CHAPTER – I

INTRODUCTION

Chapter 1

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action or both. The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. Over 20 million people are affected by diabetes in India. These numbers are expected to increase to 57 million by 2025. In the 1970s, the prevalence of diabetes among urban Indians was reported to be 2.1 per cent and this has now risen to 12.1 per cent (Pradeepa and Mohan, 2002). People with diabetes are 25 times more likely to develop blindness, 17 times more likely to develop kidney disease, 30-40 times more likely to undergo amputation, two to four times more likely to develop myocardial infraction and twice as likely to suffer a stroke as non-diabetics (Pradeepa *et al.*, 2002)

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and in a modified form, in 1985. The former classification was widely accepted and used internationally which included both staging of diabetes mellitus based on clinical descriptive criteria and a complimentary etiological classification. This disease is generally classified into two main subtypes: type-I or insulin-dependent diabetes (IDDM), and type-II or non-insulin-dependent diabetes (NIDDM).

Type 1 diabetes (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin primarily due to pancreatic islet beta cell destruction and more prone to ketoacidosis, for which neither an etiology nor a pathogenesis is known (idiopathic). This form develops most frequently in children and adolescents, but is being increasingly noted later in life and is attributed to an autoimmune process.

Type 2 diabetes (formerly named non-insulin-dependent) which results from defect(s) in insulin secretion almost always with a major contribution from insulin resistance, the body's inability to respond properly to the action of insulin. Type 2 diabetes is much

more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly ancestral, and it is noted that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene as well as by environmental factors.

People with Type 1 diabetes are totally dependent on insulin injections for survival. Such people require daily administration of insulin. The majority of people suffering from diabetes type II form do not depend on insulin for survival, but about one third of sufferers need insulin for reducing their blood glucose levels.

Other forms of diabetes include (WHO and NDDG classification):

- Gestational diabetes.
- Genetic defects of β cell function.
- Genetic defects in insulin action.
- Diseases of the exocrine pancreas.
- Endocrinopathies.
- Drug or chemical induced.
- Infections.
- Uncommon forms of immune mediated diabetes.
- Other genetic syndromes sometimes accompanied with diabetes.

Diabetes in pregnancy may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of prenatal mortality. Strict metabolic control may reduce these risks to the level of non-diabetic expectant mothers.

Diagnostic criteria for diabetes mellitus:

Fasting plasma sugar (FPS), postprandial plasma sugar (PP_2PS) and oral glucose tolerance test (OGTT) are the golden criteria for the diagnosis of diabetes mellitus. Generally FPS after overnight fasting and PP_2PS, two hour after a meal is routinely

checked for the diagnosis. OGTT is recommended in case where person is not seriously diabetic.

Recently a new classification and diagnostic criteria for diabetes were proposed by the American Diabetes Association (ADA), WHO and Japan Diabetes Society (JDS) between 1997 and 1999. Diabetes is classified in to four etiological categories; type 1, type 2, diabetes due to other specific mechanism or conditions and gestational diabetes. Following plasma glucose levels [fasting plasma glucose (FPS) 2-h plasma glucose in the 75g oral glucose tolerance test (2-hPG)] has been suggested for the diagnosis of diabetes:

Normal type - FPS < 6.1 mmol / L (110 mg/dl) and 2-hBG < 7.7 mmol/L (140 mg/dl)

Borderline type - FPS > 6.1 mmol / L (110 mg/dl) or = < 7.0 mmol / L (126 mg/dl)

PP_2PS> 7.7 mmol/L (140mg/dl) or = < 11.1 mmol / L (200 mg/dl)

Diabetic type - FPS > 7.0 mmol / L (126 mg/dl) and PP_2PS > 11.1 mmol/L (200 mg/dl)

Borderline corresponds to the sum of impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) based on ADA and WHO criteria.

Prognostic criteria: Prognosis of the disease is monitored by conducting following test:

Fasting plasma sugar (FPS) and Postprandial plasma sugar (PP_2PS): FPS > 7.0 mmol/L (126 mg/dl) and PP_2PS > 11.1 mmol/L (200 mg/dl) were recommended as high risk values for hyperglycemia.

Glycosylated Hb:

Glycosylated Hb levels provide an average picture of the patient's blood glucose concentration over the past 3 months.

Acidosis:

Decreased blood pH - < 7.4 is the indicator of acidosis.

Ketoacidosis:

Ketone bodies in serum > 1mEq/L can lead to acetone breath and ketoacidosis.

Nephropathy:

Microalbuminuria and proteinuria are the indicators of diabetic nephropathy.

Diabetic retinopathy:

Diabetic retinopathy is detected clinically by presence of visible opthalmoscopic retinal microvascular lesions and estimation of Advanced Glycated Endproducts (AGEs).

Long Term Complications of diabetes:

ORGANS AFFECTED	PATHOLOGICAL CHANGES	COMPLICATIONS
BLOOD VESSELS	Atherosclerotic plaque builds up and blocks large or medium-sized arteries in the heart, brain, legs and penis. The walls of small blood vessels are damaged so that the vessels do not transfer oxygen normally and may leak	Poor circulation causes wounds to heal poorly and can lead to heart disease, stroke, gangrene of the feet and hands, impotence and infections
BLOOD	White blood cell function is impaired	Increased susceptibility to infection, especially of the urinary tract and skin.
EYES	The small blood vessels of the retina become damaged	Decreased vision and ultimately blindness
KIDNEYS	Blood vessels in the kidney thicken; protein leaks in to the urine	Poor kidney function; kidney failure
NERVES	Nerves are damaged because glucose is not metabolized normally due to inadequate blood supply.	Sudden or gradual loss of body weight; reduced sensations, tingling and pain in the hands and feet,

		chronic damage to nerves
AUTONOMIC NERVOUS SYSTEM	The nerves that control blood pressure and digestive processes become damaged	Swings in blood pressure, swallowing difficulties and altered gastrointestinal function with bouts of diarrhoea.
SKIN	Poor blood flow to the skin and loss of feeling resulting in repeated injury	Sores, deep infections (diabetic ulcers; poor healing)
CONNECTIVE TISSUE	Glucose is not metabolized normally, causing tissues to thicken or contract.	Carpal tunnel syndrome; Dupuytrens's contractive.

Diabetes mellitus – consequence of insulin deficiency/resistance:

The defects in insulin deficiency or resistance leads to hyperglycemia – the hallmark of the X syndrome i.e. diabetes mellitus. As β cell destruction progresses in case of IDDM, plasma insulin levels fall even during the fasted state, hepatic glucose production increases, and the patient requires insulin therapy (Eisenbarth *et al.*, 1987). With more severe insulin deficiency, plasma FFA levels increase in response to enhanced lipolysis and plasma triglyceride levels may increase because of a decrease in lipoprotein lipase activity (Ong and Kern, 1989). Deficiency of insulin or increase in counter-insulin hormones is sufficiently severe to increase glycogen, protein and lipid catabolism which lead to the elevation of plasma FFA and ketone bodies. Warram *et al.*, in 1990 reported that NIDDM is a type of hyperinsulinemia associated with insulin resistance. Patients with type II diabetes and obesity have been shown to have a significant defect in glucose uptake in skeletal muscle (DeFronzo, 1992) and also there was a decrease in muscle

glycogen synthesis (DeFronzo *et al.*, 1985), and an increase in lactate production (Bogardus *et al.*, 1984). Such inhibition of glycogen synthesis causes an increase in glucose-6-phosphate, suggesting a defect in glucose uptake or decreased activity of hexokinase. Decrease in pyruvate dehydrogenase activity is also seen contributing to the decrease in glucose oxidation and increase in muscle lactate release (Mandarino *et al.*, 1986). Hyperglycemia may worsen insulin resistance leading to "glucose toxicity" (Rossetti *et al.*, 1990). Insulin has a profound effect on protein turnover, through it plays a dual role as a stimulator of protein synthesis and an inhibitor of protein degradation. Any failure in insulin function leads to impaired carbohydrate, fat and protein metabolism. As a result, elevated levels of glucose in the plasma, free fatty acids, triglycerides, cholesterol, VLDL, ketone bodies, etc. are encountered. All these consequences lead to oxidative stress, lipid peroxidation there by resulting in diabetic complications.

Lipid peroxidation:

Polyunsaturated fatty acids are important components of the membranes surrounding protoplast and cellular organelles. They are double layered structure.

Lipid peroxidation has received much attention recently because of its possible contributions to cancer, diabetes mellitus and aging. It is the oxidative deterioration of polyunsaturated fatty acids (PUFA) in which PUFA are converted to lipid hydroperoxides (LOOH). Lipid peroxidation is a free radical related process. Oxidants can react with polyunsaturated fatty acids in cell membranes, to form toxic metabolites. The reactive oxygen species (ROS) gets attached with the lipids that contain at least two or more unsaturated carbon-carbon bonds (C=C). During initiation step the removal of the allylic hydrogen from the PUFA forms a lipid radical (L°). Forming a more stable lipid radical, whose dienes are conjugated (Gutteridge, 1995). In an aerobic environment this radical reacts with oxygen, giving rise to a lipid peroxyl radical (Loo°). Because of its highly reactive properties, hydroxyl radical react non selectively with unsaturated fatty acids to form a carbon-centered radical, either by addition to double bonds or by abstraction of a hydrogen atom from the system (Mc cay *et al.*, 1984).

Carbon-centered radicals, which are produced from both the initiation and propagation processes, undergo molecular rearrangements. Lipid peroxyl radical abstracts an allylic hydrogen atom from another lipid molecule such as an adjacent PUFA, resulting in the production of lipid hydroperoxide (LOOH) and a second lipid radical (L°). This second lipid radical can proceed through the same reactions as the first, generating additional lipid hydroperoxides. The Loo• radicals are able to substract a hydrogen atom from another lipid molecule such as an adjacent fatty acid. This causes the propagation of the lipid peroxidation. The carbon radical formed can further undergo the oxygen addition reaction to form another peroxyl radical and so the chain reaction of lipid peroxidation continues.

Loo• + L-H• LOOH + L•

L• + 0_2 • Loo•

The termination events can be the result of any reaction with another radical, protein, or compound that acts as a free radical trap, forming a stable end product. These endoperoxides can start the reactions to form several biologically active, postaglandins, thromboxanes and leukotrienes via the cyclo oxygenase and lipoxygenase pathways. Non-enzymatic pathways lead to the formation of compounds such as isoprostanes, aldehydes and alkanes, which can also have concentration dependent signaling or cytotoxic effects *in vivo* (Begin 1989). Formation of these end products constitutes the termination stage of lipid peroxidation.

1. Removal of lipid peroxyl radicals

Cells have a number of ways to protect against the constant threat of the radicals produced in lipid peroxidation. There are enzymatic species that act to minimize the detrimental effect of radicals. These enzymes include superoxide dismutase, glutathione peroxidase and catalase (Das and Nair, 1980). Also there are numerous removal and repair enzymes such as phospholipase A_2 available to repair the damaged molecules (Sevanian and Kim, 1985). Cells are also known to utilize many non-enzymatic antioxidant compounds that have received considerable attention such as vitamin E (α-

tocopherol) and vitamin C (ascorbic acid) (Halliwell and Gutteridge 1989). α-Tocopherol, a fat soluble vitamin, is considered to be the major membrane-bound antioxidant used by the cell. On the other hand, ascorbic acid is regarded as the major aqueous phase antioxidant. Recent evidences suggest that α- tocopherol and ascorbic acid function together in a cyclic- type of reaction (Buettner, 1993). During this process, α- tocopherol is converted to a radical by donating liable hydrogen to a lipid peroxyl radical. The oxidized α- tocopherol radical is energetically stable and react slowly with other molecules within the membrane. Oxidized α- tocopherol can then be re-reduced to its original form by ascorbic acid. This regeneration of reduced α- tocopherol presumably occurs at the surface of the membrane where ascorbic acid and α- tocopherol can meet. Along with acting as a reducing agent for α- tocopherol, ascorbic acid is also considered as preventative antioxidant because of its ability to scavenge for reactive radicals (Buettner, 1993).

Peroxidation of lipids causes damages to cell membrane because lipids are the main components of the cell membrane. Initiation of peroxidation occurs when a radical species attacks and removes allylic hydrogen from an unsaturated fatty acid, resulting in a radical chain reaction. Once lipid peroxidation begins, iron may participate in driving the process. The possible sources of iron in body are those iron-containing proteins. There are a number of methods available to measure the markers of lipid peroxidation, either by measuring the formation of products, or the degradation of reactants. The most utilized method is TBARS test.

1. Reactive Oxygen Species

The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen ($^3O_2$). This process is mediated by enzymes such as NAD (P) H oxidases and xanthine oxidase or nonenzymically by redox reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain. In biological tissues superoxide can also be converted nonenzymically into the nonradical species hydrogen peroxide and singlet oxygen ($^1O_2$) (Steinbeck *et al.*, 1993). In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into

the highly reactive hydroxyl radical (•OH). Superoxide and NO are readily converted by enzymes or nonenzymic chemical reactions into reactive nonradical species such as singlet oxygen ($^1O_2$), hydrogen peroxide, or peroxynitrite ($ONOO^-$), which can in turn give rise to new radicals.

2. Reactive Nitrogen Species

The NO radical (NO•) is produced in higher organisms by the oxidation of one of the terminal guanidonitrogen atoms of L-arginine. This process is catalyzed by the enzyme NOS. Depending on the microenvironment, NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO^+), nitroxyl anion (NO^-) or peroxynitrite ($ONOO^-$). Some of the physiological effects may be mediated through the intermediate formation of S-nitroso-cysteine or S-nitroso-glutathione (Walker *et al.*, 2001).

Antioxidant Defense System:

To counter the harmful effects of these peroxidants, antioxidant defense mechanism operates to detoxify or scavenge these free radicals. This defense line consists of antioxidant enzymes like – superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non-enzymatic molecules like – reduced glutathione (GSH), vitamin C, vitamin E etc. Oxidative stress has been implicated in the pathogenesis of diabetes mellitus. Researchers have found that oxidative stress plays a role in the damage to tissues caused by diabetes due to hyperglycemia. Reports had shown elevated activities in tissue CuZn – SOD, glutathione reductase, catalse and decreased levels of reduced glutathione (Wohaieb and Godin, 1987) along with increased lipid peroxidation (Asayama *et al.*, 1989) in experimentally induced diabetic animals. Similarly, onset of diabetes altered the antioxidant status and defense system of diabetic patients (Dominguez *et al.*, 1998; Telci *et al.*, 2000; Sekeroglu *et al.*, 2000).

1. Superoxide dismutase (SOD) (EC 1.15.1.1)

The enzyme superoxide dismutase, or SOD, is a metalloenzyme which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

$$2O^{2}- + 2H^{+} \rightarrow O_2 + H_2O_2$$

Three isozymes of superoxide dismutase identified in mammals are mitochondrial MnSOD, which contains a manganese ion, CuZn containing extracellular SOD (EC SOD) and CuZnSOD, found primarily in the cytoplasm and nucleus of cells. The Cu-Zn enzyme is a dimer of molecular weight 32,500. The two subunits are joined by a disulfide bond (Keele *et al.*, 1971). The CuZnSOD gene, SOD1, is located on human Chromosome 21 at location 21q22.1. An alanine (GCT) to valine (GTT) substitution at position -9 in the signal peptide of human Mn-SOD has been shown to change the structural conformation of the mitochondrial targeting sequence of the enzyme leading to misdirected intracellular trafficking (Shimoda-Matsubayashi, 1996). Extracellular superoxide dismutase (EC-SOD) has an amino acid substitution Arg213Gly in the heparin-binding domain (Sandstrom *et al.*, 1994). The glycine variant of the enzyme is responsible for high EC-SOD levels in serum (Yamada *et al.*, 1997) that are correlated with a decrease in nitric oxide production in epithelial cells (Adachi and Wang, 1998) and various other metabolic cardiovascular risk factors (Marklund *et al.*, 1997).

2. Catalase (CAT) (EC 1.11.1.6)

Catalase (EC 1.11.1.6), present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The mechanism of catalysis is not fully elucidated, but the overall reaction is as follows:

$$2\,H_2O_2 \rightarrow 2\,H_20 + O_2$$

The protein exists as a dumbbell-shaped tetramer of four identical subunits (220,000 to 350,000 kD). Each monomer contains a heme prosthetic group as the catalytic center. Catalase monomers from certain species (e.g. cow) also contain one tightly bound NADP per subunit. This NADP may serve to protect the enzyme from oxidation by its H_2O_2 substrate (Eventoff, 1976). The CAT gene is located on human chromosome 11 at location 11p13. There are reports on the quantitative deficiency of catalase leading to cumulative oxidant damage of pancreatic β-cells and were at risk for atherosclerosis and diabetes (Goth and Eaton, 2000). The kind of diabetes involved with catalase deficiency

may not be classic type II diabetes in which individuals progress from impaired glucose tolerance with modest hyperglycemia and hyperinsulinemia to overt diabetes. The low insulin and C-peptide values in most of the nondiabetic hypocatalasemic subjects and in the diabetic subjects with catalase deficiency indicate that most diabetic and diabetes susceptible individuals with catalase deficiency may not have hyperinsulinemia of classic type II diabetes.

3. Glutathione Peroxidase (GPx) (EC 1.11.1.9)

The GPx gene is located on human chromosome 3 at location 3p21.3. It has a molecular weight of 84,000 and 4 subunits per mol of enzyme. Glutathione peroxidase catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione as hydrogen donor. Four distinct species of glutathione peroxidase have been identified in mammals to date, the classical cellular enzyme, the phospholipid hydroperoxide metabolizing enzyme, the gastroinestinal tract enzyme and the extracellular plasma enzyme.

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

Where GSH represents reduced monomeric glutathione and GSSG represents oxidized glutathione. Glutathione reductase then reduces the oxidized glutathione to complete the cycle:

$$GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$$

It has been suggested that this enzyme functions many times as a mechanism of protecting the cellular membrane system against peroxidative damage, and the importance of selenium as an essential trace element is further concerned with this suggested function of the enzyme. This enzyme is useful for enzymatic determination of lipid hydroperoxide.

4. Reduced Glutathione (GSH)

Glutathione (γ-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system. Glutathione often attains millimolar levels inside

cells, which makes it one of the most highly concentrated intracellular antioxidants. Glutathione exists in two forms: The antioxidant "reduced glutathione" tripeptide is conventionally called glutathione (GSH) and the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide (GSSG). The GSSG/GSH ratio may be a sensitive indicator of oxidative stress (Parris, 1997). Glutathione status is homeostatically controlled both inside the cell and outside, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), its recycling from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

The GSH can act as free-radical scavenger and as an antioxidant enzyme cofactor. Glutathione is most concentrated in the liver (10 mM), where the "P_{450} Phase II" enzymes require it to convert fat-soluble substances into water-soluble GSH conjugates, in order to facilitate their excretion. The liver parenchymal cells also export GSH to the outside, where it serves as systemic source of -SH/reducing power. GSH depletion leads to cell death, and has been documented in many degenerative conditions. Mitochondrial GSH depletion may be the ultimate factor determining vulnerability to oxidant attack.

Glutathione is an essential cofactor for antioxidant enzymes, namely the GSH peroxidases (both Se-dependent and non-Se-dependent forms exist) and the more recently described phospholipid hydroperoxide GSH peroxidases. The GSH peroxidases serve to detoxify peroxides (hydrogen peroxide, other peroxides) in the water-phase, by reacting them with GSH; the latter enzymes use GSH to detoxify peroxides generated in the cell membranes and other lipophilic cell phases. Enzymes collectively known as GSH transhydrogenases use GSH as a cofactor to reconvert dehydroascorbate to ascorbate, ribo-nucleotides to deoxyribonucleotides, and for a variety of -S-S- <--> -SH inter-conversions.

Diabetes Mellitus and carbohydrate Metabolism:

Insulin influences the intracellular utilization of glucose in a number of ways. One of the major effects of insulin is to enhance overall glucose disposal and this is achieved by stimulation of glucose uptake into the target tissues.

Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase phosphofructokinase and pyruvatekinase. Enhanced glycolysis increases glucose utilization and thus indirectly decreases glucose release in to plasma. Insulin also decreases the activity of glucose-6-phosphatase, this action of insulin results in the retention of glucose within the liver cell.

In skeletal muscles, insulin promotes glucose entry through the transporter and increases the activity of hexokinase, which phosphorylates glucose and initiates glucose metabolism. Other than this it also stimulates lipogenesis in the adipose tissue by providing acetyl-CoA and NADPH which are required for fatty acid synthesis.

The final action of insulin on glucose utilization involves another anabolic process in liver and muscle, insulin stimulates the conversion of glucose to glucose-6-phosphate which then undergoes isomerization of glucose to glucose-1-phosphate and is incorporated in to glycogen by the enzyme glycogen synthase, the activity of which is stimulated by insulin. This action is indirect and dual in nature. The actions of insulin on glycolysis and glycogenesis occur with in seconds or minutes, since they primarily involve the activation or inactivation of enzymes by phosphorylation or dephosphorylation.

Dyslipidemia and diabetes:

Abnormalities in lipoproteins are very common in both individuals with non-insulin-dependent diabetes (NIDDM) and insulin-dependent diabetes (IDDM). They are mainly induced by diabetes associated complications such as obesity and renal disease. In IDDM patients due to the obligatory requirement for insulin therapy, a variety of situation is possible with greatly elevated glucose, FFA, ketones, lipolytic hormones. Extreme elevations in VLDL levels have been recognized as being a common occurrence in diabetic ketoacidosis (Bagdade *et al.*, 1967). Elevation in VLDL triglycerides in IDDM are often correlated with the degree of diabetic control (Lopes-Virella *et al.*, 1981).

In poorly controlled diabetes, levels of triglycerides and LDL cholesterol are increased and HDL cholesterol is decreased. The hypertriglyceridaemia particularly improves rapidly with insulin therapy and an improvement in glycaemic control. Type 1 patients with nephropathy develop protein abnormalities, resulting in high LDL cholesterol even at the stage of microalbuminuria. Lipid abnormalities occur more frequently in Type 2 diabetes than in the non-diabetic state. The characteristic of dyslipidaemia is increased triglyceride level, low HDL cholesterol and high LDL cholesterol in plasma. The dyslipidaemia of Type 2 diabetes is closely related to insulin resistance and hyperinsulinaemia and is a component of Syndrome X or Reaven's Syndrome (glucose intolerance, hypertension, dyslipidaemia with accelerated atherosclerosis and truncal obesity).

It is well known that obesity is associated with insulin resistance and an increased risk for type 2 diabetes mellitus. Previously it was postulated that increased lipolysis would disrupt glucose homeostasis via Randle's hypothesis. Lipodystrophy, however, also leads to insulin resistance. Recently it has become clear that adipose tissue functions as an endocrine organ and secretes numerous proteins in response to a variety of stimuli. These secreted proteins exert pleiotropic effects which include leptin, resistin, adiponectin, acylation-stimulating protein, tumour necrosis factor-alpha and interleukin-6. None of these proteins are, however, without controversy with regard to their mechanism of action. Furthermore, some of these proteins may influence each other via common signalling pathways. A theory is presented to link the interrelationship between these adipocyte secretory products and their effect on insulin resistance (Jazet *et al.*, 2003).

Non-diabetic obese humans adapt to insulin resistance by increasing β-cell mass. In contrast, obese humans with type 2 diabetes have an approximately 60% deficit in β-cell mass. Recent studies in rodents reveal that β-cell mass is regulated in response to insulin resistance through increased β-cell supply (islet neogenesis and β-cell replication) or decreased β-cell loss (beta-cell apoptosis). Prospective studies of islet turnover are not possible in humans. The frequency of β-cell apoptosis was related to the rate of increase

of islet amyloid. These prospective studies suggest that the formation of islet amyloid is related to increased beta-cell apoptosis in a murine model of type 2 diabetes (Butler *et al.*, 2003).

Diabetic animal models:

Study of multifactorial genetics of diabetes is feasible due to availability of various types of genetically diabetic animal models. Animal models also provide unique opportunities for investigating the toxicity and efficiency of therapeutic measures developed for prevention and cure of diabetes and its complications. A large array of animal models are available for experimentation relevant to the study of diabetes especially the rats or mice like db/db mouse (obese rodent with severe diabetes), ob/ob mouse (obese rodent with mild diabetes), sand rat (nutrition induced diabetes), GK rats (Goto-kakizaki-diabetes developed by selective in breeding), NOD mouse (Non-obese diabetic-spontaneous diabetes), BB rats (Biobreeding rats-spontaneous diabetes) and experimentally induced diabetic rats/mice by chemical diabetogens like alloxan and streptozotocin. In the present study experimentally induced diabetic rat models were used. Alloxan as well as streptozotocin were used simultaneously. Alloxan cause selective β cells destruction by generating ROS (Munday, 1988; Malaisse, 1982). Low affinity glucose transporter $GLUT_2$ and glucokinase make β-cells susceptible for alloxan toxicity (Elsner *et al.*, 2002). The degree of β-cell destruction depends upon the dose, which is administrated to induce diabetes in rats. The mechanisms of STZ- induced hyperglycemia are considered as follows: (1) STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly(ADP- ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibit proinsulin synthesis and induces diabetes (Wilson *et al* ., 1990).

Therapies for Diabetes mellitus:

a. Hypoglycemic Drugs

1. Sulfonylureas (Sulpha group containing drug)

(Tollbutamide, Chlorpropamide, Glibenclamide, Glipizide, Gliclazide)

Sulfonylureas work by stimulating insulin release from the β - cells of the pancreas and may slightly improve insulin resistance in peripheral target tissues (muscle, fat). This class of compounds reduces glycosylated hemoglobin A_{1c} (HbA_{1c}) levels by 0.8 to 2.0 percent and fasting blood glucose (FBS) concentrations by 60 to 70 mg/dL (3.3 to 3.9 mmol/L). At the initiation of therapy, the greatest reduction is observed in patients with the highest FPG concentration (DeFronzo, 1999). Hypoglycemia is the most difficult side effect of the sulfonylureas. It is of particular concern with agents that are metabolized to an active metabolite with significant renal excretion. These agents include chlorpropamide (Diabinese) and glyburide, both should be avoided in the setting of impaired renal function and used with caution in elderly patients. Glipizide and glimepiride are associated with a lower incidence of hypoglycemia. All sulfonylureas have been associated with weight gain and thus, may not be the optimal choice for obese patients.

2. Meglitinides

Repaglinide (Prandin) is a new non-sulfonylurea insulin secretagogue agent, available from the meglitinide class. Nateglinide (Starlix), the newest member of the class, closely resembles that of the sulfonylureas in its action. 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 - cell, and the drug has somewhat different characteristics when compared with the sulfonylureas. Unlike the commonly used sulfonylureas, the meglitinides have a very short onset of action and a short half-life. Repaglinide has shown similar effects on HbA_{1c} and FPG levels when compared with glyburide, 0.5 to 2 percent and 65 to 75 mg/dL (3.6 to 4.2 mmol/L), respectively (Luna *et al.*, 1999). Some potential advantages of this class of agents include a greater decrease in postprandial glucose and a decreased risk of hypoglycemia.

3. Biguanides

Metformin works by reducing hepatic glucose output and, to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues. Metformin has been shown to reduce HbA_{1c} levels by approximately 1.5 to 2.0 percent and FPG levels by 50 to 70 mg/dL (2.8

to 3.9 mmol/L) (DeFronzo, 1999). Other effects include a reduction in plasma triglyceride levels and low-density lipoprotein (LDL) cholesterol levels.

On the whole, metformin has a favorable profile of side effect. Most of the related side effects (including metallic taste, gastrointestinal discomfort and nausea) are transient and commonly reported only during initiation of therapy. Slow-dosage is recommended to decrease these effects. Taking the drug with meals may also lessen the severity of the gastrointestinal side effects, as metformin does not affect insulin secretion. It is not associated with hypoglycemia when used as monotherapy, but can potentiate hypoglycemia when used in combination with a sulfonylurea or insulin. A rare, but more worrisome potential adverse effect is that of lactic acidosis. Metformin is unusual among the oral antidiabetic drugs in that therapy and has been associated with a lack of weight gain and even weight loss in some overweight patients (Hermann *et al.*, 1994). Although these effects make it an ideal first-line agent in overweight patients, results from studies have shown that metformin also improves glycemic control in patients who are not overweight.

4. Thiazolidinediones

The thiazolidinediones work by enhancing insulin sensitivity in both muscle and adipose tissue to a lesser extent by inhibiting hepatic glucose production. These agents have a notable effect on improving insulin resistance, particularly when used in combination with other antidiabetic drugs, but have no effect on insulin secretion. Monotherapy with these agents have been associated with a 0.5 to 1.5 percent reduction in HbA_{1c} levels and 25 to 50 mg/dL (1.4 to 2.8 mmol/L) reduction in FPG levels (DeFronzo, 1999). As a class, the thiazolidinediones have also shown to alter lipid profiles in patients with type II diabetes. Results from studies with troglitazone consistently showed a decrease in triglyceride levels--in some cases by 33 percent (Saltiel & Olefsky, 1996). The effects on high density lipoprotein (HDL) cholesterol levels have been either favorable or neutral, while some studies report have shown an increase in total and LDL cholesterol levels. New datas reveal that in monotherapy, rosiglitazone is associated with increase in total, LDL and HDL cholesterol levels and no change or increase in triglyceride levels.

Patients treated with pioglitazone have displayed significant decreases in triglyceride levels, mean increases in HDL cholesterol levels, and no consistent mean changes in LDL and total cholesterol levels. Thiazolidinediones have been shown to interfere with expression and release of mediators of insulin resistance originating in adipose tissue (e.g., increased free fatty acids, decreased adiponectin). Prevention of lipid accumulation in tissues critical to glycaemia such as visceral adipocytes, liver, muscle and β-cells at the expense of lipids accumulating at the less harmful subcutaneous site may be central to their net metabolic effect. Moreover, their anti-inflammatory properties also make them interesting in the prevention and treatment of atherosclerosis and possibly in other inflammatory conditions (e.g., inflammatory bowel disease) (Stumvoll, 2003).

5. Alpha-glucosidase inhibitors

Acarbose (Precose) and miglitol (Glycet) are the two agents available in this class. Alpha-glucosidase inhibitors act by inhibiting the enzyme alpha-glucosidase found in the brush border cells that line in the small intestine, which cleaves more complex carbohydrates into sugars. They inhibit the breakdown and subsequent absorption of carbohydrates (dextrins, maltose, sucrose and starch; no effect on glucose) from the gut after meals. The largest impact of this drug is on postprandial hyperglycemia. Their effect on FPG levels is modest. They have been associated with a reduction in HbA_{1c} by 0.7 to 1.0 percent and FPG levels by 35 to 40 mg / dL (1.9 to 2.2 mmol / L) (DeFronzo, 1999). Thus, these agents are most useful in patients who have mild FPG elevations or in patients with predominant postprandial hyperglycemia. The most bothersome side effects observed with these agents are gastrointestinal, including abdominal discomfort, bloating, flatulence and diarrhea but are reversible with discontinuation. Therapy with acarbose has been linked to elevations in serum transaminase levels and the use of this agent is contraindicated in patients with liver cirrhosis.

6. Insulin

Insulin treatment is necessary in case of IDDM to control hyperglycemia and development of the ketoacidosis. Maximum decline occurs in plasma glucose at 30 minutes following intravenous insulin administration and at 2-3 hours after subcutaneous

insulin administration. Various forms of insulin like rapid acting, intermediate and long acting are commercially available. Insulin administration is also associated with some side effects like hypoglycemic shock, weight gain and an increased risk of atherogenesis (Sinha *et al*, 1996; USKPD, 1998).

7. Gene and Islet therapy

Gene and islet transplantation therapy can provide an ideal solution for the treatment of IDDM. Tremendous experimental efforts are in progress to make transplanted islets more viable and functional for a longer period. Scientists are trying to make human/non-human engineered insulin producing cells suitable for graft within special immunoisolation barrier membranes. A significant number of animal studies have been demonstrated for the potential of islet cell transplantation in restoring the normoglycemia in context of immuno-regulation achieved by gene transfer of immuno-regulatory genes to allo- and xenogenic islets ex vivo. Gene and cell therapy is also used to induce tolerance to auto- and allo-antigens and to generate the tolerance state in autoimmune rodent model of type I diabetes. For human diabetics, islet transplantation is still under experimental stage. Successful clinical trails are being conducted with these advance strategies to achieve the final goal i.e. the cure of IDDM. The achievement of gene and cell therapy in type 2 diabetes is less evident. Type 2 diabetes will likely require a better understanding of the processes that determines the insulin sensitivity in the periphery (Giannoukakis and Robbins, 2002).

8. Other Additive Therapies

Exercise

Exercise helps insulin to work better and lower blood glucose, blood pressure and cholesterol levels. It also strengthens the heart and improves blood circulation and reduces body fat and thus controls body weight.

9. Vitamin E and α-Lipoic acid

Diabetes produces a state of increased free radical activity. The purported effects of vitamin E on glucose control relates to the vitamin's potent lipophilic antioxidant activity,

with possible influences on protein glycation, lipid oxidation, insulin sensitivity and secretion. Through unknown mechanisms, it may also affect nonoxidative glucose metabolism (Mooradian *et al.*, 1994) α-Lipoic acid, also known as thioctic acid, a disulfide compound synthesized in the liver is another potent lipophilic antioxidant. It is a cofactor in many multienzyme complexes and may also play a role in glucose oxidation (Konrad *et al.*, 1999). Experimental *in vitro* data have shown possible effects in enhancing glucose uptake in muscle and preventing glucose-induced protein modifications.

b. Metals

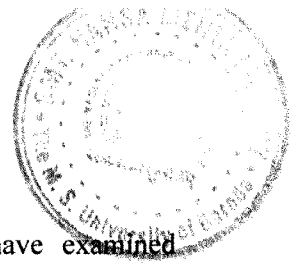
Certain metals like Chromium, Vanadium, Selenium, Manganese, Zinc and Potassium etc. have also shown the hypoglycemic activities and some of these metals are being used for the treatment of diabetes.

1. Chromium

Chromium (Cr_3), a trace element in its trivalent form, is required for the maintenance of normal glucose and lipid metabolism. Supplementation with chromium does not appear to reduce glucose levels in euglycemia, but it has shown some efficacy in reducing glucose levels in hyperglycemia (Ryan *et al.*, 2003). Experimentally, chromium deficiency is associated with impaired glucose tolerance, which can be improved with supplementation (O'Connell, 2001). Most individuals with diabetes, however, are not chromium deficient. In addition to glucose control, the supplement has been studied for its effects on weight control, lipids, and bone density. Its action is linked with glucose tolerance factor (GTF), and has been shown to increase the number of insulin receptors, to enhance receptor binding, and to potentiate insulin action. There are some suggestions that chromium picolinate is the preferred form as it is utilized more efficiently (Trow *et al.*, 2000).

2. Magnesium

Hypomagnesemia is common in patients with diabetes, especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses. Deficiency of magnesium can cause



potential states of insulin resistance (Walti *et al.*, 2003). Studies have examined magnesiums potential role in the evolution of such complications as neuropathy, retinopathy, thrombosis, and hypertension. Magnesium supplementation in diabetic patients had shown a significant fall in serum cholesterol levels, LDL cholesterol and triglycerides and a rise in HDL cholesterol levels and a reduced insulin-stimulated glucose uptake (Lal *et al.*, 2003; Djurhuus *et al.*, 2001). Magnesium is a cofactor in various enzyme pathways involved in glucose oxidation, and it modulates glucose transport across cell membranes. It may increase insulin secretion and improve insulin sensitivity and peripheral glucose uptake. It has been shown to have no effect on hepatic glucose output and nonoxidative glucose disposal (Mooradian *et al.*, 1994). It is difficult to measure the same accurately as it is an intracellular cation and the total body stores are seldom measured.

3. Vanadium

Vanadium has been described as either a nonessential nutrient or a nutrient that is required only in minute quantities. The physiological role of this trace element is yet to be assessed (Goldwaser *et al.*, 2000). There are no accurate assays in clinical settings, and there is no recommended daily allowance. Vanadium exists in several valence forms, with vanadyl (+5) sulfate and sodium metavanadate (+4) being the most common supplement forms. Its mechanism of action in glycemic control is thought to be primarily insulin-mimetic with up-regulation of insulin receptors. In animal models, it has been shown to facilitate glucose uptake and metabolism and to enhance insulin sensitivity. Clinically, it may enhance glucose oxidation and glycogen synthesis, and it may modulate hepatic glucose output (O'Connell, 2001). An organic vanadyl coordination compound was reported to concomitantly stimulate insulin secretion *in vitro* from isolated rat pancreatic islets (Conconi *et al.*, 2003). Vanadium supplementation had also been shown to up-regulate $GLUT_4$ expression in diabetic animals *in vivo* (Mohammad *et al.*, 2002).

Combination Therapy:

Diabetes mellitus is defined as a "metabolic syndrome" and can be precisely said that "type 2 diabetes mellitus represents a syndrome of metabolic and haemodynamic

abnormalities as a result of complex pathophysiological interactions between hyperglycemia, β-cell dysfunction, hypertension, insulin resistance, dyslipidaemia, endothelial cell dysfunction and proteinuria which underlie the aetiology and progression of micro- and macro- angiopathy". Keeping this in view, combination therapy for diabetes mellitus has become inevitable. In recent years it has been shown that the control of the latter has to be much stricter and that in many cases monotherapy does not achieve the established aims. At the same time, new oral anti-diabetic medicines have recently appeared with different mechanisms of action. The possible combinations of treatment with different oral anti-diabetics, or oral anti-diabetics with insulin, are very numerous and have shown their effectiveness in reducing glycemia and the glycosylated haemoglobin. Selection of the type of association will depend on the individual aims of control, on the physiopathological mechanism presumably involved in each case on the efficacy, cost and secondary effects of each medicine, as well as on the characteristics of each patient (Menendez, 2002). Several of the available oral agents have been studied in combination and have been shown to further improve glycemic control when compared to monotherapy (Riddle, 2000). Reasonable combinations of agents include a sulfonylurea plus metformin, a sulfonylurea plus an alpha-glucosidase inhibitor, a sulfonylurea plus a thiazolidinedione, metformin plus repaglinide, biguanide plus alpha-glucosidase inhibitor, and metformin plus a thiazolidinedione.

The Ayurvedic approach to diabetes:

Ayurveda, the ancient healing system from India, has steadily increased in popularity in the western world in recent years. This 5,000 year old system of medicine recommends a combination of lifestyle management (which includes diet, exercise and meditation), and treatment with specific herbs and minerals to cure various diseases. The botanicals in the Ayurvedic materia medica have been proven to be safe and effective, through several hundred to several thousand years of use. Ayurvedic physicians have treated diabetes for thousands of years using a combination of regulated lifestyle and herbal formulations. The following paragraphs summarizing the description of diabetes mellitus by two ancient Indian physicians (Dahanukar and Thatte, 1989).

"About the one transmitted genetically, he (Sushruta) says "a *person would be diabetic if his father and grandfather are diabetic*". In fact, he mentions that such type of person is clinically diabetic. The genetically transmitted entity of insulin dependent diabetes mellitus is well known today.

Charaka too agrees with the genetic origin of diabetes and adds that this type is more difficult to cure. The ancient physicians have written factors predisposing to diabetes mellitus, and these stand confirmed even today. The factors described are lack of exercise, sedentary habits, sleeping during day time and eating excessively, particularly sweet and fatty substances. These individuals lack enthusiasm, are overweight, obese and have excessive appetite." These physicians also prescribed specific herbal formulations for the treatment of diabetes. Some of these herbs, with a record of safety and efficacy, spanning several centuries, are described in the following pages. In recent times, the safety and efficacy of these herbs have been validated by laboratory experiments and clinical trials.

Herbal Therapy:

It is apparent that all the above classes of drugs mentioned above exert serious side effects when taken for a long time; hence interest is generated towards complimentary and alternative therapy. In recent years popularity of complimentary medicine such as dietary measures and traditional herbal therapy, described by ayurvedic and indigenous systems of medicine, which were commonly used in India has been increased. Herbal therapy is one of the complimentary and alternative therapies. Herbal preparations / agents are the preferred antidiabetic agents due to their easy availability, more economical and have lesser or no side effects as compared to other drugs. Enormous advances have been made in medical care but more and more people prefer using herbal or alternative remedies. In chronic conditions, patients may turn to alternative remedies that have been purported to improve glycemic control. In a survey, it was seen that among diabetic patients, 78% were taking prescribed medication for their diabetes, 44% were taking over-the-counter supplements and 31% were taking alternative medications. Diabetic patients spent almost as much as money on over-the-counter supplements and alternative medications together as they did on their diabetic medications. Plants with

purported hypoglycemic properties have been used in folk medicines and traditional healing systems around the world. Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. Among them, metformin was derived from the flowering plant, *Galega officinalis* (Goat's Rue or French Lilac), which was a common traditional remedy for diabetes (Pandey *et al.*, 1995; Oubre *et al.*, 1997). Nutritional supplements, botanicals, diet, and lifestyle considerations can decrease insulin requirements and would maintain normal blood glucose levels. In addition, they may prevent the onset of complications of hyperglycemia, including retinopathy, nephropathy, neuropathy and macro- and microangiopathy. A lot of medicinal plants are described for the treatment of diabetes of which only a few are being systematically evaluated. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara *et al.*, 1998). Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM (Ivorra *et al*, 1988; Grover *et al.*, 2002).

Scientific basis for using mixed formulations:

Ayurvedic remedies for diabetes are usually mixed formulations (Yajnik *et al.*, 1993; Nair *et al.*, 1992) containing blood sugar lowering herbs in combination with immunomodulators, diuretics and detoxicants. The rationale behind such formulations is provided by modern research, which documents that immune processes play a predominant role in the destruction of beta cells and that free radicals (Oberley, 1988) feature predominantly in the progression of the disease and its secondary complications. The inclusion of immunomodulators and detoxifying antioxidants in mixed formulations is therefore beneficial. Some traditional formulations also contain cholesterol- reducing agents and adaptogens such as *Emblica officinalis*.

Need and scope of alternative remedy:

Due to the modernization of life style, NIDDM is becoming a major health problem in developing countries. Even in the developed countries the rate of mortality due to diabetes is more alarming. It is the seventh leading cause of death in the United States. In India every fourth man is a diabetic or has the chance of becoming a diabetic patient. Life expectancy is drastically reduced by NIDDM in developing countries where its

prevalence is increasing due to modernization in the life style and inadequate treatment. With increasing incidence of diabetes mellitus in rural population throughout the world, there is a clear need for the development of alternate strategies for diabetes therapy as the current therapies are proving to be inadequate to combat all the metabolic aberrations of the disease. Due to the cost and poor availability of current therapies for many of the rural population in India, the need for the development of indigenous, inexpensive botanical sources of antidiabetic and antihyperlipidemic drugs in crude or purified form are highly essential.

In Ayurveda, drugs are classified depending on their taste, attributes, potency, taste after digestion and therapeutic effect. Four types of therapies – elimination therapy, alleviation therapy, psychic therapy and surgery, are used for the treatment of diseases. In addition to single drugs, compound formulations are generally used by Ayurvedic physicians in the form of pills, powders, decoctions, infusions, linctus, alcoholic preparations, medicated ghee, fractional distillation, collyrium, etc. Several pharmaceutical processes are followed for the preparation of medicines for easy administration; making the product delicious to taste, easily digestible and assimilable, therapeutically more efficacious, rendering them non-toxic and more tolerable and for preservation of medicines for a longer time. Ayurvedic drugs are administered both externally in the form of ointment, dusting powder, collyrium, ear drops and eye drops, and internally as tablets, pills, powder, syrups, etc. Along with medicines some regimens like sleep, walk, rest, physical exertion, etc. are also prescribed to the patients.

Herbal medicines are being used by about 80% of the world population particularly in the developing countries for primary health care. The natural products shall be considered as the best in primary health care because of its better cultural acceptability, safety, efficacy, potency, and are inexpensive with lesser side effects. Several herbal medicines and supplements have been studied as potential therapeutic agents in the management of diabetes and its related complications. Hundreds of plants have been studied for their potential blood glucose lowering properties. In recent years many developed countries have shown growing interest in alternative or complementary system of medicine for

management of diabetes. Several plant species have been used in traditional medicine to treat symptoms of diabetes since several hundred years.

None of the presently available sulfonylureas completely normalize insulin secretion and action (Back Bielsen *et al.*, 1988). A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternate drugs and therapeutic strategies. The bioactive extracts and compounds need to be standardized on the basis of active principles along with fingerprints. This can be achieved by judicious and rationally designed interdisciplinary research programmes. Cost efficiency, potency and less or no side effect of drugs of plant origin have been achieved through compound formulations either in their natural or semi processed form. But it definitely requires proper standardization, efficacy and dose regimen for therapeutic use. The herbal remedies can act as good adjuvant drug to reduce the requirement of insulin or sulphonyl urea derivatives.

The plant kingdom is a wide field to look for an effective oral hypoglycemic agents and more than 1300 plant species have been used ethnopharmacologically or experimentally to treat symptoms of diabetes mellitus throughout the world. Before the advent of insulin injection and other pharmaceutical preparations, healers relied heavily upon herbs to treat diabetes. Isolation of active principles of diverse chemical groups from plant sources such as morphine, strychnine, quinine, emetine, muscarine etc. necessitated studies of their action by animal experimentations. This was started by the French school with Francis Magendie and Claude Bernard, who studied most of the isolated principles and this was the starting point of modern pharmacology. Later, German, English and American workers and their joint endeavours in the field of pharmacology have brought this branch of science to its present stage. Plant products investigated for anti-diabetic effect have been exhaustively reviewed (Aiman, 1970; Hauda and Chawla, 1989; Ivorr *et al.* 1989; Gupta, 1994; Marles and Farnsworth, 1995). Hence discussion is limited to the work done from 1995 to 2001 except a few instances. It is observed that some of the plant extracts restrict the rise of blood sugar caused by the pituitary hormones responsible for inhibiting peripheral utilization of glucose, causing glycogenolysis in maturity onset

diabetes. Some plants have been reported to promote regeneration of β-cells of langerhans in pancreas, lowers glycosylated haemoglobin, cholesterol and triglyceride levels. Hundreds of plant species have been studied for their potential blood glucose lowering and antioxidant properties and a large number of active constituents have been isolated.

Aims and objectives of the present study:

In the present project deals with a detailed study of pharmacognosy, phytochemistry and pharmacology of the following plants.

1. *Cassia alata* Linn.
2. *Costus pictus.* D. Donn.
3. *Bauhinia purpurea* Linn.
4. *Artocarpus heterophyllus* Lam.

For all the above plants selected, the leaves were reported to possess potential antidiabetic property and therefore only the leaves are studied here. Apart from this, three herbal combinations of some other plants of interest in different ratios also are tested for their efficacy.

The formulations used are:

1. *Aegle marmelos* (L.) Correa (**LEAF**): *Catharanthus roseus* (L.)G. Don (**LEAF**): *Syzygium cumini* (L.) Skeels (**BARK**).in the ratio **(1: 1: 1).**
2. *Syzygium cumini* (L.) Skeels (**SEED**): *Trigonella foenum-graecum* L. (**SEED**): *Phyllanthus emblica* L. (**FRUIT**). In the ratio **(1: 1: 2).**
3. *Terminalia arjuna Roxb* (**BARK**): *Gymnema sylvestre* Retz (**LEAF**): *Syzygium cumini* (L.) skeels (**SEED**). In the ratio **(1: 1: 1).**

Objectives:

1) To conduct pharmacognostic studies including Micromorphological characters to find out the pharmacognostic characters and biomarkers on the leaves of all the four plants.

2) To analyse all the four plants phytochemically for plant products such as flavonoids, phenolic acids, quinones and steroids so that the chemical spectra of these plants and the phytochemical biomarkers could be understood.

3) To study the optimum dose determination of individual plant extract and herbal formulation and its efficacy in hypoglycemic activity (to check the lowering of blood glucose in normal rats).

4) To evaluate the effect of the extracts in improving glucose tolerance and their safety profile in normal and diabetic rats.

5) To study the antihyperglycemic activity of the extracts in diabetic rats.

6) To study the effect of the extracts on enzymatic antioxidants (SOD, CAT, and GPX)

7) To study the effect of the extracts on non-enzymatic antioxidants and lipid peroxidation (TBARS, GSH, vitamin C and vitamin E)

8) To study the effect of the extracts on carbohydrate metabolizing enzymes (hexokinase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase).

9) To evaluate the effect of the extracts to retrieve the renal toxicity caused due to diabetes [urea, uric acid, creatinine and blood urea nitrogen (BUN)]

10) To study the antihyperlipidaemic effect of the extracts on diabetic rats. (Cholesterol, free fatty acids, triglycerides, phospholipids, LDL, VLDL and HDL).