1. Diabetes:

Diabetes continues to be a devastating and daunting health scourge spreading across geographical and genetic boundaries. It is a devastating disease that is characterized by high glucose levels in the blood and has been recorded in the medical literature since as early as 1500 BC (Brar et al., 2007). The causes were not well understood until the 19th century when Paul Langerhans, a German medical student discovered what have become known as the islets of Langerhans which produce insulin. Insulin is a protein hormone that promotes the uptake of glucose by the body’s cells (Kelley & Rouchka, 2007). The World Health Organization has estimated that 347 million people worldwide are reported to have different types of diabetes and there is a gradual increase in the number of patients. It was observed that 2.8% world population was suffering from diabetes in 2000, which increased to 6.8% in 2010 and is expected to further rise to 7.7% in 2030 (Shaw et al., 2010). The prevalence of diabetes is rising all over the world due to population growth, aging, urbanisation, and the increase of obesity due to physical inactivity (Brahm Kumar et al., 2013).

The synthetic hyperglycemic agents used in clinical practices have serious side effects like hematological effects, coma, disturbance of the function of liver and kidney. In addition they are not suitable for use during pregnancy. Compared with synthetic drug, drugs derived from plants are frequently considered to be less toxic with fewer side effects. Therefore the search for more effective and safer anti diabetic agents has become an area of active research (Suresh et al., 2014). Herbal medicines have been used for thousands years to fight diseases and improve body functions. Phytochemicals present in these herbal remedies are considered as a major nutrients responsible for improving general health and for providing cure for certain specific pathological conditions (Pandey & Rizvi, 2010). Most of the drugs from plant sources are secondary metabolites, which have no role in plant metabolism but play a significant role in plant defense mechanism. There are about 200 pure compounds from plant sources reported to show blood glucose lowering effect. The phytochemical classes include: Alkaloids, Carbohydrates, Coumarins, Cyanogenic glycosides, Flavonoids, Steroids, Inorganic salts, Iridoids, Lipids, Saponins, Peptides, Phenolics (simple), Terpenoids, Xanthenes, Glycopeptides (Ghani, 2003).
1.1 Insulin:

In 1921, Dr. Frederick Banting discovered how to extract insulin from cattle that could be used as an injectable form for human diabetics which revolutionized the treatment of the disease and made it more manageable. Insulin is a hormone that is important for metabolism and utilization of energy from the ingested nutrients especially glucose. Insulin is a protein chain or peptide hormone. There are 51 amino acids in an insulin molecule. It has a molecular weight of 5808 Da. Insulin is produced in the islets of Langerhans in the pancreas. The name insulin comes from the Latin "insula" for "island" from the cells that produce the hormone in the pancreas. Insulin's structure varies slightly between species of animals. Both porcine (from pigs) and bovine (from cows) insulin are similar to human insulin but porcine insulin resembles human insulin more closely.

Insulin has several broad actions including:

- It causes the cells in the liver, muscle, and fat tissue to take up glucose from blood and convert it to glycogen that can be stored in the liver and muscles.
- Insulin also prevents the utilization of fat as an energy source. In the absence of insulin or in conditions where insulin is low glucose is not taken up by body cells, and the body begins to use fat as an energy source.
- Insulin also controls other body systems and regulates the amino acid uptake by body cells.
- It has several other anabolic effects throughout the body as well.

1.2 Secretion of insulin:

Insulin is synthesized in significant quantities only in beta cells in the pancreas. It is secreted primarily in response to elevated blood concentrations of glucose. Insulin thus can regulate blood glucose and the body senses and responds to rise in blood glucose by secreting insulin. Other stimuli like sight and taste of food, nerve stimulation and increased blood concentrations of other fuel molecules, including amino acids and fatty acids, also promote insulin secretion.
1.3 The Role of Insulin:

Insulin is in essence an anabolic hormone that acts primarily on the liver, adipose tissue, and skeletal muscle but has many other functions, some of which are still being discovered. The primary function of insulin is facilitation of glucose into peripheral tissue cells. Insulin also inhibits gluconeogenesis in the liver, stimulates glycogen formation in the liver, converts fatty acids to triglyceride, discourages lipolysis, and stimulates protein synthesis. In addition, insulin encourages the production of nitrous oxide in the endothelial cells lining blood vessels throughout the vascular tree. In turn, nitrous oxide has multiple functions that protect against the formation of atherosclerosis.

Most cells in the body have cell surface receptors for insulin. Once insulin binds to the insulin receptor, glucose is taken into the cell, and different enzyme-controlled reactions occur. Interestingly, there are two organs that do not have insulin receptors- the brain and the liver. However, the cells in these organs are permeable to glucose, and it passes into the cells of these two organs readily via diffusion.

Endogenous secretion of insulin has both a basal component and bolus component. The basal secretion of insulin limits lipolysis and gluconeogenesis in the liver while maintaining a blood level sufficient for cerebral metabolism. Non obese, healthy adults secrete basal insulin at the rate of 0.5-1 U/hour, which maintains the plasma insulin concentration at 35-104 pmol/L. The basal insulin at normal levels results in a fasting plasma glucose of 70-110 mg/dL.

Insulin secretion rises rapidly following a meal. The response to food is a fivefold to tenfold increase in insulin release as bolus insulin. This bolus of insulin inhibits gluconeogenesis in the liver and stimulates peripheral glucose utilization by muscle. The plasma concentrations of insulin rise to the peak of 417-556 pmol/L within 30-60 minutes of eating.

The release of bolus insulin occurs in a two peak process with an initial rise immediately after the meal and a second rise that lasts up to 6.5 hours, depending on the type of food consumed. The first peak of insulin is short, limiting the circulating glucose level of the quickly absorbed carbohydrates and simple sugars in the food. The second peak is longer and is present to process the blood glucose levels that occur
as carbohydrates are absorbed from the gastrointestinal tract over time. Up to 6.5 hours may be needed in the post absorptive state—even more if the intake was high in fact.

The insulin response to a meal is further affected by other factors, including the carbohydrate, fat, and protein content of the meal, transit time in the gastrointestinal system, insulin, and glucagon effect on glucose metabolism in the peripheral tissues and the liver. Obese adults who are otherwise healthy show a several fold higher rate of basal and bolus insulin response depending on the degree of obesity. Both healthy weight and obese individuals who have normal glucose metabolism will have a postprandial blood glucose level at or below 140 mg/dL. In healthy individuals, the plasma glucose level returns to basal level within 2 to 3 hours after eating.

The chemical imbalance of insulin leads to:

(a). **Hyperglycemia** (High blood glucose level): It occurs when pancreas fail to produce adequate insulin, the hormone needed to convert glucose into energy.

(b). **Polyuria** (excessive urination): Hyperglycemia may exceed renal threshold and result in Polyuria.

(c). **Polydypsia** (excessive drinking): Polyuria results in water loss leading to dehydration of the body leading to polydypsia

(d). **PolYPHAGIA** (excessive eating): Lose of glucose via urine causes a demand of more fuel in the body. As a result, a diabetic gets a voracious appetite, i.e., Polyphagia.

(e). **Wasting**: To meet the rising demands of the fuel in the body, endogenous proteins and fats are catabolized. As a result, a diabetic loses weight (i.e., wasting) despite of hearty meals.

Long term complication of diabetes can lead to degenerative changes in the blood vessels, atherosclerosis (hardening of the arteries); it may also result in microangiopathy (thickening of the capillary walls), paralysis, tiredness, recurrent infections, problems with visions, peripheral neuritis, heart attacks and gangrene are complications that commonly diabetics face.
1.4 Classification:

Diabetes mellitus is a genetically and clinically heterogeneous group of disorders that share glucose intolerance in common. The evidence in favour of this heterogeneity is overwhelming: (1) there are more than 30 distinct, mostly rare, disorders in which glucose intolerance is a feature; (2) ethnic variability in prevalence and clinical features; (3) genetic heterogeneity in diabetic animal models; (4) clinical variability between thin, ketosis-prone, insulin-dependent diabetes and obese, non-ketotic, insulin-resistant diabetes; (5) genetic and immunologic studies that show "juvenile" and "adult-onset" diabetes to be distinct entities; and (6) demonstration

The type of mild diabetes in young people, which is inherited in an autosomal dominant fashion, is clearly different from the classic acute-onset diabetes of juveniles. The class of diabetes mellitus is divided into three distinct types, in each of which subtypes have been identified. Heterogeneity within the diabetic syndrome has important implications for research and for the clinical management of diabetes: first, those different genetic and environmental etiologic factors can result in similar diabetic phenotypes; and second, that the distinct disorders grouped together under the rubric diabetes may differ markedly in pathogenesis, natural history, and responses to therapy and prophylactic measures (Goldenberg & Punthakee, 2013).

1.5 Type I, Insulin-dependent diabetes mellitus:

The first subclass of diabetes, type I or insulin-dependent diabetes mellitus (IDDM), is usually characterized clinically by abrupt onset of symptoms, insulinopenia and dependence on injected insulin to sustain life, and proneness to ketosis. Classically, this type of disease occurs in juveniles, and it was formerly termed juvenile diabetes. However, it can be recognized and become symptomatic for the first time at any age; hence, diagnosis based on age at onset is inappropriate. In addition to the ketosis-prone stage, this type of diabetes can also be recognized in a preketosis-prone stage. For example, prospective testing in siblings of insulin-dependent diabetics has disclosed patients with normal fasting plasma glucose (FPG) levels but with abnormal glucose tolerance who progress rapidly to the ketotic form, usually within 2 yr after recognition, but occasionally after longer periods of time. IDDM appears to be heterogeneous in terms of genetics and environmental factors that precipitate the disease. Genetic determinants are thought to be important in most
patients, as expressed by the associated increased or decreased frequency of certain histocompatibility antigens (HLA) on chromosome 6. Abnormal immune responses and autoimmunity are also thought to play an etiologic role, and islet cell antibodies are frequently present at diagnosis in this type of diabetes (American Diabetes Association, 2004).

1.6 Idiopathic Diabetes:

Some forms of type 1 diabetes are less well understood, and can present with various degrees of β-cell dysfunction. This form of diabetes is inherited and is not human leukocyte antigen (HLA) associated. Only a minority of patients fall under this category, which appears to be more common in individuals from African-Caribbean origin. The function of β-cell is variable, and while the disease is often manifested by severe insulinopenia and/or ketoacidosis, β-cell function often recovers, rendering almost normal glucose levels. These patients should be treated initially with insulin, but insulin replacement therapy may not always be necessary after the recovery phase.

1.7 Type II, noninsulin-dependent diabetes mellitus:

The second subclass of diabetes, type II or noninsulin-dependent diabetes mellitus (NIDDM), frequently presents with minimal or no symptoms referable to the metabolic aberrations of diabetes. Patients with NIDDM are not dependent on insulin for prevention of ketonuria and are not prone to ketosis. However, they may require insulin for correction of symptomatic, or persistent, fasting hyperglycemia if this cannot be achieved with the use of diet or oral agents. Such patients may develop ketosis under special circumstances, such as severe stress precipitated by infections or trauma. There may be normal levels of insulin, mild insulinopenia, or above normal levels of insulin associated with insulin resistance. The whole range of insulin responses to glucose from low to supranormal has been found in patients of this subclass, many of whom do not have fasting hyperglycemia. Patients with NIDDM may be asymptomatic for years or decades and show only slow progression of the disease. However, the typical chronic associations and complications of diabetes, namely, macroangiopathy, microangiopathy, neuropathy, and cataracts, may be seen in this type. NIDDM undoubtedly is also heterogeneous in nature. Although in most patients who develop NIDDM the onset is after age 40, the NIDDM type also occurs in young persons who do not require insulin and are not ketotic. Consequently, age at
onset is again not recommended as a criterion by which to classify an individual, and the terms adult-onset diabetes and variations of this phrase, should be abandoned as classifying terms.

NIDDM also has a genetic basis, which appears to be stronger than in IDDM, as evidenced by a more frequent familial pattern of occurrence. Indeed, included within this type are families in whom diabetes presents in children, adolescents, and adults in which autosomal dominant inheritance has been well established (formerly referred to as maturity-onset-type diabetes of the young). Environmental factors superimposed on genetic susceptibility are undoubtedly involved in onset of the NIDDM types. Intake of excessive calories leading to weight gain and obesity is probably an important factor in its pathogenesis. Although small changes in weight may be important, NIDDM has been subdivided according to the absence or presence of obesity, as 60% to 90% of all NIDDM patients are obese in Western societies. Hyperglycemia and glucose intolerance are usually improved by weight loss. In persons with this type of diabetes, characteristic aggregation of HLA types and islet cell antibodies have not been found.

1.8 Gestational Diabetes Mellitus (GDM):

Gestational diabetes complicates ~4% of all pregnancies in the United States, but the true prevalence may range from 1 to 14% depending on the population studied. It now represents nearly 90% of all diabetes in pregnancy. Due to its high incidence, the previous recommendation of screening only high-risk patients has now been changed to the early screening of all pregnant women unless they are defined as low risk. This low-risk group includes women who:

- Are less than 25 years of age
- Have a normal body weight
- Have no family history (i.e., first-degree relative) of diabetes
- Have no history if abnormal glucose metabolism
- Have no history of poor obstetric outcome
- Are not members of an ethnic/racial group with a high prevalence of diabetes (e.g., Hispanic American, Native American, Asian-American, African-American, and Pacific Islander)
Evaluation for GDM should be done early in pregnancy, particularly in women at high risk (marked obesity, personal history of GDM, glycosuria, or a strong family history of diabetes). If a woman is found not to have GDM at the initial screening, she should be retested between 24 and 28 weeks of gestation. Women of average risk should be tested at 24-28 weeks of gestation. Early screening and diagnosis is crucial as proper monitoring and initiation of therapy reduces perinatal morbidity and mortality. The relationship between high blood sugar levels and poor maternal and fetal outcomes was studied normal (non-gestational diabetes) pregnant women in the Hyperglycemia and Adverse Pregnancy Outcome Study. Preliminary results have shown a direct correlation between high blood sugar and poor outcome for both mother and baby without a glycemic threshold. Women with gestational diabetes mellitus need to be evaluated after delivery, as they may have antecedent diabetes diagnosed at the time of pregnancy. Even with a negative postpartum test these patients need further monitoring as they remain at an increased risk of developing type 2 diabetes and cardiovascular disease.

1.9 Other types of diabetes:

In this subclass, diabetes forms part of certain other conditions and syndromes that often have many clinical features not generally associated with the diabetic state. In some instances the co-occurrence of glucose intolerance and the other features is known to be etiologically related. In others, the frequency of co-occurrence indicates that there is an, as yet unknown, causal relationship. Thus, this subclass has been divided according to the known or suspected etiologic relationships. For example, diabetes may be secondary to (1) pancreatic disease or removal of pancreatic tissue, (2) endocrine diseases such as acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, somatostatinoma, and primary aldosteronism, or (3) the administration of certain hormones, drugs, and chemicals that cause hyperglycemia.

Diabetes may also be associated with defects of insulin receptors, which may be caused by either abnormalities in numbers or affinity of insulin receptors or antibodies to receptors with or without associated immune disorders. Diabetes (or carbohydrate intolerance) is found in increased frequency with a large number of genetic syndromes. Finally, this class contains room for certain special types of diabetes that occur only under specific, well-described environmental and clinical conditions, e.g., diabetes associated with malnourished populations.
1.10 Pathophysiology of type 2 diabetes:

The development of alterations in glucose metabolism results from the gradual fall in b-cell function occurring within a background of insulin resistance. The two principal components of the blood glucose regulation pathway are insulin secretion and insulin sensitivity (Defronzo, 2009).

1.11 β-Cell function:

Type 2 diabetes is progressive, and one main factor responsible for this is a continued decline in β-cell function. Diabetes and prediabetes do not develop until the β-cell fails to compensate appropriately to the peripheral insulin resistance state. The ability of the β-cell to secrete sufficient insulin to adequately respond to the peripheral insulin resistance state depends on multiple factors, including β-cell mass and secretory capacity (Kahn et al., 2006), which are influenced by genetic and environmental factors (Lyssenko et al., 2008). In fact, although the progressive loss of β-cell function could be due to different metabolic derangements (insulin resistance, lipotoxicity), several studies have suggested that β-cell dysfunction depends also on a pre-existing and perhaps genetically determined risk, which is crucial for β-cell dysfunction to occur.

1.12 Insulin resistance:

Insulin resistance plays an important role in its development and occurs 10–20 years before the onset of the disease and that it is the best predictor of whether or not an individual will later become diabetic (Shulman, 2000). In addition, insulin resistance, by placing an increased demand on the β-cell to hypersecrete insulin, influences the progressive β-cell failure of type 2 diabetes. The precise mechanism(s) by which insulin resistance leads to β-cell failure remain(s) unknown, however a possible hypothesis is that the cause of insulin resistance is also directly responsible for the β-cell failure (i.e., lipotoxicity) (Kahn et al., 2006; Defronzo, 2009).

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young and are characterized by impaired insulin secretion with minimal or no defects in insulin action. The most common form is
associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell.

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes.

1.13 Epidemiology:

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga & Rothman, 2006). Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe. Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes, which accounts for more than 90% of all diabetes cases. Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000 (Wild et al., 2006). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree et al., 2004). It affects 366 million people worldwide (6.4% of the adult population) and is expected to rise to 552 million by 2030 (International Diabetes Federation, 2011).

1.14 Evolution of the diabetes epidemic in India:

The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council Medical Research (ICMR, New Delhi). Screening was done in about 35,000 individuals above 14 yrs of age, using 50 g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1% in urban population and 1.5% in the rural
population while in those above 40 yrs of age, the prevalence was 5 % in urban and 2.8 % in rural areas. Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in south India reported a prevalence of 5%. The prevalence of impaired glucose tolerance in the same study was 2 %. A national rural diabetes survey was done between 1989 and 1991 in different parts of the country in selected rural populations (Sridhar et al., 2002). This study which used the 1985 WHO criteria to diagnose diabetes, reported a crude prevalence of 2.8 %. The Eluru survey which looked at the prevalence of known diabetes in four villages in Andhra Pradesh showed a prevalence of 1.5 per cent. The prevalence of known diabetes was 6.1 per cent in individuals aged above 40 yrs which was unexpectedly high at that time for a rural area with low socio-economic status and decreased health awareness. A study done in 1988 in Chennai reported a prevalence of 8.2 % in the urban and 2.4 % in the rural areas (Ramachandran et al., 1992).

1.15 Diabetic Complications:

Oxidative stress has been considered to be a pathogenic factor of diabetic complications including Acute and Chronic complications.
1.16 Acute complications:

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. Low insulin levels cause the liver to turn to fat for fuel (ie, ketosis); ketone bodies are intermediate substrates in that metabolic sequence. This is normal when periodic, but can become a serious problem if sustained. Elevated levels of ketone bodies in the blood decrease the blood’s pH, leading to DKA.

Non-ketotic Coma is a medical emergency in which a person with diabetes mellitus is comatose (unconscious) because of one of the acute complications of diabetes. In which extreme hyperglycemia and dehydration alone are sufficient to cause unconsciousness.

Lactic acidosis consists of elevated lactic acid (lactic acidemia, 2.0 mmol/L) with acidosis (pH 7.3) and without ketoacidosis. There may be low levels of ketones present (1:4 on serum dilution, or beta hydroxybutyrate >0.4 but <0.6 mmol/L). Approximately half of the reported cases of LA have occurred in patients with diabetes.

Hypoglycemia, or abnormally low blood glucose, is an acute complication of several diabetes treatments. It is rare otherwise, either in diabetic or non-diabetic patients. The patient may become agitated, sweaty, weak, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic.

1.17 Chronic complications:

Diabetes - when it results in high blood sugar over a period of years can affect almost every organ system of the body. We refer to these effects as the long-term complications of diabetes. These include effects on the heart, brain, kidneys, eyes, stomach, bowel, bladder, sexual organs, peripheral nerves, and others. The whole purpose of treating diabetes is to prevent the damaging effects of high sugars on these organs. If this can be done, a person with diabetes will live a longer, healthier and more pleasant life.
The chronic complications of diabetes are typically classified as

1. Macrovascular

2. Microvascular

Macrovascular complications of diabetes include coronary artery disease (CAD) and peripheral vascular disease. They result from accelerated atherosclerotic changes in the walls of the coronary arteries and the large and medium blood vessels in the legs and feet.

Microvascular complications result from the thickening of capillary and arteriole basement membranes. Although these changes occur in the small blood vessels throughout the body, they most commonly affect the eyes and kidneys, resulting in retinopathy and nephropathy, respectively.

1.18 Renal function:

The kidneys are found on each side of the backbone, situated between the thick muscles of the back and abdomen and are shaped like a bean.

- They filter the metabolic wastes from the blood plasma and excrete it from the body.
- They participate in the maintenance of the constant extracellular environment, required for proper cell functioning.
• They excrete waste products of metabolism - such as urea, creatinine and uric acid.
• They excrete water and electrolytes to match water intake and endogenous production.
• They are able to regulate the excretion of water and solutes by changing the tubular re-absorption or excretion.
• They secrete hormones that participate in systemic and renal hemodynamic regulation (renin, prostaglandins, bradykinin), red blood cell production (erythropoietin), as well as calcium, phosphorus and bone metabolism (vitamin D).
• They also perform other functions such as catabolism of peptide hormones and synthesis of glucose (glyconeogenesis) when fasting.

Each kidney contains about 1,000,000 to 1,300,000 nephrons, which is the basic functioning component of the kidney, and are specialized in such a way that it can move material back and forth between blood plasma and urine in order to conserve essential materials while still eliminating wastes. Each nephron consists of a glomerulus (it is a tuft of capillaries between two arterioles) and a series of tubes. The blood plasma is filtered across the specialized glomerular membrane (in the glomerulus) into the nephron tubes. By active and passive membrane transport the nephron tubule reabsorb essential materials from the filtrate and return it to the circulating blood.
1.19 Diabetic nephropathy:

Diabetic nephropathy (DN) is a glomerular sclerosis and fibrosis caused by the metabolic and hemodynamic changes of diabetes mellitus. It manifests as slowly progressive albuminuria with worsening hypertension and renal insufficiency. Pathogenesis begins with small vessel disease and is complex, involving glycosylation of proteins, hormonally influenced cytokine release (eg, transforming growth factor-β), deposition of mesangial matrix, and alteration of glomerular hemodynamics. Hyperfiltration, an early functional abnormality, is only a relative predictor for the development of renal failure.

2. Oxidative stress:

Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko et al., 2001, Maritim et al., 2003). ROS include free radicals such as superoxide ('O$_2^-$'), hydroxyl ('OH), peroxyl ('RO$_2^-$), hydroperoxyl ('HRO$_2^-$') as well as non radical species such as hydrogen peroxide (H$_2$O$_2$) and hydrochlorous acid (HOCI). RNS include free radicals like nitric oxide (\'NO) and nitrogen dioxide (\'NO$_2^-$), as well as non radicals such as peroxynitrite (ONOO$^-$), nitrous oxide (HNO$_2$) and alkyl peroxynitrates (RONOO) (Turko et al., 2001, Evans et al., 2002). Of these reactive molecules, \'O$_2^-$', \'NO and ONOO$^-$ are the most widely studied species and play important role in the diabetic cardiovascular complications.

\'NO is normally produced from L-arginine by endothelial nitric oxide synthase in the vasculature. \'NO mediates endothelium-dependent vasorelaxation by its action on guanylate cyclase in vascular smooth muscle cells (VSMC), initiating a cascade that leads to vasorelaxation. \'NO also displays antiproliferative properties and inhibits platelet and leukocyte adhesion to vascular endothelium (Turko et al., 2001). Therefore, \'NO is considered as a vasculoprotective molecule. However, \'NO easily reacts with superoxide generating a highly reactive molecule ONOO$^-$ and triggering a cascade of harmful events (Vega-Lopez et al., 2004). Therefore its chemical environment, i.e. presence of \'O$_2^-$, determines whether \'NO exerts protective or harmful effects. Production of one ROS or RNS may lead to the production of others through radical chain reactions.
'\( \text{O}_2^- \) is produced by one electron reduction of oxygen by several different oxidases including NAD(P)H oxidase, xanthine oxidase, cyclooxygenase and even eNOS under certain conditions as well as by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation, which is essential for generating ATP (Evans et al., 2003, Griendling and Fitz, 2003, Taniyama and Griendling, 2003). Under normal conditions, '\( \text{O}_2^- \) is quickly eliminated by antioxidant defense mechanisms. '\( \text{O}_2^- \) is dismutated to \( \text{H}_2\text{O}_2 \) by manganese superoxide dismutase (Mn-SOD) in the mitochondria and by copper (Cu)-SOD in the cytosol (Evans et al., 2003).

\( \text{H}_2\text{O}_2 \) is converted to \( \text{H}_2\text{O} \) and \( \text{O}_2 \) by glutathione peroxidase (GPx) or catalase in the mitochondria and lysosomes, respectively. \( \text{H}_2\text{O}_2 \) can also be converted to the highly reactive 'OH radical in the presence of transition elements like iron and copper.

While ROS are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation, excess generation in oxidative stress has pathological consequences including damage to proteins, lipids and DNA. ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier et al., 2001). Since it will be presented and discussed in greater detail in the next section, '\( \text{O}_2^- \) can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro and macrovascular complications. '\( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) stimulate stress-related signaling mechanisms such as NF-κB, p38-MAPK and STAT-JAK resulting in vascular smooth muscle cell migration and proliferation. In endothelial cells, \( \text{H}_2\text{O}_2 \) mediates apoptosis and pathological angiogenesis (Taniyama & Griendling, 2003). Furthermore, '\( \text{O}_2^- \) immediately reacts with 'NO generating cytotoxic ONOO⁻ and this reaction itself has several consequences. The first consequence is ONOO⁻ alters function of biomolecules by protein nitration as well as causing lipid peroxidation (Turko et al., 2001). For example, potassium channels, which regulate the vasorelaxation response, are inhibited by nitration (Liu & Gutterman, 2002). As recently reviewed by Turko et al. (2001), reported that increased levels of
nitrotyrosine are associated with apoptosis of myocytes, endothelial cells and fibroblasts in diabetes. The second consequence is ONOO− causes single-strand DNA breakage which in turn activates nuclear enzyme poly(ADP-ribose) polymerase (Soriano et al., 2001). The third one is, it decreases NO bioavailability causing impaired relaxation and inhibition of the antiproliferative effects of NO. Furthermore, ONOO− oxidizes tetrahydrobiopterin (BH4), an important cofactor for nitric oxide synthases (NOS), and causes uncoupling of NOS, which produces O2− instead of NO. Reactive oxygen species (ROS) induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which ultimately affects function (Maritim et al., 2003; Taniyama & Griendling, 2003). All these pathological modifications contribute to the pathogenesis of vascular dysfunction.

2.1 Sources of oxidative stress in diabetes:

Direct evidence of oxidative stress in diabetes is based on studies that focused on the measurement of oxidative stress markers such as plasma and urinary F2-isoprostane as well as plasma and tissue levels of nitrotyrosine and O2− (Vega-Lopez et al., 2004). There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways. Thus, we will first discuss these mechanisms and conclude with the recently proposed working plan for the initiation of oxidative stress and related vascular complications in diabetes. Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate OH radicals (Turko et al., 2001). In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of O2−. Enzymatic sources of augmented generation of reactive species in diabetes include nitric oxide synthases, NAD(P)H oxidase and xanthine oxidase. All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH4 and Ca2+-calmodulin. If nitric oxide synthases lacks its substrate L-arginine or one of its cofactors, NOS may produce O2− instead of NO and this is referred to as the uncoupled state of NOS (Aliciguzel et al., 2003). NAD(P)H oxidase is a membrane associated enzyme that
consists of five subunits and is a major source of 'O2·' production (Etoh et al., 2003). Guzik et al., investigated 'O2·' levels in vascular specimens from diabetic patients and probed sources of 'O2·' using inhibitors of NOS, NAD(P)H oxidase, xanthine oxidase and mitochondrial electron transport chain. This study demonstrated that there is enhanced production of 'O2·' in diabetes and this is predominantly mediated by NAD(P)H oxidase. Furthermore, the NOS-mediated component is greater in patients with diabetes than in patients who do not have diabetes (Guzik et al., 2002).

NAD(P)H oxidase activity is significantly higher in vascular tissue (saphenous vein and internal mammary artery) obtained from diabetic patients (Ergul et al., 2004). There is plausible evidence that PKC, which is stimulated in diabetes via multiple mechanisms, i.e. polyol pathway and Ang II, activates NAD(P)H oxidase (Amiri et al., 2002). The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and FADH2, through four complexes in the inner mitochondrial membrane, to oxygen, generating ATP in the process (Green et al., 2004). Under normal conditions, 'O2·' is immediately eliminated by natural defense mechanisms. A recent study demonstrated that hyperglycemia-induced generation of 'O2·' at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (Nishikawa et al., 2000, Brownlee et al., 2001). When endothelial cells are exposed to hyperglycemia at the levels relevant to clinical diabetes, there is increased generation of ROS and especially 'O2·', which precedes the activation of four major pathways involved in the development of diabetic complications. Nishikawa and colleagues elegantly demonstrated that generation of excess pyruvate via accelerated glycolysis under hyperglycemic conditions floods the mitochondria and causes 'O2·' generation at the level of Complex II in the respiratory chain. The mitochondrial 'O2·' is the initiating snowball that turns oxidative stress into an avalanche in diabetes by stimulating more ROS and RNS production via downstream activation of NF-κB-mediated cytokine production, Protein kinase C (PKC) and NAD(P)H oxidase. Thus, inhibition of intracellular free radical formation would provide a causal therapy approach in the prevention of oxidative stress and related vascular complications in diabetes.
2.2 Natural defense against oxidative stress and antioxidants:

Reactive oxygen species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. Natural defense mechanism exists against oxidative stress through endogenous or exogenous antioxidant substances. However, chronic diabetes is shown to disturb antioxidant defense system as shown: alteration in antioxidant enzymes (Strain, 1991), impaired glutathione metabolism (McLennan et al., 1991) and decreased ascorbic acid levels. Superoxide Dismutase (SOD) is the most important antioxidant enzyme because it is found virtually in all aerobic organisms. It immediately converts 'O$_2^\cdot$' to H$_2$O$_2$, which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria. Another enzyme that is important is glutathione reductase, which regenerates glutathione that is used as a hydrogen donor by glutathione peroxidase during the elimination of H$_2$O$_2$. Maritim and colleagues recently reviewed in detail that diabetes has multiple effects on the protein levels and activity of these enzymes, which further augment oxidative stress by causing a suppressed defense response (Maritim et al., 2003). Catalase catalyses the decomposition of hydrogen peroxide to water (H$_2$O) and oxygen (O$_2$).

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

H$_2$O$_2$ is a powerful oxidizing agent and is potentially damaging to the cells. By preventing excessive H$_2$O$_2$ build up, catalase allows important cellular processes which produce H$_2$O$_2$ as a byproduct to take place safely. Glutathione peroxidase enzyme is a well-known first line of defense against oxidative stress, which in turn requires glutathione as a cofactor. Glutathione peroxidase is considered the major detoxification enzyme for H$_2$O$_2$. Glutathione reductase is an ancillary enzyme to limit the amounts of ROS via its reduction of GSSG in the presence of an adequate supply of NADPH. Thus, the ratio of GSH/GSSG is maintained at a high level so that the cell maintains the capacity to combat oxidative stress.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADPH}
\]

Glutathione-S-transferase (GST) enzyme catalyzes the conjugation of a molecule of Glutathione (GSH) to an electrophilic or other reactive species (Kodavanti, 1999). This activity is useful in the detoxification of endogenous compounds such as
peroxidised lipids as well as the metabolism of xenobiotics. As an enzyme, GSTs may also bind toxins and function as transport proteins. Because of this, an early term coined for GSTs was “ligandin”. GSH is the major cellular reductant present in millimolar quantities in many cell GSH reduces hydrogen and organic peroxides via a reaction catalyzed by GPx; it serves as a scavenger of OH• and singlet oxygen (O2•−); and GSH is believed to reduce tocopherol radicals, either directly or indirectly by reducing DHA radical there by prevent lipid peroxidation.

Nonenzymatic antioxidants include vitamins A, C and E; glutathione; α-lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q10 (CoQ10); and cofactors like folic acid, uric acid, albumin, and vitamins B1, B2, B6 and B12. Alterations in the antioxidant defense system in diabetes have recently been reviewed (Vega-Lopez et al., 2004). Glutathione (GSH) acts as a direct scavenger as well as a cosubstrate for GSH peroxidase. It is a major intracellular redox tampon system.

Vitamin E is a fat-soluble one that prevents lipid peroxidation. It exists in 8 different forms, of which α-Tocopherol is the most active form in humans. Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back to the phenol by ascorbate and NAD(P)H dependent reductase enzymes (Hensley et al., 2000, 2004).

CoQ10 is an endogenously synthesized compound that acts as an electron carrier in the Complex II of the mitochondrial electron transport chain. Brownlee et al., 2001 reported that this is the site of O2•− generation under hyperglycemic conditions (Nishikawa et al., 2000, Brownlee et al., 2001). CoQ10 is a lipid soluble antioxidant, and in higher concentrations, it scavenges O2•− and improves endothelial dysfunction in diabetes (Watts et al., 2002; Hodgson et al., 2002, 2003).

Vitamin C (ascorbic acid) increases NO production in endothelial cells by stabilizing NOS cofactor BH4. α-Lipoic acid is a hydrophilic antioxidant and can therefore exert beneficial effects in both aqueous and lipid environments. α-lipoic acid is reduced to another active compound dihydrolipoate. Dihydrolipoate is able to regenerate other antioxidants such as vitamin C, vitamin E and reduced glutathione through redox cycling (Heller et al., 2001). Thus, both experimental and clinical studies summarized in the next sections utilized these naturally occurring
antioxidants, especially vitamins C, E and α-lipoic acid, in order to delineate the role of oxidative stress in the development of vascular complications of diabetes.

3.0 Management of Diabetes:

Studies have shown that there was significant reduction in the incidence of type 2 DM with a combination of maintenance of body mass index of 25 kg/m², eating high fiber and unsaturated fat and diet low in saturated and trans-fats and glycemic index, regular exercise, abstinence from smoking and moderate consumption of alcohol (Willi et al., 2007; Chen et al., 2011). Majority of type 2 DM can be prevented by lifestyle modification. Patients with type 2 DM should receive a medical nutrition evaluation; lifestyle recommendations should be tailored according to physical and functional ability (Chiniwala & Jabbour, 2011).

Bi guanides, of which metformin is the most commonly used in overweight and obese patients, suppresses hepatic glucose production, increases insulin sensitivity, enhances glucose uptake by phosphorylating GLUT-enhancer factor, increases fatty acid oxidation, and decreases the absorption of glucose from the gastrointestinal tract.
Meglitinides have a rapid onset and a short duration of action (4-6 hrs) and thus lower risk of hypoglycemia. Meglitinides are given before meals for postprandial blood glucose control. Preprandial administration allows flexibility in case a meal is missed without increased risk of hypoglycemia.

Thiazolidinedione is an insulin sensitizer, selective ligands transcription factor peroxisomes proliferator-activated gamma. They are the first drugs to address the basic problem of insulin resistance in type 2 DM patients, whose class now includes mainly pioglitazone after the restricted use of rosiglitazone recommended by Food and Drug Administration recently due to increased cardiovascular events reported with rosiglitazone. Alpha-Glucosidase Inhibitors are most effective for postprandial hyperglycemia and should be avoided in patients with significant renal impairment. Their use is usually limited due to high rates of side-effects such as diarrhoea and flatulence.

Glucagon-like peptide 1 (GLP-1) analogues are the foundation of incretin-based therapies which are to target this previously unrecognized feature of DM pathophysiology resulting in sustained improvements in glycemic control and improved body weight control.

Dipeptidyl-peptidase IV inhibitors inhibit dipeptidyl peptidase-4, a ubiquitous enzyme that rapidly inactivates both GLP-1 and GIP, increase active levels of these hormones and, in doing so, improves islet function and glycemic control in type 2 DM.

Insulin is used alone or in combination with oral hypoglycemic agents. Augmentation therapy with basal insulin is useful if some beta cell function remains. However, these drugs have side effects. Thus, it is essential to search for a new class of compounds to overcome the diabetic problems (Noor et al., 2008). Much attention has focused on the protective function of natural antioxidants in dietary plants.

Supplementation with exogenous antioxidants has been proved as a complementary treatment of diabetes and some of the antidiabetic agents are reported to have antioxidant properties, independent to their role in glucose control (Vedavanam et al., 1999). Recently, there has been a considerable interest in finding natural antioxidants from plant materials. Plants have been used as remedies and still
they play an important role in health care for about 80% of the world's population from the beginning of civilization. The therapeutic basis of herbal medication has formed by the presence of diverse bioactive compounds like steroids, terpenoids, flavonoids, alkaloids, phenols, glycosides etc. in plants. For the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agents. It has been reported that both antioxidant nutrients and phytochemicals can be useful in alleviating diabetes and diabetic complications (Lean et al., 1999). *Xanthium indicum* is one of the hypoglycaemic plants and it has more medicinal values.

4.0 Xanthium indicum:

*Xanthium indicum* (Asteraceae) is a coarse annual plant, which grows to about a meter high and is found in India and the wild fallow lands of Bangladesh, Malaysia and Indonesia. It grows as a gregarious weed in fallow paddy fields and by the canal or ditch banks in all areas. It is known as ‘burweed’ in English and ‘marulamathangi’ in Telugu. It is commonly known as Ghagra, Banokra, Bichaphal or cocklebur is a coarse annual about a meter or more in height. Leaves are numerous, 5-7.5 cm long and almost as broad as long, broadly triangular-ovate or suborbicular, acute, often 3-lobed, rough with appressed hairs, irregularly serrate. Heads in terminal and axillary racemes. Fruits are ovoid; about 1.6 cm long, with 2 erect mucronate beaks, thickly clothed with usually hooked prickles.

The roots, leaves and fruits of the plant are used in Ayurvedic preparations. The leaves have diaphoretic, sedative and sudorific activity and useful in longstanding causes of malaria. Root is bitter, tonic and useful in strumous diseases and different cancers like urinary cancer. Fruits are rich in vitamin C and have cooling and demulcent properties. It is given in small pox and for eye ailments as ointment. Leaf, boiled in water is given in dysentery. Tender stems and petioles of the plant are used as vegetables (Uddin, 2006). The plant is reported to contain alpha and gamma tocopherols, polyphenols, glucoside, xanthostrumarin and xanthonolides as the main constituents (Ghani, 2003).

*Xanthium indicum* has been reported to be used by the Lohit community of Arunachal Pradesh, India for treatment of inflammation-related diseases (Namsa et al., 2009). Ethnomedicinal uses in Bangladesh include using the plant to control blood sugar in diabetic patients and for treatment of rheumatic pain (Rahmatullah et al.,
A recent report has demonstrated anti-bacterial (Obayed et al., 2013) and cytoxic activities in methanolic extract of the plant leaves (Ullah et al., 2013). It was also to be noted that antinociceptive activity of leaf extract of the plant has been reported before (Raquibul et al., 2009; Farhad et al., 2011), improve the performance of growth and cocoon characteristics of silkworm larvae, (Pardeshi and Bajad, 2014), inhibitory activity of prostaglandins (Sadhu et al., 2003) antioxidant and anti diarrhoeal activity (Akter et al., 2009). The recent reports have been demonstrated that X. indicum stem has hypoglycemic activity (Ezazul et al., 2013).

5.0 Bioinformatics:

Bioinformatics is the application of computer technology to the management of biological information. Computers are used to gather, store, analyze and integrate biological and genetic information which can then be applied to gene-based drug discovery and development. The need for Bioinformatics capabilities has been precipitated by the explosion of publicly available genomic information resulting from the Human Genome Project. The goal of this project is to determination of the sequence of the entire human genome (approximately three billion base pairs) will be reached by the year 2002.
The science of Bioinformatics, which is the melding of molecular biology with computer science, is essential to the use of genomic information in understanding human diseases and in the identification of new molecular targets for drug discovery. Bioinformatics tools have become very important to pinpoint the targets for different ligands and unravelling the structure and function of complex biological mechanisms. The analysis of primary gene products has further been considered as diagnostic and screening tool for disease recognition. Such strategies aim at investigating all gene products simultaneously in order to get a better overview about disease mechanisms and to find suitable therapeutic targets.

6.0 Drug discovery:

Traditional drug discovery generally requires innovation of lead compounds by medicinal chemists. Lead compounds will then be synthesized and experimentally tested until a compound with the desired pharmacological properties has been developed. This trial and error process can be expensive and time consuming. In modern drug discovery, computational methods are generally involved in identifying and modifying lead compounds. For lead discovery and lead optimization, 3D structural information on the ligand, the protein receptor, or both, is highly desirable. A commonly used method in 3D computer-aided drug design is molecular docking. Docking involves two processes:

1. Geometric sampling of potential ligand/protein binding models and
2. Scoring, usually using an equation and specific parameters to estimate a ligand’s binding affinity.

Therefore, success not only depends on the experience and intuition of the medicinal chemist, but also on the accuracy of both the computational modeling methods and the structural information, especially for the protein–ligand active site.

In the past twenty years, about thirty docking programs have been developed (Shoichet & Peishoff, 2006) and each of them adopts different docking approaches with their own advantages and limitations. Studies have demonstrated that the performance of docking program greatly depends on the type of protein-ligand system, especially the characteristics of the protein active site and the ligand (Schulz-Gasch & Stahl, 2003).
7.0 Molecular docking:

7.1 Common terms used in docking:

**Posing:** The process of determining whether a given conformation and orientation of a ligand fits the active site. This is usually a fuzzy procedure that returns many alternative results.

**Scoring:** Both posing and ranking involve scoring. The pose score is often a rough measure of the fit of a ligand into the active site. The rank score is generally more complex and might attempt to estimate binding energies.

**Ranking:** A more advanced process than pose scoring that typically takes several results from an initial scoring phase and re-evaluates them. This process usually attempts to estimate the free energy of binding as accurately as possible. Although the posing phase might use simple energy calculations (electrostatic & vander Waals), ranking procedures typically involve more elaborate calculations (perhaps including properties such as entropy or explicit solvation).

**Torsional entropy:** Entropy associated with a rotatable bond in a molecule. Immobilization of a rotatable bond on binding leads to loss of its torsional (rotational) entropy.

**Regression analysis:** Determination of parameter values for a chosen (linear or nonlinear) function to best fit a set of observations.

**Potential of mean force (PMF):** In the context of docking and scoring, PMFs are derived from statistical analysis of experimentally observed distributions and frequencies of specific atom-pair interactions in a large collection of protein–ligand structures. Interaction potentials between each atom pair in two molecules (for example, ligand and protein) approximate the free energy of each pair-wise interaction as a function of inter-atomic distance.

**Linear discriminant analysis:** Mathematical analysis based on two classes of data and two independent variables (a, b) that attempts to find a line that best separates the data. This line is orthogonal to the discriminant function that is a linear combination of the original variables, in this case: \( F = c_1a + c_2b \) (\( c_1, c_2; \) coefficients).
**Pharmacophore:** The spatial arrangement of atoms or groups in a molecule known or predicted to be responsible for specific biological activity.

The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site. In general, there are two aims of docking studies: accurate structural modelling and correct prediction of activity. However, the identification of molecular features that are responsible for specific biological recognition, or the prediction of compound modifications that improve potency, are complex issues that are often difficult to understand and even more so to simulate on a computer.

In view of these challenges, docking is generally devised as a multi-step process in which each step introduces one or more additional degrees of complexity (Brooijmans & Kuntz, 2003). The process begins with the application of docking algorithms that pose small molecules in the active site. This in itself is challenging, as even relatively simple organic molecules can contain many conformational degrees of freedom. Sampling these degrees of freedom must be performed with sufficient accuracy to identify the conformation that best matches the receptor structure, and must be fast enough to permit the evaluation of thousands of compounds in a given docking run. Algorithms are complemented by scoring functions that are designed to predict the biological activity through the evaluation of interactions between compounds and potential targets. Early scoring functions evaluated compound fits on the basis of calculations of approximate shape and electrostatic complementarities. Relatively simple scoring functions continue to be heavily used, at least during the early stages of docking simulations. Pre-selected conformers are often further evaluated using more complex scoring schemes with more detailed treatment of electrostatic and vander Waals interactions, and inclusion of at least some solvation or entropic effects (Gohlke & Klebe, 2002). It should also be noted that ligand-binding events are driven by a combination of enthalpic and entropic effects, and that either entropy or enthalpy can dominate specific interactions.
7.2 Molecular representations for docking:

To evaluate various docking methods, it is important to consider how the protein and ligand are represented. There are three basic representations of the receptor: atomic, surface and grid (Halperin et al., 2002). Among these, atomic representation is generally only used in conjunction with a potential energy function (Burnett, & Taylor, 2000) and often only during final ranking procedures (because of the computational complexity of evaluating pair-wise atomic interactions). Surface-based docking programs are typically, but not exclusively, used in protein–protein docking. These methods attempt to align points on surfaces by minimizing the angle between the surfaces of opposing molecules (Norel et al., 1999). Therefore, a rigid body approximation is still the standard for many protein–protein docking techniques. The use of potential energy grids was pioneered by Goodford, and various docking programs use such grid representations for energy calculations. The basic idea is to store information about the receptor’s energetic contributions on grid points so that it only needs to be read during ligand scoring. In the most basic form, grid points store two types of potentials: electrostatic and van der Waals.

7.3 Search methods and molecular flexibility:

The algorithms used to treat ligand flexibility and, to some extent, protein flexibility. Treatment of ligand flexibility can be divided into three basic categories (Brooijmans & Kuntz, 2003): systematic methods (incremental construction, conformational search, databases); random or stochastic methods (Monte Carlo, genetic algorithms, tabu search); and simulation methods (molecular dynamics, energy minimization).

7.4 Systematic search:

These algorithms try to explore all the degrees of freedom in a molecule, but ultimately face the problem of combinatorial explosion (Leach, 1996). Therefore, ligands are often incrementally grown into active sites. A stepwise or incremental search can be accomplished in different ways for example, by docking various molecular fragments into the active-site region and linking them covalently (which is most popular as a de novo ligand-design strategy) or, alternatively, by dividing
docked ligands into rigid (core fragment) and flexible parts (side chains). In the latter case, once the rigid cores have been defined, they are docked into the active site.

Next, flexible regions are added in an incremental fashion (Klebe, & Rarey, 1996). For example, DOCK 4.0 (Ewing et al., 2001) poses the core fragment by steric complementarity, and flexible side chains are grown one bond at a time by systematically exploring each bond’s pose space. A pruning algorithm is applied to remove unfavourable conformations early on, thereby reducing the complexity of the problem. FlexX differs from DOCK in that the placement of the rigid core fragment is based on interaction geometries between fragments and receptor groups. Interacting groups are primarily hydrogen-bond donors and acceptors, as well as hydrophobic groups. FlexX further differs from DOCK in that it uses a pose-clustering algorithm to classify the docked poses (Kramer et al., 1999). The Hammerhead algorithm, in common with other incremental search algorithms, also divides ligands into fragments. However, Hammerhead docks each fragment and then rebuilds the ligand from fragments that have acceptable initial scores. During the fragment-growing stage, energy minimization is performed after each new addition (Welch et al., 1996).

Another method of systematic search is the use of libraries of pre-generated conformations. Library conformations are typically only calculated once and the search problem is therefore reduced to a rigid body docking procedure. For example, FLOG generates database conformations on the basis of distance geometry. Once acceptable conformations have been generated, the algorithm explores them in a manner similar to DOCK (Kearsly et al., 1999).

7.5 Random search:

These algorithms (often called stochastic methods) operate by making random changes to either a single ligand or a population of ligands. A newly obtained ligand is evaluated on the basis of a pre-defined probability function. Two popular random approaches are Monte Carlo and genetic algorithms. Alternative implementations of Monte Carlo search have been reported, including a popular form in AutoDock (Olson et al., 1993). By contrast, several other programs (including DOCK and GOLD) have implemented genetic algorithms (Morris et al., 1998). The basic idea of a tabu search algorithm is to take into consideration already explored areas of conformational space. To determine whether a molecular conformation is accepted or
not, the root mean square deviation is calculated between current molecular coordinates and every molecule’s previously recorded conformation. For example, PROLEADS makes use of a tabu search algorithm (Westhead et al., 1997: Baxter et al., 1998).

7.6 Simulation methods:

Molecular dynamics is currently the most popular simulation approach. However, molecular dynamics simulations are often unable to cross high-energy barriers within feasible simulation time periods, and therefore might only accommodate ligands in local minima of the energy surface. Therefore, an attempt is often made to simulate different parts of a protein–ligand system at different temperatures (Di Nola et al., 1994). Another strategy for addressing the local minima problem is starting molecular dynamics calculations from different ligand positions. In contrast to molecular dynamics, energy minimization methods are rarely used as stand-alone search techniques, as only local energy minima can be reached, but often complement other search methods, including Monte Carlo (Trosset et al., 1995). DOCK performs a minimization step after each fragment addition, followed by a final minimization before scoring.

7.7 Protein flexibility:

The treatment of protein flexibility is less advanced than that of ligand flexibility, but various approaches have been applied to flexibly model at least part of the target (Carlson, & McGammon, 2000), including molecular dynamics and Monte Carlo calculations, rotamer libraries and protein ensemble grids (Knegtel et al., 1997). The idea behind using aminoacid side-chain rotamer libraries is to model protein conformational space on the basis of a limited number of experimentally observed and preferred side-chain conformations. To reduce the number of discrete protein conformations arising from combinations of rotamers, a dead-end elimination algorithm is often used. This algorithm recursively removes side-chain conformations that do not contribute to a minimum energy structure. Another method of treating protein flexibility is to use ensembles of protein conformations (rather than a single one) as the target for docking and to map these ensembles on a grid representation. One approach generates an average potential energy grid of the ensemble, as first
implemented in DOCK; other maps various receptor potentials to each grid point and subsequently scores ligand conformations against each set of receptor potentials.

7.8 Scoring:

The evaluation and ranking of predicted ligand conformations is a crucial aspect of structure-based virtual screening. Even when binding conformations are correctly predicted, the calculations ultimately do not succeed if they do not differentiate correct poses from incorrect ones, and if ‘true’ ligands cannot be identified. So, the design of reliable scoring functions and schemes is of fundamental importance. Free-energy simulation techniques have been developed for quantitative modelling of protein–ligand interactions and the prediction of binding affinity (Simonson et al., 2002). However, these expensive calculations remain impractical for the evaluation of large numbers of protein–ligand complexes and are not always accurate. Scoring functions implemented in docking programs make various assumptions and simplifications in the evaluation of modelled complexes and do not fully account for a number of physical phenomena that determine molecular recognition, for example, entropic effects. Essentially, three types or classes of scoring functions are currently applied: Force-field-based, empirical and knowledge-based scoring functions.

7.9 Structures of target sites:

The choice and preparation of the structural model of a targeted binding site are important variables. Experimentally determined (X-ray or nuclear magnetic resonance) structures are generally preferred. However, as the number of proteins of pharmaceutical interest has grown faster than the number whose structures have been determined, homology modelling has risen in popularity. A recent study compared the quality of docking results when either crystal structures of holo- or apo-enzymes or homology models were used as templates (McGovern et al., 2003). Perhaps surprisingly, homology models yielded enrichments factors of ten or better in eight of ten test cases studied, and apo-enzymes and homology modelled structures performed comparably well. However, by far the best performance was observed when ligand-bound protein conformations were used as starting points. The study demonstrated that even subtle protein conformational changes that result from ligand binding were sufficient to significantly influence the quality of docking results. Nevertheless,
homology models built in the presence of high sequence similarity provided reasonable docking templates.

7.10 Pre-screening: three-dimensional filtering:

In addition to conventional one/two-dimensional filters such as the rule-of-five (Lipinski & Christopher, 1997), three-dimensional filter functions have been implemented to efficiently pre-screen very large databases and reduce the final number of docking and scoring steps. For example, shape similarity methods can be applied for filtering. The heuristic is based on identifying similar molecular shapes on the basis of signatures, triplets, quartets or higher-order groups of atoms (Zauhar et al., 2003). However, these shape filters are usually limited to pre-screening of databases that contain single molecular conformations, which can be a source of false-negatives. In addition, pharmacophore-based screening can be carried out where predefined chemical and geometric features in compounds are matched (Rastelli et al., 2003). Signatures and bit strings derived from triplets of distances and surface triplets and histograms have been used to identify preferred candidates. Recently, a ray-tracing-based approach has been applied to calculate shape signatures of molecules for database searching. These types of descriptors are also highly conformation-dependent and therefore limited in their predictive value when only a single (hypothetical) molecular conformation is used.

7.11 Hit identification:

The ultimate measure of success for the methods is their ability to produce significant hit rates while reducing the number of compounds that need to be tested. It is interesting to note that nearly all groups performed pre-filtering and used two-dimensional similarity methods and shape or drug-like filters to reduce the number of database compounds for the time-consuming steps of flexible docking, elaborate scoring and visual analysis. Hits with at least low-micromolar potency were usually found, often without biasing search calculations towards previously identified hits. The results also mirror the general trend that hits in the micromolar range are much more frequently identified than nanomolar hits in these calculations (this is similar to the situation in biological screening). Major reasons for this are that newly identified active compounds are rarely optimized for potency against a given target and that
nanomolar potency is typically only obtained after chemical modification in the course of hit-to-lead transition and lead optimization.

7.12 Structure-based lead optimization:

In addition to hit identification, docking techniques are increasingly used to support lead optimization efforts. Here, the scenario changes: to facilitate a hit-to-lead transition, the compound potency typically has to be increased by two to three orders of magnitude and relatively small chemical modifications can lead to significant changes in binding. The requirement to estimate the effects of relatively small chemical changes further complicates the calculations and therefore distinguishing a micromolar compound from a nanomolar analogue often requires much greater accuracy than typical docking and scoring can provide. However, once hits or leads have been co-crystallized with their targets and exact binding conformations have been established, docking of analogues can be facilitated by the application of algorithms such as ‘anchored search’ (Ewing et al., 2001) that model compound modifications on pre-defined core fragments of leads. These ‘conservatively’ predicted complexes usually involve only a limited number of analogues, and so alternative and consensus scoring schemes can be easily explored.

7.13 Simulations:

Free-energy simulations are applicable to evaluate limited numbers of analogues. Various approximations have been proposed for reducing the complexity of perturbation calculations for these purposes. For example, the OwFeg method performs a free-energy simulation over bound and unbound states of ligands but maps energy changes to a grid (Pearlman. & Charifson, 2001), which greatly simplifies calculations for transforming one functional group into another. Grid points those are energetically relevant for various chemical modifications can be monitored during analogue design. Moreover, linear response approximations that utilize ligand-interaction energies with the protein and solvent environment are now more commonly applied in lead optimization (Aquist et al., 1994). These methods require the availability of at least a few experimental data points across the range of activities considered to be significant.
A quantitative structure–activity relationship (QSAR) is applied to combine non-bonded interactions that occur within the simulated system. Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) (Kollman et al., 2000) calculations are another molecular dynamics based simulation technique involving both force-field and solvation terms that are important for binding. Solvation effects are estimated using a continuum Poisson–Boltzmann model (Sitkoff et al., 1998). A major difference between MM/PBSA and linear response methods is the treatment of the ligand in its unbound state: MM/PBSA uses normal mode analysis to calculate enthalpic and entropic contributions to the ligand free energy.

### 7.14 Active-site analysis:

Graphical computational analysis of binding sites has greatly contributed to structure-based drug design since its early days. Docking and simulation techniques have also been applied to analyse features of the active site, including various hydrophobic and hydrophilic molecular fields that can identify promising areas for ligand docking and/or de novo design. Surface maps and molecular fields are mostly stored on grids that are used to semi quantitatively compare active sites in homologous enzymes to explore differences in specificity (Sheridan et al., 2002). The evaluation of potential interactions in active sites can complement docking analyses. Another recent approach generates structural interaction fingerprints (SIFts) that allow pre-screening for potential ligands in databases prior to docking (Deng et al., 2004). Active-site analysis techniques can also be applied in combination with quantitative methods.

### Absorption, distribution, metabolism and excretion properties:

Docking techniques are currently also applied to aid in structure-based absorption, distribution, metabolism and excretion (ADME) evaluation.

### 8.0 PROGRAM OF THE PRESENT STUDY:

Oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes. In diabetic conditions, the production of free radicals and to counter these free radicals on antioxidant enzymes systems alterations would be expected in the study. Several authors reported that Streptozotocin (STZ)-induced diabetic rats showed reduced activities of antioxidant
enzymes, oxidative enzymes and enhanced the lipid peroxidation levels. The therapeutic role of *Xanthium indicum* leaves on diabetic condition with reference to antioxidant and lipid peroxidation have been reported extensively. However the nature of *Xanthium indicum* leaves on diabetic condition has not been reported so far.

Keeping in view of this above literature and background of the research the following program of work has been planned to elucidate the role of crude extract of *Xanthium indicum* leaves and its bioactive compound on diabetic condition employing male albino rat as an animal model.

8.1 Objectives of the present study:

1. To screen the anti diabetic bioactive compounds from the leaves of *Xanthium indicum* by *in silico* studies with specific molecular target proteins.

2. To study the effect of crude leaf extract of *Xanthium indicum* and its bioactive compound, α-tocopherol on blood glucose levels, body weights and insulin levels changes in STZ induced diabetic rats.

3. To evaluate antidiabetic activity of crude leaf extract of *Xanthium indicum* and its bioactive compound, α-tocopherol on antioxidant defense system in STZ induced diabetic rats.

4. To study the effect of *Xanthium indicum* and its bioactive compound, α-tocopherol on lipid peroxidation by measuring the MDA levels in diabetic induced rats.

5. To estimate the activity of *Xanthium indicum* crude extract and its bioactive compound, α-tocopherol on kidney markers like creatinine and cystatin C levels in serum, creatinine levels in serum and urine, microalbumin levels in urine in STZ induced diabetic rats.

6. To study the histopathological changes of the kidney tissue, under the influence of crude leaf extract of *Xanthium indicum* and its bioactive compound, α-tocopherol in normal as well as STZ induced diabetic rats.