonylureas

Fig. 1.18. PPAR-γ agonist.

Fig. 1.19. Biguanides

Fig. 1.20. α-Glucosidase inhibitors
1.12. Medicinal plants with antidiabetic effects

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. According to WHO, about 80% of the world’s population rely on traditional medicine for their primary health care. About 45,000 plants species have been reported in Traditional Health Care System. Plants possess large number of compounds, having innumerable pharmacological profiles. Wide range of plants have been used to treat various diseases and complications in the ancient medicinal systems, because natural products have safer side over synthetic drugs having little or no side effects (‘O’ Hara et al. 1998, Sani et al. 2009). Biological activities of various plants have been proven by phytochemical studies; among these alkaloids, glycosides, polysaccharide, peptidoglycanes, guanidine, steroids, glycopeptides, terpenoides, amino acids, inorganic ions have demonstrated pharmacological activity including treatment of diabetes and its complications. The mode of antihyperglycemic action of these phytochemicals is described in table 3 (Grover et al. 2002).

Table 3. Mode of action of phytochemicals derived from plant

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Constituents</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Inhibit α-glucosidase and decrease glucose transport through the intestinal epithelium</td>
</tr>
<tr>
<td>2.</td>
<td>Imidazoline compounds</td>
<td>Stimulate insulin secretion</td>
</tr>
<tr>
<td>3.</td>
<td>Polysaccharides</td>
<td>Increase serum insulin levels, reduce blood glucose levels and improve tolerance of glucose</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>Suppress glucose level, reduce plasma cholesterol and triacylglycerol significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic beta cell islets</td>
</tr>
<tr>
<td>5.</td>
<td>Saponin, Triterpenoid and steroidal glycosides</td>
<td>Effectively stimulates the absorption of glucose, retard glucose diffusion and inhibit the activity of alpha-amylase and may be responsible for decreasing the rate of glucose absorption and concentration of postprandial serum glucose</td>
</tr>
<tr>
<td>6.</td>
<td>Ferulic acid</td>
<td>Stimulatory effects on insulin secretion</td>
</tr>
</tbody>
</table>

Various studies have been conducted on many plants and important findings with some of these plants are briefly mentioned below:
1.12.1. *Acacia arabica* (Mimosaceae; Common name-Babul)

It has been used for the treatment of high cholesterol, diabetes, cancer, gingivitis, stomatitis. The seeds of *A. arabica* treatment produced significant hypoglycemic effect in alloxan-diabetic rabbits (p<0.05). It induces release of insulin from pancreatic beta cells (Bajpai et al. 1991).

1.12.2. *Aegle marmelos* (Rutaceae; Common name-Bael)

Alcoholic leaf extract of *A. marmelos* showed significant improvement in the utilization of external glucose load in glucose induced hyperglycemic rats. Aqueous extract of fruits of *A. marmelos* showed hypoglycaemic effect in streptozotocin induced diabetes in rats either by increasing utilization of glucose or by direct stimulation of glucose uptake through increased insulin secretion (Sachdewa et al. 2001).

1.12.3. *Allium sativum* (Alliaceae; Common name-Lahasun)

Antihyperglycemic effect of ethanolic extract of garlic was studied in streptozotocin-induced diabetic rats for 14 days showed significant glucose lowering effects in DM (Eidi et al. 2006). The antidiabetic effect of garlic is thought to be due to the formation of a colloidal type suspension in the stomach and intestines when the mucilaginous fiber of garlic is hydrated, therefore affecting gastrointestinal transit and slowing glucose absorption. Daily oral feeding of garlic extract at 100mg/kg bw increased plasma insulin level with concomitant decrease in plasma glucose level. It also potentiates glucose induced insulin secretion from pancreas (Mustafa, et al. 2007).

1.12.4. *Annona squamosa* (Annonaceae; Common name-Sharifa)

Aqueous leaf extract has shown hypoglycemic activity in streptozotocin-nicotinamide induced diabetic rats and rabbits (Mukherjee et al. 2006). It also plays important role in the reduction of oxidative stress of pancreas in STZ induced diabetes (Singh 2011).

1.12.5. *Azadirachta indica* (Meliaceae; Common name-Neem)

Studies showed that petroleum ether extract of neem showed significant protection against the oxidative damage induced by STZ in heart and erythrocytes of rats. The decrease in the activities of superoxide dismutase (SOD) and catalase (CAT) and increase in lipid peroxidation (LPO) of erythrocytes as observed in diabetes was regained after treatments (Gupta et al. 2004). A bitter principle isolated from seeds of the tree was effective at a dose of 200 mg/kg in reducing blood glucose and this effect
was due to blocking the action of epinephrine on glycogenolysis and peripheral utilization of glucose (Chattopadhyay 1996).

**1.12.6. *Camellia sinensis* (Theaceae; Common name-Tea)**

The hot water extract of *C. sinensis* significantly reduced the blood glucose level and was found to possess both preventive and curative effects on experimentally produced diabetes in rats. The green tea as well as black tea both possess antidiabetic activity and antioxidant effects (Gomes et al. 1995).

**1.12.7. *Cassia auriculata* (Cesalpinaceae; Common name-Tanner’s cassia)**

Extract of flowers of *C. auriculata* suppressed the elevated blood glucose and lipid levels in diabetic rats. Extract also significantly reduces tissue lipids (Pari et al. 2002). Gupta et al. 2010 also reported the antihyperglycemic effects of *Cassia auriculata* in mild and severe diabetes.

**1.12.8. *Catharanthus roseus* (Apocynaceae; Common name-Sadabahar)**

Administration of aqueous extracts of *V. rosea* flower and leaf has been found to regulate the blood sugar level in alloxan diabetic male albino rats (Ghosh et al. 2001). Ethanolic extract of *V. rosea* promotes significant wound healing and closure in diabetic rats (Nayak et al. 2006). Alkaloids isolated catharanthine, vindoline and vindolinine lower blood sugar levels (Mukherjee et al. 2006).

**1.12.9. *Cinnamom zeylanicum* (Lauraceae;)*

*In vitro* incubation of pancreatic beta cell islets with *C. zeylanicum* resulted in enhanced insulin release. The insulinotropic effect of cinnamaldehyde isolated from *C. zeylanicum* was due to increase in the glucose uptake through glucose transporter 4 (GLUT4) translocation in peripheral tissues (Anand et al. 2010).

**1.12.10. *Citrullus colocynthis* (Cucurbitaceae)**

*C. colocynthis* pulp extract at 300 mg/kg was found to significantly increase insulin and decrease plasma glucose levels in alloxan induced diabetes. Immunohistochemistry of insulin in beta cells of the pancreas is higher in *C. colocynthis* treated diabetic rats (Al-Khateeb et al. 2009). Aqueous extract of *C. colocynthis* showed dose dependent increase in insulin release from isolated islets (Bnouham et al. 2006). The extract of *C. colocynthis* showed the presence of free amino acids which induces insulin secretion from isolated pancreatic islets (Singh 2011).
1.12.11. *Eugenia jambolana* (Myrtaceae; Common name-Jamun)

Bark, leaves, seed and fruits of this plant are astringent. Juice of the fruit is stomachic, astringent, diuretic and antidiabetic (Grover et al. 2000). It also increased cathepsin B activity and had insulin secretagogue effects (Achrekar et al. 1991). Two active compounds have been isolated from pulp of *E. jambolana* showed significant glucose lowering effects (Sharma et al. 2006).

1.12.12. *Ficus bengalensis* (Moraceae; Common name-Bargad)

Water extract of *F. bengalensis* was given orally to diabetic rats showed significant fall in FBG and improvement in glucose tolerance (Shukla et al. 1994, Edwin et al. 2008). It also increases insulin levels in normal and diabetic rats. The increased insulin secretion is mainly due to inhibited insulinase activity from liver and kidney (Bnouham et al. 2006; Singh 2011). Blood sugar lowering activity of a dimethoxy derivative of leucocyanidin 3-0-beta-d-galactosyl cellobioside at a dose of 250 mg/kg bw isolated from bark of *F. bengalensis* in normal and diabetic rats and showed insulinomimetic activity (Ayodhya et al. 2010, Bnouham et al. 2006).

1.12.13. *Ficus racemosa* (Moraceae; Common name-Gular)

Amyrin acetate, isolated from the fruits of *F. racemosa* at the dose of 100 mg/kg body weight, significantly lowered the blood glucose levels after 5 and 24 hour, in sucrose challenged streptozotocin induced diabetic rat. Its p-chlorobenzoic acid derivative and nicotinic acid derivative showed potent antihyperglycemic activity at 100 mg/kg body weight (Narender et al. 2009).

1.12.14. *Momordica charantia* (Cucurbitaceae; Common name-Karela)

The anti-diabetic potential of *M. charantia* is well established in streptozocin or alloxan induced diabetic animals. *M. charantia* displays insulin-like properties, remarkably stimulates glycogen storage by the liver and improves peripheral glucose uptake (Reyes et al. 2006).

1.12.15. *Musa sapientum* (Musaceae; Common name-Kela)

Pari et al. (1999) reported the significant improvement in glucose tolerance in *M. sapientum* treated animals and the effect was compared with glibenclamide. The
antihyperglycemic and antihyperlipidemic effect of *M. sapientum* in STZ induced diabetes was reported by Dikshit et al. (2012). *M. sapientum* also have hepatoprotective effect in CCl₄ induced liver toxicity (Dikshit et al. 2011).

1.12.16. *Ocimum sanctum* (Lamiaceae; Common name-Tulsi)

Alcoholic extract of leaves of *O. sanctum* reduced blood sugar levels in normal and diabetic rats. In addition, the extract also showed favorable effect on glucose disposition in glucose fed hyperglycemic rats (Vats et al. 2002).

1.12.17. *Trigonella foenum graecum* (Fabaceae; Common name-Fenugreek)

Antihyperglycemic effect of extract of *T. foenum* have been linked to delayed gastric emptying caused by the higher fiber content, inhibition of carbohydrate digestive enzymes and stimulation of insulin secretion (Chauhan et al. 2010). Murthy et al. 1989 also reported the glucose lowering effect of *T. foenum* in diabetic rats. Galactomannan, extracted from *T. foenum* reported to reduce postprandial blood glucose response. The uptake of low or high concentration of glucose was significantly and progressively reduced by increasing concentrations of galactomannan in both lean and obese rats (Srichamroen et al. 2009). 4-hydroxyleucine, novel amino acid from fenugreek seeds showed increased glucose stimulated insulin release by isolated beta cell islets (Broca et al. 1999). A specific amino acids hydroxyisoleucine isolated from fenugreek also showed insulin stimulating effects. The seed of *T. foenum* may help to improve insulin sensitivity, which is presumed to be the effect of fiber, which slow carbohydrate metabolism (Kaczmar 1998).

1.13. **WITHANIA COAGULANS**

1.13.1. Distribution

*W. coagulans* is a small genus of shrubs, which are distributed in the East of the Mediterranean region and extend to South Asia i.e. drier parts of north and west India namely Punjab, Gujarat, Simla, Kumaon etc as well as Iran, Pakistan, Afghanistan etc.
1.13.2. Synonyms

English: Vegetable Rennet, Indian Cheese-maker, Unani-desi Asgandh, Kaaknaj-e-hind, Paneer, Paneer-band, Akri (fruit) etc.

Local name: Paneer phool, Dodi paneer, Puni-k-bij, Khamjira, Punir-ja-fota etc. (Naz 2002)

**Usable parts:** Whole plant, roots, leaves, stem, green berries, fruits, seeds and bark are usable.

**Fruits:** Carminative, depurative, used for dyspepsia, flatulence and strange. The properties are attributed to the pulp and husk of the berry. The berries contain a milk coagulating enzyme, esterase, free amino acids, fatty oil, essential oil and alkaloids (Khare 2007) ([Fig. 1.21](#)).

1.13.3. Phytochemistry

The main components of berries are esterases, fatty oil, amino acids such as proline, hydroxyproline, valine, tyrosine, aspartic acid, glycines, aspargines, cysteine and glutamic acid and alkaloids are the phytoconstituents (Atal and Sethi 1963). *W. coagulans* is rich in steroidal lactones, which are known as withanolides ([Fig. 1.22](#)). Withanolides are naturally occurring polyhydroxy C28 steroidal lactones (Mirjalili et al. 2009). In the basic structure of all withanolides a six- or five-membered lactone or lactol ring is attached to an intact or rearranged ergostane skeleton (Khodaei et al. 2012). They give a positive Dragendorff’s test even though they are not N-containing. On spraying the TLC with H$_2$SO$_4$–MeOH they give a characteristic blue colour spot (Maurya et al. 2010). This class of compounds does not occur in all members of the Solanaceae family. However, the occurrence of withanolides is not restricted to Solanaceae. They have also been reported from marine organisms (soft corals) and from members of plant families Taccaceae and Leguminoseae (Khodaei et al. 2012).

1.13.4. Active compound(s)

One of the characteristic features of the plants that produce withanolides is their extraordinary ability to introduce oxygen functions at almost every position of the carbocyclic skeleton and side chain. Modifications either of the carbocyclic skeleton or of the side chains result in many novel structural variants of withanolides.
**Fig. 1.21.** Fruits of *Withania coagulans*.

**Fig. 1.22.** Basic skeleton of Withanolides.
Phytochemical examination of the whole plant resulted in the isolation of 75 compounds, including 24 withanolides (Khodaei et al. 2012, Maurya et al. 2010). Withanolides having regular $17\beta$-oriented as well as unusual $17\alpha$-oriented side chains have been identified from *W. coagulans*.

### 1.13.5. Pharmacological properties

The berries of the plant are used for milk coagulation (Chadha, 1976) It has always had a prominent place in Ayurvedic, Unani, and ancient Indian systems of medicine. The fruits of the plant have been reported to have sedative, emetic, alterative and diuretic effects. They are useful in complaints of the liver, blood purifier, dyspepsia, flatulent colic and other intestinal infections (Hemalatha et al. 2008). These are also used for the treatment of asthma, biliousness and strangury (Kirtikar and Basu 1933).

#### 1.13.5.1. Antihyperglycemic / antidiabetic activity

*W. coagulans* exhibited hypoglycemic activity which is an effective and safe alternative treatment for diabetes (Budhiraja et al. 1977 & 1987, Hemalatha et al. 2004). Isolated alkaloids and steroids from plant sources are responsible for their hypoglycemic activity (Adebajo et al. 2006). The aqueous and chloroform extracts of the fruits decreased the blood glucose (55%) in diabetic rats (Hoda et al. 2010). Coagulin L isolated from fruits of *W. coagulans* showed significant glucose lowering effect in diabetic rats and the effects are comparable with standard antidiabetic drug metformin-treatment (Maurya et al. 2008). The Mg and Ca in *W. coagulans* are also responsible for diabetes management (Rai et al. 2007, Giugliano et al. 2000). It has been reported already that higher concentrations of Mg and lower concentrations of K play vital roles in diabetes management (Fox et al. 2001).

#### 1.13.5.2. Antihyperlipidemic and antioxidant activity

The aqueous extract of *W. coagulans* fruits in high fat diet induced hyperlipidemic rats, significantly reduced elevated serum cholesterol, triglycerides, lipoprotein and the LPO levels. This drug also showed hypolipidemic activity in triton induced hypercholesterolemia (Hemalatha et al. 2006). The extracted coagulin L from fruits of *W. coagulans* has antidysslipidemic effect on mice (Maurya et al. 2008). Aqueous and chloroform extracts of the fruits decreased triglyceride, total cholesterol, LDL and VLDL increased the HDL levels (Hoda et al. 2010, Lalsare and Chutervedi 2010).
1.13.5.3. Cardioprotective effect

The biosynthetic pathway leads from phytosterol precursors to the cardiac glycosides (important compounds in the treatment of cardiac insufficiency in humans) basically deduced from studies using radiolabelled precursors (Kreis and Muller-Uri 2010). An isolated new withanolide with a special chemical structure that was similar to the aglycones of the cardiac glycosides was examined for its cardiovascular effects of *W. coagulans* fruits. The withanolide caused a moderate drop of blood pressure in dogs (34 +/- 2.1 mm Hg) which was blocked by atropine and not by mepyramine or propranolol. In rabbits Langendorff preparation and ECG studies, produced myocardial depressant effects but in per-fused frogs hearts it caused mild positive inotropic and chronotropic effects (Budhiraja et al. 1983).

1.13.5.4. Hepatoprotective effects

The aqueous extract of fruits of this plant has been shown to exert hepatoprotective activity. Since the steroidal compounds (glucocorticoids) having anti-inflammatory properties are used in some hepatic disorders, 3-b-hydroxy-2,3 dihydrowithanolide F has been screened for its hepatoprotective effect (Budhiraja et al. 1986).

1.13.5.5. Antifungal and antibacterial activity

The volatile oil from the fruits of *W. coagulans* showed antibacterial and antifungal activity (Choudhary et al. 1995, Khan et al. 1993, Atta-ur-Rahman and Choudhary 1989, Gaind and Budhiraja 1967). Withanolide D has antifungal cytotoxic activity on thirteen fungi which are responsible for human infectious (five dermatophytes, one nondermatophyte mold, six yeasts and *Pneumocystis carinii*) (Roumy et al. 2010). Lalsare et al. (2010) showed antioxidant and antimicrobial activities of various extracts of *W. coagulans* fruits.

1.13.5.6. Immunomodulating activity

Withaferin A and withanolide E were reported to have specific immunosuppressive effects on human B and T lymphocytes as well as on mice thymocytes (Shohat et al. 1978). Various withanolide were evaluated for its effect on various cellular functions related to immune responses including lymphocyte proliferation, interleukin-2 (IL-2) cytokine expression. Coagulin-H was found to have a powerful inhibitory effect on lymphocyte proliferation and the Th-1 cytokine production. The inhibition of the phytohemaglutinin (PHA) activated T-cell proliferation by coagulin-H (Mesaik et al. 2006).