CHAPTER – I
GENERAL INTRODUCTION

Non - communicable diseases (NCD) have emerged as a major health problem and accounts for 60% of all deaths worldwide. An estimated 8 to 14 million people die prematurely every year in developing countries due to preventable NCDs such as diabetes mellitus (DM), cancer, cardiovascular and respiratory diseases. DM is one of the fastest growing NCDs whose prevalence is seen in all the six inhabited continents of the globe and is the seventh leading cause of death worldwide. According to the International diabetes federation (IDF), 285 million people worldwide had diabetes in 2010. It causes about 5% of all deaths that are now occurring on low and middle income countries (WHO, 2010). The total number of diabetic subjects in India is around 41 million and this is further set to rise to 70 million by the year 2025 (Sicree et al., 2006).

Epidemiology of diabetes

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga and Rothman, 2006) over the past 30 years. The status of diabetes has changed from being considered as a mild cause of morbidity and mortality affecting the youth and middle aged
people. The major proportion of the increase occur in developing
countries of the world where the disorder predominantly affects
younger adults in the economically productive age group (Mather et
al., 1987).

**Diabetes in the world**

The World Health Organization (WHO) has predicted that the
major burden of diabetes will occur in the developing countries and
there will be a 24% increase (from 51 to 72 million) in the developed
countries and 170% (from 84 to 228 million) in the developing
countries (Wild et al., 2004). In recent years pronounced changes in
human environment, human behavior, life style and accompanying
globalization have resulted in the escalating rate of diabetes (Pari and
Sarvannan, 2007).

**Diabetes mellitus**

Diabetes mellitus is a complex and multifactorial disease
indulging severe insulin dysfunction in conjunction with gross
abnormalities in glucose homeostasis, lipid and protein metabolism.
The diabetes mellitus describe several syndromes of abnormal
carbohydrate metabolism that are characterized by hyperglycemia.
Diabetes mellitus mostly affects aged people and approximately 6% of
the world’s population is affected (Adeghate et al., 2006). Diabetes
mellitus long being considered as a disease of minor significance to world health is now talking its place as one of the main threats to human health in the 21st century (Zimmet et al., 2001).

Chronic supra physiological glucose concentration that affects the secretion of β cells, brings metabolic imbalances and pathological disturbances in several tissues such as pancreas, eye, liver, muscle, adipose tissue, kidney and nerves (Bruner et al., 2009). These pathophysiological changes in multiple organ system impose a tremendous burden on the individuals with diabetes and on the health care system.

Diabetes mellitus may lead to absolute deficiency or lack of insulin and be defined as a disorder of metabolism. Such hypo effect of insulin leads to chronic hyperglycemia with or without glucosuria. Diabetes is a chronic endocrine disorder affecting the body’s metabolism and resulting in structural changes affecting the organs of the vascular system. Serious complications resulting from diabetes include coronary heart disease, stroke, retinopathy, renal failure, peripheral artery disease and neuropathy. The two main forms of diabetes are type I diabetes and type 2 diabetes. Type 1 diabetes is a result of pancreatic islet β-cell destruction usually due to an autoimmune response. This results in insulin deficiency requiring
exogenous insulin to prevent serious complications. In type 1 diabetes, the body does not produce insulin, and hence daily insulin injections are required. Over 700,000 people in the United States have type 1 diabetes which is 5-10% of all cases of diabetes mellitus. Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children. Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity and eventually oral medications or insulin. Type 2 diabetes is believed to affect more than 15 million adult, 50% of whom are undiagnosed. It is typically diagnosed during adulthood. However with the increasing incidence of childhood obesity and concurrent insulin resistance, the number of children diagnosed with type 2 diabetes has also increased worldwide.

1.1 Classification of Diabetes Mellitus

Diabetes mellitus regardless of its underlying cause is divided into idiopathic and secondary types (WHO, 1999). Recent advances in understanding the etiology and pathogenesis of diabetes have led to a revised classification.
1.1.1 Type 1 (or) Insulin dependent diabetes mellitus (IDDM)

Type 1 diabetes is mainly due to the destruction of pancreatic β-cells usually leading to absolute insulin deficiency. It is a leading cause of end stage renal disease, blindness, amputation and cardiovascular disease. Insulin is required for survival to prevent the development of keto acidosis and coma.

A) Immune mediated diabetes (IDM)

Type 1A is defined as “immune-mediated” DM that results from cell mediated immune destruction of pancreatic β-cells.

B) Idiopathic

This form of diabetes is also characterized by insulin deficiency as well as a tendency to develop ketosis. Type 1B also is immune mediated but characterized by absolute insulin deficiency (insulinopenia).

1.1.2 Type 2 diabetes (or) Non insulin dependent diabetes (NIDDM)

Type 2 diabetes begins with insulin resistance, a condition in which the cells fail to respond to insulin properly (WHO, 2013). As the disease progress a lack of insulin may also develop (Text book of diabetes mellitus, 2012). This form was previously referred to as non insulin dependent diabetes mellitus (or) adult onset diabetes.
Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several aspects, involving a combination of relative insulin secretion and responsiveness. It occur in about 2-10% of all pregnancies and may improve or disappear after delivery. However after pregnancy, approximately 5-10% women with gestational diabetes are found to have diabetes mellitus. Mostly type 2 diabetes management may include dietary changes, blood glucose monitoring and in some cases insulin untreated gestational diabetes can damage the health of the fetus or mother. Risks to the body include macrosomia (high birth weight), congenital cardiac and nervous system anomalies and skeletal muscle malformations.

Prediabetes

Prediabetes indicates a condition that occurs when a person’s blood glucose level are high enough for the diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

1.2 COMPLICATIONS OF DIABETES MELLITUS

Several clinical studies including the diabetes control and complication trials (DCCT) and United Kingdom prospective diabetes study (UKPSS) have shown that the severity of these complications are
directly associated with hyperglycemia, regardless of the different pathogenesis of type 1 or 2 diabetes. The complications can be divided into 2 types (Drazin, 1993)

(i) Acute metabolic complication

(ii) Chronic complication

**Acute metabolic complication**

In diabetes mellitus severe hyperglycemia may result from absolute or relative deficiency of insulin. Under some circumstances the condition may culminate into life threatening complications. The acute status of diabetes includes ketoacidosis (DKA), hyperosmolar non-ketotic coma (HNC), acidosis (LA) and hypoglycemia (Ohkubo et al., 1995).

**Diabetic ketoacidosis (DKA)**

Diabetic ketoacidosis (DKA) is a complex metabolic disorder state that develops when absolute insulin deficiency and excess counter regulatory hormones (glycogen, catecholamine, cortisol and growth hormone) increase hepatic glucose production, decrease peripheral glucose utilization and stimulate the release of fatty acids from fat cells and produce ketones by the liver (Chiasson et al., 2003; English and Williams, 2004). Excess glycogen decrease the activity of pyruvate kinase whereas insulin deficiency increase the activity of
phosphoenol pyruvate carboxy kinase (PEPCK). The hepatic changes shift the handling of pyruvate towards glucose synthesis (gluconeogenesis) and away from glycolysis (Umpierrez et al., 1996).

Since the introduction of insulin in 1922, recent evidences still indicate a mortality rate ranging between 3.4 and 4.6 % among diabetics (Wallace et al., 2001).

**Hyperosmolar non ketotic coma (HNC)**

Hyperosmolar non ketotic coma is a syndrome characterized by severe hyperosmolarties (>330 mosm/Kg), hyperglycemia (<600 mg/dl) with mild acidosis (serum bicarbonate> 15 mmoL/L ) and mild ketonuria (≤15mg /dL) (ADA, 2001). The syndrome is usually a complication of NIDDM and produces severe dehydration with hypotension and variable degrees of CNS depression including seizures and coma. Hypothermia may also occur in some patients.

**Insulin therapy**

Insulin therapy is used to maintain blood glucose at normal levels. It plays an important role in achieving glycemic control and decreasing the risk of choronic complications in patients with type II diabetes (Elder, 2005). Insulin therapy is indicated when glycemic control cannot be achieved and maintained with lifestyle intervention including diet and exercise and treatment with oral antidiabetic agents.
For type 2 diabetes insulin replacement therapy is required and however is not always necessary for the treatment and control of diabetes in type 2 diabetes (Buse, 1999). Insulin binds to specific plasma membrane receptors of target tissues (muscle and adipose tissue) and activate receptors to release a series of biochemical signaling pathways (While and Khan, 1994).

**Impact of insulin metabolism**

Insulin stimulates the uptake of glucose, fatty acids and amino acids from the blood for the storage and utilization. Glucose is stored as glycogen in the liver, fatty acids are stored as triglycerides in the adipose tissue and amino acids are stored as proteins in the muscle tissues. The abnormalities in insulin secretion and action result in disordered metabolism of carbohydrates, lipids, ketones and amnio acids (Yeh, 1998; Mc Donald, 2006). Insulin also inhibits the actions of other hormones which enhance the breakdown of glycogen, lipids and proteins (Khan and Sccehter, 1989).

**Diabetes and carbohydrate metabolism**

Alterations in glucose metabolism in diabetes are accompanied by changes in the activities and gluconeogenesis in liver and muscle, such that the latter process becomes favoured (Gerich, 1993). Insulin deficiency leads to hyperglycemia in diabetic patients. Untreated
diabetes leads to more liver glucose output. Stored glycogen in the liver is used to produce glucose (Alane et al., 2001). Increased rate of hepatic glucose production results in the development of overt hyperglycemia especially fasting hyperglycemia in patients with type 2 diabetes (Defronzo et al., 1992).

Insulin exerts direct effects on the liver (Micheal et al., 2000) as well as influences the substrate availability and fluxes of free fatty acids (FFA) (Bergman and Ader, 2000). Some of them are directly controlled by insulin, via phosphorylation and de-phosphorylation (Zhang, 2002). Uncontrolled diabetes leads to increased hepatic glucose output. Glycogen stores in the liver are mobilized and hepatic gluconeogenesis is used to produce glucose (Alan, 2001).

**Diabetes and lipid metabolism**

Alterations or secretion of insulin and glycogen also have profound effect on lipid and ketone metabolism. At concentration below those required to stimulate glucose uptake, insulin inhibits the hormone sensitive lipase in adipose tissue and thus inhibits the hydrolysis of triglycerides stored in the adipocyte. Lipid abnormality is a major problem in patients with diabetes in which 97% had at least one lipid abnormality (Fagot Compagna et al., 2000). Insulin favours the synthesis of triglycerides from glucose through activating the
enzyme acetyl Co-A, the carboxylase key enzyme for fatty acid synthesis (Satyanarayana and Chakrapani, 2007).

Insulin reduces the concentration of glycerol (a substrate for gluconeogenesis) and free fatty acids (FFAs) a substrate for the production of ketone bodies and necessary fuel for gluconeogenesis and ketogenesis (Lizuka and Morose, 1997). In the diabetic patients, particularly with IDDM, the consequence of insulin deficiency and excess glycogen provide a hormonal imbalance that favours hepatic ketogenesis which in turn leads to ketonemia and ketoacidosis (Chiassion et al., 2003; English and Williams, 2004).

Insulin also enhances the transcription of lipoprotein lipase (LPL) in the capillary endothelium. This enzyme hydrolyze triglycerides present in very low density lipoprotein (VLDL) and chylomicrons, resulting in the release of intermediate density lipoprotein (IDL) particles. In addition deficiency of insulin may be associated with increased production of VLDL (Kirpichnikov and Sowers, 2001).

**Diabetes and protein metabolism**

Insulin enhances the protein synthesis and decreases the degradation of protein. Insulin stimulates the amino acids uptake and thus it decrease the circulation of amino acids (Some amino acids are
precursors for glucose production, e.g. glutamine and alanine). Proteins are an important target for oxidative challenges. The reactive oxygen species (ROS) modify amino acid side chains of protein such as arginine, lysine, threonine and protein residues to form carbonyls (Chevion et al., 2003). Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol (-SH) group and low molecular weight thiols, in particular GSH (glutathiolation) (Donne et al., 2005). A correlation between glauciated albumin, amatory modified plasma protein and diabetic nephropathy and other complications has also been reported (Schalkeijk et al., 2002). In type 2 diabetic patients after 2 weeks of poor glycemic control, glauciated albumin increased with significant increase in the glycation of several plasma proteins including transferrin, immunoglobulin (Ig) and fibrin (Jaleel et al., 2005).

1.3 ANTIOXIDANTS

Antioxidants are defined as compounds that inhibit and/or delay the oxidation of other molecules by inhibiting the initiation and/or propagation of oxidizing chain reactions. They are also known as oxidation inhibitors (Pokorny, 2001). One antioxidant molecule can react with a single free radical and is capable to neutralize free radical(s) by donating one of their own electrons ending the carbon-
stealing reaction. Antioxidants prevent cell and tissue damage as they act as scavengers. Various components can act against free radicals to neutralize them from both endogenous and exogenous origin ( Jacob, 1999). These include endogenous enzymatic antioxidants and non enzymatic, metabolic and nutrient antioxidants. During normal metabolic functions, highly reactive compounds called free radicals are produced in the body. However, they may also be added from the environment. These molecules are unstable as they have a lone pair of electron and thus can become highly reactive. They react with cellular components such as proteins, lipids and carbohydrates and denature them.

Antioxidant enzymes are capable of stabilizing or deactivating free radicals before they attack cellular molecules. Antioxidants mainly act by reducing the energy of the free radicals or by giving up some of their electrons for neutralizing the radicals, thus stabilizing the free radicals. In addition, they may also inhibit the oxidizing chain reaction for minimize the damage induced by free radicals. It has been reported that a substantial association exists between free radicals with more than sixty different health conditions, such as the process, aging process, cancer, diabetes, Alzheimer’s disease, strokes, heart attacks and atherosclerosis. By lowering the exposure to free radicals and by
increasing the level of antioxidant enzyme rich foods or antioxidant enzyme supplements, the body’s risk of free radical associate health problem is reducable..

Currently there has been an increasing interest in understanding free radicals in biological systems and their important role as causative agents in a variety of pathological physiologies. Free radicals can be described as any species, which is capable of independently exist and contain one or more unpaired electrons, making them highly reactive species. They prime role of free radicals is to promote beneficial oxidation to generate energy as well as kill microbial invaders. But in excess they can lead to harmful oxidation that can damage of cell membrane and even lead to cell death. Antioxidant nutrients have the ability to scavenge free radicals in the system as well as neutralize them before they can do any damage to body cells. Most plants have compounds with protective biochemical functions which are naturally occurring antioxidants in the cells. Various secondary compounds and enzymes of higher plants have been reported with in vitro experiments to protect against oxidative damage by inhibiting and/or quenching free radicals as well as reactive oxygen species.

Naturally, existing antioxidants in plant cells are i) enzymatic and peptide defence mechanisms (catalases, peroxidases, superoxide
dismutases, glutathione and other proteins), ii. Non enzymatic mechanisms such as phenolic defence compounds (vitamin E, flavonoids, phenolic acids and other phenols), nitrogen compounds (alkaloids, amino acids and amines), carotenoids and chlorophyll derivatives. Both the enzymatic and non-enzymatic antioxidants are responsible for playing a major role as natural antioxidants. Ascorbate oxidase is a component of the multicopper oxidase family which catalyzes the one-electron oxidation of ascorbate with the concomitant four-electron reduction of dioxygen to water. Catalase a tetrahedral protein, constituted by four heme groups, catalyze the dismutation of hydrogen peroxide in water and oxygen. Peroxidases are heme containing enzymes, which are capable to oxidise organic and inorganic compounds using hydrogen peroxide as co-substrate.

1.3.1 Non enzymatic antioxidants

Reduced glutathione (GSH)

GSH is an important cellular thiol radical scavenger, present in high concentration in lung cells. It function as a substrate for several enzymes including GPx. GSH is a reduced tripeptide compound of three amino acids. The GSH redox system include glutathione reductase, glutathione peroxidase, glutathione 5-transferase and glucose-6-phosphate dehydrogenase (Rahuman et al., 1999; Meiste,
Exposure to oxidants such as \( \text{H}_2\text{O}_2 \), xanthine/xanthine oxidase, ozone, ionizing radiation, lipid peroxidation by products, hyperopia or heat stock results in an intracellular decrease in GSH associated with a concomitant increase in GSSG levels (Rahman et al., 2000).

**Vitamin C**

Vitamin C is an important water soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. Vitamin C is an outstanding antioxidant in the aqueous phase and spontaneously reacts and scavenges a wide variety of free radicals.

**Vitamin E**

Vitamin E is a group of eight lipid soluble compounds consisting of four tocopherols and four tocotrienols exhibiting similar biological properties. Among these, \( \alpha \) tocopherol is the most common and essential antioxidant capable of functioning as an efficient antioxidant. A tocopherol is believed to function as an antioxidant primarily to protect unsaturated fatty acids of membrane lipids from oxidation. It is located mostly in cell membrane and extracellular fluids. Vitamin E can terminate chain reactions initiated by lipid peroxidation, particularly in cellular and subcellular membranes (Van der Vilet et al., 1999).
1.3.2 Enzymatic antioxidants

Glutathione peroxidase (GPx)

Glutathione peroxidase is an important enzyme in the glutathione systems. It contains selenium which functions in the mechanism of action of this enzyme and thereby contributes to the cellular protection. It plays a critical role in the protection of membrane lipids against oxidation. Formation of ROS results in the production of organic peroxides. Glutathione peroxidase by catalyzing the reduction of $H_2O_2$ to water interrupts the propagation of peroxidation through membrane lipids. The subsequent rejuvenation of GSH, via. glutathione reductase -mediated reduction of GSSG is dependent on proteins and NADPH. Thus extensive oxidation of pyridine nucleotides can impair the regeneration of GSSH thereby lowering the resistance of the cell to oxidation stress.

Superoxide dismutase (SOD)

Superoxide dismutase are metallo- enzymes which catalyze the protonation of $O_2$ from $H_2O_2$. SOD is ubiquitous in aerobic cells and has been located from bacteria, plants, birds and mammals. The enzyme is absent from obligate anaerobes and is therefore assumed that the lack of SOD contributes to inability of such organisms to tolerate oxygen. In mammalian cells, there are two isoenzymes of
SOD which contain different metals at their active sites and also differ in molecular weight, amino acid sequences and number of subunits. Mammalian cytosolic SOD contains one copper and zinc atom per molecule. Hence SOD has more important sites of inflammation than glutathione peroxidase and catalase, both of which are rapidly inactivated by hypochlorous acid.

**Catalase (CAT)**

Catalase is another antioxidant enzyme present mostly in peroxisomes. It is important in the dismutation of H$_2$O$_2$ to water and oxygen. It is also found in the cytoplasm, mitochondria and bronchoalveolar fluid. The catalase gene is located in the chromosomes (Valko, 2007). Catalase is a haem-containing tetrameric enzyme, with a high molecular weight of greater than 22,000. Catalase is relatively inactive at lower concentrations of H$_2$O$_2$ and become more active at higher concentrations.

**Oxidative stress in diseases**

Oxidative stress is thought to contribute to the development of a wide range of diseases including heimer’s disease, rheumatoid arthritis (Christen, 2000; NUNOMURA, 2006; Wood-Kaczmar, 2006) parkinson’s disease (Davi, 2005), the pathologies caused by diabetes and neurode generation in motor neuron disease (Hitchon, 2004). In many of these
cases, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage (Cookson, 1999). One case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. However low density lipoprotein (LDL) oxidation appears to trigger the process in cardiovascular disease.

**Oxidative stress in diabetes mellitus**

Oxidative stress is an imbalance in the general and exogenous oxidants and the antioxidant system. It is a condition that has been associated with cell injury seen in many pathologic conditions. Free radical mediated damage contributes to such varied processes as chemical and radiations injury, ischemia – reperfusion injury, cellular aging and microbial killing by phagocytes (Wulf Dorage, 2002).

Oxidative stress is the term denoting an imbalance between the production of oxidants and the respective defense system of an organism (Halilwell and Gutteridge, 1994). In diabetes mellitus, oxidative stress seems to be caused by both increased production of reactive oxygen species, sharp reduction in antioxidant defence system and altered cellular redox status (West, 2000).
**Lipid peroxidation**

Lipid peroxidation refers to oxidative damage to polyunsaturated fatty acids that contain two or more carbon-carbon double bonds. Lipid peroxidase is an intermediate in the process.

**Initiation**

Lipid peroxidation is initiated with the abstraction of hydrogen from the allylic bond of the polyunsaturated fatty acids (PUFA). This requires a powerful oxidant. Hydrogen abstraction leaves a carbon radical in the lipid. This generally rearranges to form a conjugated diene, followed by the reaction with \( \text{O}_2 \) to form a peroxyl radical.

**Propagation**

At this point, the propagation phase of lipid peroxidation begins. The peroxyl radical can abstract hydrogen from another lipid. Ultimately, chain scission occurs with the release of reactive aldehydes, ketones or hydrocarbons. Prostaglandin-like compounds formed during peroxidation can be used as markers of lipid peroxidation that cause toxic effect.

**Termination**

A lipid radical can also react with another lipid radical leading to chain termination. However, this can cause cross-linking which impairs proper membrane function. Nitric oxide can also terminate
lipid peroxidation protecting against cytotoxicity (Wink et al., 1995; Gupta et al., 1997; Padmaja et al., 1993).

1.4 GAS CHROMATOGRAPHY AND MASS SPECTROMETRY (GC-MS)

Gas chromatography ("GC") and mass spectrometry ("MS") make an effective combination for chemical analyses. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. Gas chromatography (GC), is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance. In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating
column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph.

**Mass spectrometry (MS)**

Mass spectrometry (MS) is an analytical chemistry technique that helps to identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions. A mass spectrum (plural spectra) is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra are used to determine the isotopic signature of a sample, the masses of particles and molecules and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing the chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

**1.5 IN-SILICO DOCKING ANALYSIS ON DIABETIC ALBINO WISTAR RAT AGAINST Pimenta dioica**

Computational (In silico) methods have been developed and widely applied to pharmacology hypothesis development and testing. The in silico methods include database searching, quantitative structure-activity relationships, similarity searching, pharmacophore identification, computational modeling and docking. Such methods
have frequent use in the discovery and optimization of novel molecules with the affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization (Ekis, 2007). In the field of the molecular modeling, docking is a method which can predicts the possible as well as preferred orientation of one molecule to a second when they are bound to each other to forming a stable complex (Lengauer, 1996). Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets for predicting the affinity as well as activity of the small molecule under study. Thus, docking plays a vital role in the rational design of drugs (Kitchen, 2004). It is essential to find out the binding energy between the ligands and the receptor. Molecular docking is an important tool in structural molecular biology and computer assisted drug designing. Structure based design (SBD) and the related fragment based design (FBD) are well established strategies in the rational development of small molecular drugs. Knowledge of how a small molecule binds into a proteins affords considerable advantages. Both in terms of prioritizing compounds for early stage screening, through optimizing potency and selectivity, the in silico docking study was performed to prevent diabetes in albino wistar rats using phenol, 2-
methoxy-3-(2-propenyl)-from *Pimenta dioica* as inhibitor against diabetes associated Dipeptidyl peptidase 4 protein. Antidiabetic drug (glibenclamide) was also included in the docking study to perform comparative study and to prove whether phenol, 2-methoxy-3-(2-propenyl)- could be a potent inhibitor for diabetes.

**1.6 BONE MASS DENSITY**

Finite element method (FEM) is a computer numerical analyzing tool used to analysis the model in the various applications ranges from engineering, medical, structural, aviation, etc. Recently this tool is used to analyze the biomedical problems. In specific, the flow analysis of blood flow, strength analysis of metal implant, corrosion behaviour of metal implants, strength analysis of bone, etc, can be effectively analyzed using FEM. The impact of diabetes on bone strength and the stress distribution is critical to analyze by using biological experiments. So an alternate technique is required to find the complete strength mapping of bones.

**Mechanism and present drug for the therapy of diabetes mellitus**

Achieving glycemic control is a primary therapeutic goal for patients with diabetes mellitus (Kadhe and Arasan, 2002). The present treatment of diabetes is focused on controlling and lowering blood
glucose to a normal level (Lebovitz, 1999; Bohannon, 2002). The
effect is only aimed to lower the level of blood glucose. Moreover in
most cases, side effects such as hypoglycemia, lactic acid intoxication
and gastrointestinal upset appear after patients took these medicines (Li
et al., 2004).

**Sulfonylurea**

Sulfonylurea are insulin secretagogues, triggering insulin release
by direct action on the kATP channel of the pancreatic β-cells. The
sulphonylureas are divided traditionally into two groups. Acetohexamide, tolazanide and chlorpropamide constitute the first
generation sulphonylureas, whereas glyburide or glibenclamide
glipizide are widely known as second generation sulphonylureas that
potentiates the insulin action on peripheral tissues. It is completely
metabolized and causes the β-cells to become more sensitive to
glucose and increase hexokinase activity (UK prospective diabetes
study group, 1998; Fister et al., 2004)

**Bigunanides**

Bigunanides are agents that do not lower the blood sugar in
normal subjects but then do so in all types of diabetic patients (Bailey,
1992). The main action is probably by increasing the glucose uptake
across the cell membrane in skeletal muscle. Gastrointestinal adverse
effects including diarrhoea, nausea, abdominal pain, abdominal bloating, flatulence, dyspepsia and anorexia occur in 50% patients receiving metformin therapy (Bailey, 1992; Setter et al., 2003). Biguanides decrease the amount of glucose produced and released by the liver by inhibiting hepatic gluconeogenesis (Hermann et al., 1994).

**Glibenclamide (GLY)**

Glibenclamide a member of the second generation sulfonylurea is an important drug for the management of hyperglycemic condition during diabetes. It often normalizes blood glucose directly by increasing insulin secretion and decreasing the hepatic glucose production and also has an extra pancreatic effect which directly contributes toward maintaining blood glucose homeostasis during diabetes. The enhancement of glucose transport in rat showed that the GLY was capable of excreting direct insulin like and insulin potentiating effects on non-pancreatic tissues in vitro and in vivo.

**1.7 ANIMAL MODELS IN DIABETES**

Suitable animal models are required for the investigations of diabetes and the complications resulting from both types of diabetes (Chaltopadhyay et al., 1997). The use of animals rather than human subjects has many advantages as various aspects of the diseases like the etiology, its multifunctional genetics, pathogenesis and its
complications can be explicitly understood (Bell and Hye, 1983). The introduction of experimental diabetes in rodents using chemicals which selectivity destroy pancreatic β-cells is very conventional and simple to use. Diabetes is a choronic disease with late vascular sequences. Complete understanding of diabetes requires metabolic and immunogenic studies of many stages of the disease (Graeme and Kenneth, 2001). Several metabolic functions and structural changes in alloxan and streptozotocin diabetic rats have fundamental similarities with those in diabetic patients (Thora et al., 1997; Heidari et al., 2003). Streptozotocin induced hyperglycemia in rodents is considered to be a good experimental model since it is less toxic than the other chemicals inducing diabetes (Zhang and Tan, 2000). Rats treated with streptozotocin display many of the features seen in human subjects with uncontrolled diabetes and provide valuable information about the underlying pathophysiological changes that lead to choronic diabetic complications (De Angelis et al., 2002).

**Streptozotocin (STZ)**

Streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent (White, 1963). It is widely used to induce experimental diabetes due to its ability to target and destroy insulin producing β-cells and its diabetogenic action has been
ascribed to the enhancement of intracellular methylation reactions and the production of free radicals (Sukudelski, 2001). Again this insulinopenia syndrome called “steptozotocin diabetes” (Suchein et al., 1967) caused has been the agent of choice for the induction of diabetes mellitus in animals ever since (Arison et al., 1967).

![Fig.1. Streptozotocin - Structure.](image)

STZ [2-deoxy-2-(-3-methyl-3-nitro soureido)-D- glucopyranose] is a glucosamine- nitrosourea compound with the molecular structure similar to that of 2-deoxy-D-glucose with a replacement at C2 with a N-methyl-N-nitrosourea group. The nitro sources moiety and methyl group are attached at one end and a glucose molecule on the opposite side (Bolzan and Bianchi, 2002).

It is soluble in alcohol and highly soluble in water. The chemical structure of STZ comprise a glucose molecule with a highly reactive nitrosourea side chain that is thought to initiate its cytotoxic actions. The glucose moiety directly attach this agent to the pancreatic
β-cells where it binds to a membrane receptor to generate a structural damage. A decrease in diabetic induction efficacy after the substitution of glucose by other sugar supports the presence of a stereo specific receptor or receptor recognition site on the plasma membrane of the β-cells identified as probably being the glucose transporter (GLuT2) (Schned et al., 1994; Yousef et al., 2004).

**Traditional Indian medicine**

Plants have been the major sources of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants is found in Rigveda (2500 - 1800 BC). Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs (Kirtikar, 1975). Informations on medicinal plants in India has been systematically organized (Kirtikar, 1975). The World Health Organization expert committee on diabetes has listed in one of its recommendations that traditional methods of treatment of diabetes should be further investigated (WHO Expert Committee, 1980). Traditional Indian system of medicine mainly consists of three major systems namely Ayurveda, Siddha and Unani (ASU) (Mukherjee et al., 2006).
1.8 MEDICINAL PLANTS IN DIABETES

There are many antidiabetic plants that provide useful sources for the development of drugs which can be used in the treatment of diabetes mellitus. Upto now, many kinds of antidiabetic medicines have been developed for the diabetic patients and most of them are chemical or biological agents aiming at controlling and lowering blood glucose to a normal level. Despite the impressive advances in health science and medical care, there are many patients who are using alternative therapies alone or complementary to the prescribed medication. Traditional plant remedies or herbal formulations exist from ancient times and are still widely used, in spite of all the controversy concerning their efficacy and safety (Huxtable, 1990; Fugh-Berman, 2000), to treat hypoglycemic and hyperglycemic conditions all over the world. It must be noted that many ethno-botanical surveys on medicinal plants used by the local population have been undertaken in many parts of the world and still there are a considerable number of plants. The effect of feeding along with diet of different fractions obtained from the seeds of *Syzygium cumini* was tried on fasting blood glucose and glucose tolerance in normal and alloxan diabetic rats (Pandey et al., 2002). The observations indicated the hypoglycemic effect of *Syzygium cumini* seeds. In order to identify the underlying
mechanism of the hypoglycemic activity of the aqueous extract of
*Spergularia purpurea* it was tested in diabetic mice after its
administration. The obtained data indicated that the aqueous extract of
*Spergularia purpurea* inhibited endogenous glucose production in
mice. This inhibition was at least one mechanism explaining the
observed hypoglycemic activity of this plant in diabetic animals
(Eddouks et al., 2003). The fruit of *Tetrapleura tetraptera* is
frequently used in tropical African traditional medicine for the
management and/or control of an array of human ailments, including
arthritis and other inflammatory conditions, asthma, diabetes mellitus,
hypertension and epilepsy. The above study was undertaken to
examine the anti-inflammatory and hypoglycemic effects of
*Tetrapleura tetraptera* fruit aqueous extract in rats. *Sutherlandia
frutescens* is widely used in South African traditional medicine for the
management and/or control of a plethora of human ailments. In order
to scientifically apprise some of the ethnomedical uses of *Sutherlandia
frutescens*, a study was undertaken to investigate the analgesic, anti-
inflammatory and antidiabetic properties of the plant shoot aqueous
extract in experimental animal models (Ojewole, 2004).
Objectives of the Present Study

Diabetes mellitus is thus a chronic metabolic disorder in human beings characterized by chronic hyperglycemia associated with absolute or relative deficiencies in insulin secretion or function in pancreas. Before the introduction of therapeutic use of insulin, the major source of treatment for the disease was the use of traditional medicines mainly derived from medicinally and economically important plants. Hence keeping in view of the detailed review of literature and the works done earlier employing varied medicinal plants to treat diabetes, the present investigation was carried out focusing the following objectives.

1. To study the anti-diabetic activity of *Pimenta dioica* methanolic leaf extract in STZ - induced diabetic rats.

2. To elucidate the anti-hyperlipidemic activity of *Pimenta dioica* methanolic leaf extract in STZ - induced diabetic rats.

3. To validate the anti-oxidant activity of *Pimenta dioica* methanolic leaf extract in STZ - induced diabetic rats.

4. To analyse the histopathological alterations by *Pimenta dioica* methanolic leaf extract in STZ - induced diabetic rats.
5. To carry out GC-MS analysis on *Pimenta dioica* methanolic leaf extract in order to screen and identify potential phyto-components.

6. To undergo in-silico molecular docking studies of phenol 2-methoxy-3-(2-propenyl)- from *Pimenta dioica*.

7. To perform the biomechanical studies of bones in STZ – induced diabetic rats.