INTRODUCTION
CHAPTER 1
INTRODUCTION

Diseases adversely affected the health and well-being of human population. In 21st century non-communicable disease have become the main public concerns. Among hundred years diseases were caused by infectious agents (Zimmet et al., 2001). Nature gave unexhaustive bounties to explore, experiment, analysis and utilize to cure and treat human and animal ailments successfully such as hepatitis, malaria, jaundice, inflammation, skin disorders and depression. Recently extensive research still going to find remedy to cure many diseases like diabetes, hepatitis, cancer etc. which is not only effective in complete irradiation of disease but also safe and economical. Day by day there is an increasing demand of medicine and treatment for many diseases. There is an important need to optimize the possibilities of using plants to develop numerous drugs to treat human ailments. The synthetic drugs which are used continously in some individuals may lead to side effects or drug interactions when used to treat such ailments. There is a new research look forward to use plants and herbal preparations for their therapeutic values because synthetic drug cause side effects. e.g., sulfonamides inhibit metabolism or excretion of antidiabetic drug sulfonylureas thereby producing hypoglycemia, while rifampicin increases their metabolism to reduce their hypoglycemic effect.

DIABETES

The Egyptians was first documented Diabetes. It was characterised by weight loss and polyuria. However, Aertaeus the Greek physician who coined the term diabetes mellitus (DM). In Greek, diabetes means “to pass through” and mellitus is the Latin word for honey (referring to sweetness). Diabetes is an important cause of prolonged ill health and premature mortality, and claims more lives per year than HIV-AIDS with nearly 1 death every 10 seconds. Diabetes mellitus (DM) is a metabolic disease due to defects in insulin secretion and/or action characterized by hyperglycemia. Diabetes is one of the main threats to human health which has been a disease of minor significance to world health until a few decades ago (Alberti et al., 2004). In developed countries, diabetes is the fourth to fifth major cause of mortality and it is most common non-communicable disease worldwide. The current estimate
revealed that among 150 million to 220 million in 2010 and 300 million in 2025 will affect by diabetes worldwide. Developing countries like India had maximum increases in last few years. In India is 2.4% in the rural population and 11.6% in the urban population affected by Type-2 diabetes. It has been estimated that there will have the largest number of diabetic subjects in the world on 2025 (Tripathi et al., 2006). Diabetes mellitus is one of the most important major killers in Asian and Western Pacific peoples which were indicated by the World Health Organization (Tiwari et al., 2002). India 43.2 million people were affected by diabetes which is next to China. These estimates are based on lost productivity, resulting primarily from premature death.

Insulin improperly regulated the homeostasis of carbohydrate and lipid metabolism which is defined as diabetes. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normal and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus (Kameswar et al., 2003). Major complications of diabetes include hyperglycemia, dyslipidemia or hyperlipidemia which leads to the development of micro and macrovascular complications of diabetes leads to morbidity and death.

Recently there was numerous research revealed that physiological and biochemical derangement of the diabetic state. Several derangements have been characterized in hyperglycemic animals. Lipid metabolism and structure also significantly changed in diabetes. Naturally there occur oxidative stress due to the structural changes which are associated with development of vascular disease in diabetes (Baynes et al., 1999). Antioxidant will be reduced due to oxidative stress in diabetes, it will enhance the deleterious effects of free radicals. The diabetogenic activity was induced by streptozotocin by inducing oxygen free radicals and thereby damaging the pancreas (Szkudelski 2001). It also increased lipid peroxidation which causes Hyperlipidemia in diabetic rats (Saravanan 2005). Glucose and lipid homeostasis mainly occur in insulin dependent tissue liver, which was severely affected during diabetes. Free fatty-acids, synthesis of cholesterol, phospholipids and triglycerides were uptake, oxidation and metabolic conversion by the organs liver and kidney (Satynarayana 2004). The concentration
and composition of lipid will be profoundly altered during diabetes. Diabetes is treating with many traditional plants throughout the world. The herbal formulation and drugs from plants are frequently considered to be less toxic and more free from side effects than synthetic one (Mitra et al., 1996). Traditional medicines are very important to treat hyperglycemic which is recommend by world health organization. Because there will be side effects and toxicity due to continuous use of the synthetic antidiabetic drugs (Ashok et al., 2010).

Diabetes mellitus is one of the most important chronic diseases affecting more than 1 in 20 people. In this disorder, either the pancreas produces insufficient amounts of the hormone insulin or body cells become resistant to the hormone’s effects. Normally, insulin is produced by the pancreas and enables the body’s cell to absorb the sugar glucose (their main energy source) from blood stream. In Diabetes mellitus, the cells have to use other source of energy, which may lead to a buildup of toxic by products in the body. Unused glucose accumulates in the blood and urine, causing excessive urination and thirst. Treatment is designed to control glucose levels in the blood. Among people treated for diabetes mellitus, 1 in 10 depends on self administered injections of insulin for life. The rest need a carefully managed diet and often oral drugs. These measures enable most affected people to lead normal lives. However in many cases, complications eventually develop, although their onset may be delayed by treatment. Complications include problems with the eyes, kidneys, cardiovascular system and nervous system and thus increase susceptibility to infections such as cystitis. There are two forms of diabetes mellitus designated as Type 1 diabetes and Type 2 diabetes.

Type 1 Diabetes (Insulin Dependent Diabetes Mellitus) occurs when the pancreas produces far too little insulin or produces none at all. The disorder usually develops suddenly in childhood or adolescence although dietary measures are also important, it must be treated with insulin injections. Type 1 Diabetes is usually caused by an abnormal bodily reaction, in which the immune system destroys insulin-secreting cells in the pancreas. Type 1 Diabetes may cause weight loss and Ketoacidosis is an acute complication.
Type 2 diabetes (Non Insulin Dependent Diabetes Mellitus) is a change in metabolic condition which is characterized by hyperglycemia (high blood sugar). In this condition, the pancreas continues to secrete insulin, but cells in the body become resistant to its effects. This form of diabetes mainly affects people over age 40 and is more common in over weight people. The classic symptoms are excess thirst, frequent urination, and constant hunger. Type 2 diabetes in people is thought be of obesity, who are genetically predisposed to the disease. Increasing exercise and dietary changes help to manage Type 2 diabetes. Metformin or insulin may be needed, if blood sugar levels are not adequately lowered by exercise and food control. Routine check up of blood sugar levels in those on insulin is typically required. Since 1960, there have been rates of Type 2 diabetes increased markedly in parallel with obesity. In 1985 around 30 million of people with diabetes, but in 2010 there were approximately 285 million people diagnosed with the disease. The life span of an individual become ten year shorter in their life expectancy due to chronic disease. Long term complications of Type 2 diabetes from high blood sugar which can include, diabetic retinopathy where eyesight is affected, heart disease, strokes, kidney failure (dialysis require), nephropathy, wound unhealing and poor blood flow in the limbs leading to amputations. Other symptoms that are commonly present at diagnosis include a history of blurred vision, itchiness, peripheral neuropathy, recurrent vaginal infections and fatigue. In the first few years people with diabetes, have no symptoms and are diagnosed on routine testing. Hyperosmolar hyperglycemic state (a condition of very high blood sugar associated with a decreased level of consciousness and low blood pressure) rarely present in people with Type 2 diabetes.

There is a number of complications for Type 2 diabetes which including: two to four time increase the risk of cardiovascular disease and stroke, a 20-fold increase in lower limb amputations and increased rates of hospitalizations. Type 2 diabetes is the largest cause of nontraumatic blindness and kidney failure in the developed world and increasingly elsewhere. Increased risk of Type 2 diabetes also been associated with Alzheimer's disease and vascular dementia of cognitive dysfunction. Wound healing is delayed due to hyperglycemia, acanthosis nigricans, sexual dysfunction and frequent infections with most common complications. Recent studies revealed that lifestyle and genetic factors combinedly involved in the development of Type 2 diabetes.
While some of these factors are under personal control such as diet and obesity, other factors are not, such as increasing age, female gender, and genetics. Type 2 diabetes people feel lack in sleep due to alteration in regular metabolism. The nutritional status of a mother during fetal development may also play a role, with one proposed mechanism being that of altered DNA methylation.

Type 2 diabetes is developed due to change in a number of life style factors, including obesity and being overweight (defined by a body mass index of greater than 25), lack of poor diet, stress and physical activity. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60-80% of cases in those of European and African descent, and 100% of cases in Pima Indians and Pacific Islanders. Those who are not obese often have a high waist–hip ratio. Smoking also appears to increase the risk of Type 2 diabetes mellitus. Most cases of diabetes involve many genes, with each being a small contributor to an increased probability of becoming a Type 2 diabetic. If one identical twin has diabetes, the chance of the other developing diabetes within his lifetime is greater than 90%, while the rate for nonidentical siblings is 25–50%. As of 2011, more than 36 genes had been found that contribute to the risk of Type 2 diabetes.

![Fig. 1. Complications of Type 2 diabetes](image)
All of these genes together still only account for 10% of the total heritable component of the disease. There are a number of rare cases of diabetes that arise due to an abnormality in a single gene known as monogenic forms of diabetes or "other specific types of diabetes". These include maturity onset diabetes of the young (MODY), Donohue syndrome and Rabson-Mendenhall syndrome, among others. Maturity onset diabetes of the young constitute 1–5% of all cases of diabetes in young people. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (Subramanian et al., 2008).

Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. On the other hand, mammalian α-glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion. An effective means of lowering the levels of postprandial hyperglycemia have been offered by α-amylase and α-glucosidase inhibitors. Several inhibitors of α-amylase and α-glucosidase has been isolated from medicinal plants to serve as an alternative drug with increased potency and lesser adverse effects than existing synthetic drugs.

Alpha glucosidase inhibitors are used as oral anti diabetic drugs for treating Type 2 diabetes mellitus. They act by preventing the digestion of carbohydrates such as starch. Carbohydrates are normally converted into simple sugars which can be absorbed through the intestine. Alpha glucosidase inhibitors act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrates. The intestinal alpha glucosidases hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates. Less amounts of glucose is absorbed
because the carbohydrates are not broken down into glucose molecules. In diabetics the short term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating etc.

There are a number of medications and other health problems that can predispose to diabetes. Some of the medications include: glucocorticoids, thiazides, beta blockers, atypical antipsychotics, and statins. Those who have previously had gestational diabetes are at a higher risk of developing Type 2 diabetes. Testosterone deficiency is also associated with Type 2 diabetes. Type 2 diabetes is due to insufficient insulin production from ß cells in the setting of insulin resistance. Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue. In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood. The proportion of insulin resistance versus ß cell dysfunction differs among individuals, with some having primarily insulin resistance and only a minor defect in insulin secretion and others with slight insulin resistance and primarily a lack of insulin secretion.

**Glucose Uptake by Skeletal Muscle**

![Diagram of Glucose Uptake by Skeletal Muscle](image)

Fig.2. Glucose uptake by skeletal muscle
Glucose is an essential substrate for the metabolism and homeostasis of all eukaryotic cells, with skeletal muscle being critical for glucose disposable and blood glucose regulation. Glucose cannot passively diffuse into a cell and must be transported through the cell membrane by glucose transporters (GLUTs). GLUT1 and GLUT3 are located in the plasma membrane of cells throughout the body, as they are responsible for maintaining a basal rate of glucose uptake. Basal blood glucose level is approximately 5 mM (5 millimolar). The Km value (an indicator of the affinity of the transporter protein for glucose molecules, a low Km value suggests a high affinity) of the GLUT1 and GLUT3 proteins is 1 mM, therefore GLUT1 and GLUT3 have a high affinity for glucose and uptake from the bloodstream is constant. GLUT2 in contrast has a high Km value (15-20 mM) and therefore a low affinity for glucose. They are located in the plasmamembranes of hepatocytes and pancreatic cells.

GLUT4 transporters are insulin sensitive, and are found in muscle and adipose tissue. As muscle is a principle storage site for glucose and adipose tissue for triglyceride (into which glucose can be converted for storage), GLUT4 is important in post-prandial uptake of excess glucose from the bloodstream. During fasting, some GLUT4 transporters will be expressed at the surface of the cell. However, most will be found in cytoplasmic vesicles within the cell. After a meal and at the binding of insulin (released from the islets of Langerhans) to receptors on the cell surface, a signalling cascade begins by activating phosphatidylinositol kinase activity which culminates in the movement of the cytoplasmic vesicles toward the cell surface membrane. Upon reaching the plasmalemna, the vesicles fuse with the membrane, increasing the number of GLUT4 transporters expressed at the cell surface, and hence increasing glucose uptake.

GLUT4 is the major glucose transporter isoform expressed in skeletal muscle, and has a large capacity to increase glucose transport across the cell membrane through facilitative diffusion (Bryant et al., 2002). GLUT4 is located intracellularly; therefore, its translocation to the cell surface to facilitate glucose transport into the cell is essential for the maintenance of whole body glucose homeostasis in response to acute perturbations in blood glucose. Although insulin and contraction both facilitate glucose uptake into skeletal muscle by increasing GLUT4 translocation to the cell
membrane, the mechanism(s) through which they signal GLUT4 translocation and glucose uptake, although not yet fully elucidated, are known to be independent (Ryder et al., 2001). Skeletal muscle glucose uptake during dynamic exercise can increase as much 50-fold, and is regulated through three important steps; delivery to the muscle cell, transport through the cell membrane, and flux through intracellular metabolism Fig. 2. Under normal submaximal exercise conditions, and providing that adequate extracellular glucose is available, skeletal muscle glucose uptake appears to be limited by glucose transport though the cell membrane. The mechanism(s) through which contraction/exercise stimulates GLUT4 translocation and glucose uptake appear to arise from local factors within skeletal muscle such as calcium (Ca\(^{2+}\)), CaMK, reactive oxygen species (ROS), nitric oxide (NO), and AMP-activated protein kinase (AMPK).

**FREE RADICALS**

Oxygen is important for life processes to occur an excess of oxygen could result in oxidative damage, which may even lead to death. The damage is not due to the presence of oxygen, but rather due to its role in the oxidation of certain products to toxic free radicals. These free radicals are produced within living cells and are part of the cell’s normal metabolic processes, including detoxification processes and immune system defenses. It is the excessive generation of the free radicals, reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals and hydrogen peroxide that contribute to the development of various diseases such as cancer, rheumatoid arthritis, certain neurodegenerative diseases, tissue damage and also ageing, especially if free radical production exceeds the capacity of tissues to remove them (Selby et al., 1985). Free radicals also play an important role in radiation injury, inflammation, atherosclerosis, ischemia of the heart, brain, small intestine, kidney and liver, diabetes mellitus and disorders of prematurity. Free radicals seem to be one of the final common pathways of cell damage and affect the cell membrane and the nuclear DNA. The cell membrane damage is by cross-linking of proteins and by critical alterations of lipids (Woo, 1992). In aerobic organisms, the defense system against these free radicals is provided by free radical scavengers which act as anti-oxidants.
Free radical scavengers function by donating an electron to the free radical, the latter of which pairs with the unpaired electron and thereby stabilising it. Antioxidant defense involves both enzymatic mechanisms, which utilise specific enzymes such as superoxide dismutase, catalase and glutathione peroxidase as well as non-enzymatic mechanisms, which utilise nutrients and minerals (Lee, 1995). ROS is a collective term including both oxygen radical-centered free radicals and non-radical oxidants. By definition, free radicals are atomic or molecular species with at least one unpaired electron on the outer shell (Lieber, 1988). ROS include superoxide (‘O₂), hydroxyl (‘OH), hydroperoxyl (HOO’), alkoxy radicals (RO’) and peroxyl radicals (ROO’). Examples of non-radical oxidants, which can shift to be oxidized into radicals relatively easily, are hydrogen peroxide (H₂O₂) and singlet oxygen (’O₂) (Katewa et al., 2001).

**Source of production of reactive oxygen species**

ROS come into contact with the metabolizing organism as the result of either endogenous or exogenous production. Oxygen metabolism, energy generation in the mitochondria and detoxification reactions in the liver are some of the main sources of endogenous ROS generation, while exposure to environmental pollutants and cigarette smoke, ionizing radiation, alcohol consumption and fungal or viral infections are examples of exogenous sources. A certain amount of ROS is beneficial for controlling antimicrobial activity and regulating cell proliferation in the body, however, when free radicals and other oxidative molecules are generated beyond the capacity of the body defense network, they are not effectively detoxified leading to oxidative stress.

**Oxidative stress**

As mentioned above, several pathological situations, including atherosclerosis, cardiovascular disease, stroke, cancer, arthritis, Alzheimer's disease and age-related disorders are thought to be caused by free radicals and ROS, which generate oxidative stress (Larkins, 1999). An equilibrium between oxidants and antioxidants in the body is essential to avoid oxidative stress. Oxidative stress is imposed on the cell as a result of decreased levels of antioxidants. This can be caused intrinsically, for example, by DNA mutations that have altered the
cellular antioxidant defense system activity, or extrinsically by a deficiency in dietary minerals (cofactors), or by toxins and other factors which deplete the antioxidant defenses. An increased level of oxidants in the cell can also result in oxidative stress. Oxidative stress may result in adaptation of the cell or organism by triggering up-regulation of the immune defense system; however, this can also result in cell injury and cell death. Cellular interaction with ROS results in damage to DNA molecules, indicating that oxidative stress likely to play an important role in increasing the risk of cancer through enhanced mutagenesis, carcinogenesis and aging. Oxidative stress can also elicit structural and compositional alterations to enzymes, receptors and transport proteins that affect their functions. These defective proteins are degraded and removed from the cell.

Oxidative stress is the outcome of an imbalance between the production and neutralization of Reactive Oxygen and Nitrogen Species (RONS) such that the antioxidant capacity of cell is overwhelmed. Ordinarily, the peculiar molecular configuration of oxygen (O₂) confers a very slow reactivity between O₂ and biomolecules. Two main factors make O₂ kinetically insert; the spin restriction imposed by its triplet state, and the negative standard potential for one electron reduction of O₂ to superoxide radical (O₂⁻). However, O₂ possesses the attributes of free radicals in that it has two unpaired electrons with parallel spin in different π-anti-bonding orbitals that is responsible for its paramagnetic properties and relative stability. Spin restriction can be overcome by single electron exchange that converts it to strong oxidizing agent. Therefore, the activation of O₂ by specific enzymes is achieved by the presence, at the active site, of either flavins or reduced transition metals such as iron (Fe²⁺) and copper (Cu²⁺), which donates single electron to O₂. In the case of metalloproteins, a varying degree of electron transfer from the metallic moiety to O₂ is possible. On this basis, metalloproteins can behave either as O₂ carriers (hemoglobin, hemocyanin, hemerythrin, myoglobin), where reversible interaction with O₂ occurs, or as O₂ reductants. Studies showed that autoxidation of oxy-hemoglobin elicit the generation of free radicals. Free radical production and oxidative stress Electron transfer to O₂ is catalyzed by oxidases for production of chemical energy or oxidation of substrates (Acworth et al., 1997).
These enzymes, located in different subcellular compartments (mitochondria, endoplasmic reticulum, peroxisomes) are potential sources of partially reduced Cu2+ derivatives in biological milieu. Cytosolic enzymes xanthine oxidase, NADPH oxidases, lipoxygenase, cyclooxygenase (COX), cytochrome P450 enzymes and aldehyde oxidase, uncoupled endothelial nitric oxide synthase (eNOS), and other hemoproteins also produce O2 during catalysis. The mitochondrial electron transport chain reduces O2 to O2 at ubiquinone and NADH dehydrogenase sites whereas; microsomal cytochrome P450 and its reductases produce O2 during xenobiotic biotransformation. The “leaky” inner mitochondrial membrane electron transport chain reacts with O2 directly to generate O2, which dismutates to form hydrogen peroxide (H2O2), which can further react to form the hydroxyl radical (OH). Additionally, the mitochondrial outer membrane enzyme monoamine oxidase catalyzes the oxidative deamination of biogenic amines and is a quantitatively large source of H2O2 that contributes to increase in the steady state concentrations of reactive species within both the mitochondrial matrix and cytosol. Specifically, O2 is the primary radical formed by the reduction of O2 leading to secondary radicals or reactive oxygen species (ROS) such as H2O2 and OH in the mitochondria. Although the cause-effect relationship remains tentative, there appears to be a strong association between mitochondrial dysfunction and chronic metabolic diseases such as Type 2 diabetes mellitus and obesity.

Antioxidants

Antioxidants are compounds which, when present in low concentrations compared to oxidizable substrates, can quench free radicals and significantly delay or inhibit oxidation of the substrate and protect biological systems against potential harmful effects of free radicals (Bhendes, 2002). Antioxidants are categorized as synthetic or natural. Synthetic antioxidants are compounds with phenolic structures of varying degrees of alkyl substitution, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Aggarwal et al., 2005). Their usage is being restricted, as they are suspected to cause negative health effects such as carcinogenicity and there is increasing interest in replacing synthetic antioxidants with naturally occurring antioxidants (Halliwell et al., 2004).
Antioxidants can also be categorized as either free radical scavengers (nonenzymatic) that trap or decompose free radicals, or cellular and extracellular enzymes (enzymatic) that inhibit peroxidase reactions involved in the production of free radicals. Free radical scavengers or non-enzymatic antioxidants include ascorbate (Vit. C) (Kojo, 2004), tocopherols (Vit. E) (Pryor, 2000), carotenoids, flavonoids, polyphenols, α-lipoic acid and glutathione. Antioxidant enzymes include glutathione peroxidase, superoxide dismutase and catalase Fig. 3. Enzymatic antioxidants are important for intracellular defenses, while non-enzymatic antioxidants are the major defense mechanism against extracellular oxidants. Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, anthocyanins and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines) or carotenoids as well as vitamin C and E, and phospholipids (Suh et al., 2003). Most of these antioxidant compounds are present in foods as endogenous constituents and are referred to as dietary antioxidants.

The Food and Nutrition Board of the National Academy of Sciences (National Academy of Science, 1998) defined a dietary antioxidant as a substrate in foods that significantly decreases the adverse effects of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS) or both on normal physiological function in humans (Ayiram, 2005). Free radicals are molecules or molecular fragments containing one or more unpaired electrons (Niles, 2004). The presence of unpaired electrons confers a considerable degree of reactivity to free radicals. Free radicals are ubiquitous in the body and can be generated by normal physiological processes, including aerobic metabolism and inflammatory responses (Holmquist et al., 2007) to eliminate invading pathogenic microorganisms. Reactive oxygen species can be produced from endogenous sources such as mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation. It was reported that imbalance between ROS/RNS and antioxidant defense systems may lead to chemical modification of biologically relevant macromolecules like DNA, proteins, carbohydrates or lipids (Giustarini et al., 2008).
Defense provided by the anti-oxidant systems is crucial for survival and can operate at different levels within the cells through the prevention of radical formation, intercepting formed radicals, repairing oxidative damage, increasing the elimination of damaged molecules and recognition of excessively damaged molecules (Valko et al., 2004) which are not being repaired but rather eliminated to prevent mutations from occurring during replication. Non-enzymatic anti-oxidants are classified as being either water-soluble or lipid-soluble, depending on whether they act primarily in the aqueous phase or in the lipophilic region of the cell membranes. The hydrophilic anti-oxidants include Vitamin C and certain polyphenol flavonoid groups, while the lipophilic anti-oxidants include ubiquinols, retinoids, carotenoids, apocynin, procyanidins, certain polyphenol flavonoid groups and tochopherols (Middleton et al., 2000). Other non-enzymatic anti-oxidants include antioxidant enzyme cofactors, oxidative enzyme inhibitors and transition metal chelators such as ethylene diamine tetra-acetic acid (EDTA). Synthetic anti-oxidants, such as BHA and BHT have been developed, but their uses are limited due to their toxicity. In search for sources of novel anti-oxidants with low toxicity, over past few years medicinal plants have been studied extensively for their radical scavenging activity (Molyneux, 2004). As plants produce a large number of anti-oxidants to control the oxidative stress caused by sunbeams and oxygen, it is clear that plants may represent a source of new compounds with antioxidant activity (Scartezzini et al., 2000).

**Diabetes and oxidative stress**

It is accepted that oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism’s antioxidant potential. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids and nucleic acids. In both insulin dependent (Type 1) and non-insulin-dependent diabetes (Type- 2) there is increased oxidative stress (Rahimi et al., 2005).
During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by nonenzymic and enzymic catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses (Moussa, 2008). The peroxidation of lipoproteins is believed to play an important role in atherosclerosis. First, aldehyde products of lipid peroxidation are believed to react with the amino groups of low density lipoprotein (LDL), causing it to become modified and prone to uptake by scavenger receptors. Secondly, accumulation of oxidized phospholipids in the various fractions of lipoprotein may cause inappropriate, pathophysiological, responses within the cell types with which they come in contact (Zaheed et al., 1996). Precise measurement of lipid hydroperoxides would appear critical to the scrutiny of this oxidative stress hypothesis of atherosclerosis.

Fig. 3. Enzymatic and Non-Enzymatic Antioxidants
Oxidative stress has been related to the etiopathogenesis of several chronic diseases and plays a paramount role in the aging process. Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. These products are mainly aldehyde, with the ability to exacerbate oxidative damage. Longevity and high reactivity allow these molecules to act inside and outside the cells, interacting with biomolecules such as nucleic acids and proteins, often irreversibly damaging the delicate mechanisms involved in cell functionality. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. Since the 1960s several methods have been developed to assess this molecule in order to quantify the level of oxidative stress in vivo and in vitro (Rio et al., 2005). Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of ROS, including superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH) or reduction of antioxidant defense system. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides. Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction (Sarkhail, 2007).

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Oxygen free radical activity can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, basement and other membranes and play a role in the long term complications of diabetes. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals (Sabu et al., 2002). Antioxidants have been shown to reduce the risk of diabetes onset (Letitia et al., 2007) improve glucose disposal and improve some of the associated complications. It is possible that a population prone to diabetes using
sources of antioxidants kept diabetes in a preclinical state and reduced the occurrence of diabetic complications that may have arisen with fluctuating glucose levels.

Diabetic patients are exposed to oxidative stress and complications of diabetes seem to be mediated by oxidative stress. Hyperglycemia is one of the main causes of oxidative stress in Type 2 diabetes. Under hyperglycemia, the increased blood level of various reducing sugars promotes protein glycation and advanced glycation end products. ROS are formed in this process and trigger tissue damage. Recently, the progressive deterioration of β cell function in Type 2 diabetes has been accounted for in the oxidative stress-induced tissue damage. Due to a relatively low expression level of antioxidant enzymes, β cells are implicated to be vulnerable to oxidative stress as compared with other tissues (Kimoto, 2003).

WOUND

Skin, being the first barrier in the body defense plays a very important role in preventing the body from various infections, diseases and disorders. Any injury to this protective layer, that is, skin lead to different types of wounds which depend upon the degree of the injury caused. Healing of such injuries is a natural process which begins soon after the injury and completely recovers the injured or wounded tissue. This is a general phenomenon which includes various interlinked and overlapping phases: Hemostasis and inflammation, Re-epithelialization and Remodeling.

Hemostasis and Inflammation

Hemostasis and Inflammation stage includes the initial breakage of the epithelial tissue lining which activates the clotting factors. Fibrinogen begins the clotting of blood through the fibrin formation. Inflammation starts within 24 hours of hemostasis and can be extended upto 49-72 hours. This phase causes dilation of the blood vessels, thus initiating the infiltration of the inflammatory cells. Platelets, itself being released from thrombus, release the growth factors like Platelet derived growth factor (PDGF) and Transforming growth factor – β (TGF-β). The above mentioned infiltration of inflammatory cells includes the chemotactic movement and the proliferation of the macrophages and the neutrophils. Both, neutrophils and macrophages eat away the debris of the necrotic tissue (Kondo et al., 2010). Unlike
neutrophils, the macrophages release certain growth factors and cytokines such as interleukins (IL-1, IL-6, and IL-8), PDGF, TGF-β, Insulin growth factor (IGF), Vascular Endothelial Growth Factor (VEGF), and Fibroblast Growth Factor (FGF), which in turn helps in the revival and proliferation of the cells near the wound site like endothelial cells and the fibroblast cells along with more macrophages (George et al., 2006).

**Proliferative**

Proliferative stage completely rotates around the fibroblast cells which produce collagen, not only to provide structural support but also replaces the matrix formed from the composite of fibrin and fibronectin. This fibroblasts proliferation continues with the formation of blood vessels, commonly known as angiogenesis (Kelly et al., 2003). Apart from the major role played by the fibroblast cells, epithelialization of the keratinocytes over the wound also occur during this phase.

**Remodeling**

Remodeling stage begins with the advancement of the synthesis of collagen and the degradation of the same. Fibroblasts producing collagen crosslink it, thus increasing the wound toughness accompanied by the contraction of the wounds. This takes two to three weeks or may be upto 2 years, depending upon the type of wound.

The wounds can also be classified as

- Acute wounds
- Chronic wounds

**Acute wounds** are such wounds which get healed up within a time of repair whereas the chronic wounds are those which are hard to heal wounds and do not follow the normal physiological process of healing (Diegelmann et al., 2004). This improper healing in chronic wounds in turn leads to chronic inflammations causing the development of various ulcers like diabetic foot ulcer, pressure ulcers and venous ulcers.
**Chronic wounds** like those mentioned above results from various factors due to the reason that these factors delay the wound closure and thus the healing of the wounds. The factors affecting wounds include: Infections, Moisture in the wound site, Age, Stress, Obesity, disorders like Diabetes, non steroidal anti-inflammatory drugs, smoking and Alcohol intake (Goldman, 2004). These major criterions decide how early or how late the wounds get healed.

The major cause of such hard to heal wounds is:

- Chronic Inflammation
- Insufficient Extracellular Matrix (ECM)
- Insufficient Angiogenesis
- Insufficient Adipogenesis
- Special category of ulcers.

ECM plays a very important role in tissue regeneration and wound healing. It provides communication between the cells causing them to adhere, proliferate and differentiate over the surface of ECM. Insufficient ECM at the wound delays the healing process as it provides the structure and adhesion to the cells and the growth factors embedded in it. Growth factors rely over the ECM for their activity and in the absence of ECM, the repairing process of the injured tissue gets disturb (Hodde *et al.*, 2007). Therefore the external addition of ECM can be a preferable option as in chronic wounds, the high level of inflammatory cells can in turn release proteases which degrade the ECM components and growth factors which is crucial for healing.

Like ECM, Angiogenesis is a crucial factor in Tissue Engineering. Insufficient angiogenesis can cause hypoxia to occur which is unfavorable for the healing of wounds. The reason behind is known to be the inflammatory responses which delays the healing process due to release of oxygen free radicals. The overproduction of proteases from the inflammatory cells promotes the tissue necrosis and delays the normal healing and repair process. It has been recently found that the adipocytes interact or communicate with the fibroblast cells and repopulate during the second phase, that is, proliferative phase of the wound healing process. Adipogenesis is the process through which the adipocytes proliferate and migrate from the non injured site to the injured one, thus increasing their population. Thus, insufficient adipogenesis can lead to improper proliferation and interaction of adipocytes with the fibroblasts (Hodde *et al.*, 2007).
Beside these causes, there are some distinct types of ulcers which come in the category of the chronic wounds. These include: tubercular ulcers, leishmanial ulcers, and ulcers due to the severe effects of diabetes, cancer and AIDS. These are some of the major cause of the persisting wounds.

**Diabetes and Wound healing**

Wound healing is impaired in diabetic patients with infection or hyperglycemia (McMurry, 1984). Wound healing is a complex (but orderly) phenomenon involving a number of processes, including induction of an acute inflammatory process by wounding, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix proteins, remodeling of connective tissue and acquisition of wound strength. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. Diabetic wounds are slow, non-healing wounds that can persist for weeks despite adequate and appropriate care. Such wounds are difficult and frustrating to manage Fig. 4. Diabetic wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. The exact pathogenesis of the poor wound healing with the wound is not clearly understood, but evidence from studies involving both human and animal models reveal several abnormalities in the various phases of the wound healing process. Diabetes mellitus is one of the major contributors to chronic wound healing problems (Goodson *et al.*, 2010). When diabetic patients develop an ulcer, they are exposed to high risk for major complications including infection and amputation. It has been suggested that diabetes impairs wound healing through disruption of local cytokine production, notably platelet derived growth factor (PDGF), tumor necrosis factor α (TNFα), interleukin 1β, and vascular endothelial growth factor (VEGF), reduced biosynthesis or accelerated degradation of newly synthesized collagen (Rosenberg *et al.*, 2008). The heat shock proteins (HSPs), originally identified as heat- inducible gene products, are a highly conserved family of proteins that respond to a wide variety of stress.
**Fig.4. Schematic representation of normal wound healing and diabetic wound healing process.**

The wound bed contains abundant inducible HSP70 which contributes to protein homeostasis and cell survival within the healing wound. HSP functions are compromised under conditions of diabetes. Both type 1 and Type 2 diabetes are characterized by an increased risk for the development of microvascular and macrovascular complications. In diabetes, endogenous defence systems are overwhelmed, causing various types of stress. Uncontrolled oxidative stress represents a characteristic feature of diabetes (Mustafa, 2009). Among the other important conditions related to diabetes are dyslipidemia, modification of proteins and lipids, and perturbations in the tissue antioxidant defence network.

**HERBAL MEDICINE**

Plant kingdom had played vital role in man’s existence on this earth. Nature has always been stands as a golden mark to amplify the outstanding phenomenon of symbiosis. Medicinal plants existing even before human being made their appearance on the earth. Natural products have been derived from higher plants, microbes or animals and those can be of either terrestrial or marine or aquatic origin. Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian Medical system Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. Also, Siddha and Unani are traditional health
care systems have been flourishing for many centuries in the country. Apart from these systems there has been a rich heritage of ethanobotanical tradition of herbs by a diversity of tribal communities in the country.

The medicinal preparations based on these raw materials were in the form of crude drug. Many of these reputed medicinal plants came under chemical investigation leading to the isolations of active principles with the advent of scientific methods (Sujatha et al., 2012). There was continuous activity in this area since 1800 AD and many of the well known medicinal plants were chemically analyzed and their active principles were characterized. Soon after their isolation and characterization of these compounds, either in pure state or in the form of extracts, became part of pharmacopoeias of several countries. This is where herbal medicine and modern medicine have a common link (Ashok et al., 2007).

**Herbal wealth of India**

Now-a-days natural products are becoming an integral part of human health care system, because of popular concern over toxicity and resistance of modern drugs. India is one of 12 leading bio-diversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora 15,000 to 20,000 have good medicinal value (Aswatha et al., 2009).

WHO currently encourages, recommends and promotes traditional / herbal remedies in national health care systems because such drugs are easily available at low cost, are comparatively safe and the people have faith in such remedies. WHO defined total health, is not just the absence of disease, but a state of physical, mental, social and spiritual well-being. Today we are more concerned with life style diseases like depression, cancer and heart troubles caused by faulty nutrition and stress. Because these diseases have a mental or emotional component, there is a growing conviction that allopathy is largely unable to cure them, all of it offers is temporary relief from symptoms (Agarwal et al., 2005). There is a need of alternative therapy, to cover a good health for all. Herbal therapy will be one of the best practices to overcome the illness.
In India use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous systems of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. These systems of medicines cater to the need of nearly 70% of our population residing in villages. Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in the drug development (Rao, 2000). Due to these facts over the past ten years a considerable revival of interest in the use of herbal medicine in the world has come up. The WHO has also appreciated the fact that most of the world population depends on traditional medicine and therefore WHO has evolved guidelines to support the member states in their efforts to formulate remedies on traditional medicine and to study their potential usefulness, safety and efficacy (Elsa et al., 1992). The importance and challenges conducting clinical research in herbal drugs, simple bioassays for biological standardization, pharmacological and toxicological evaluation, toxic herbal drugs in use, various animal models for toxicity and safety evaluation were dealt with in detail by various experts in the field (White et al., 1996).

For pharmaceutical proposes, the quality of medicinal plant material must be as high as that of their medicinal preparations. However, it is impossible to assay for a specific chemical entity when the bioactive ingredient is not known. In practice, assay procedures are not carried out even for those medicinal plant materials where there are known active ingredients. Standardization of the presumed active compounds of drug in general does not reflect reality. Only in a few cases does drug activity depend upon single component. Generally, it is the result of concerted activity of several active compounds as well as of inert accompanying substances. Though these inert accompanying components do not directly affect pathological mechanism, it is reasonable to use the complex mixtures of components provided by a medicinal plant because these inert components might influence bioavailability and excretions of the active component (Alam et al., 2009). Further, by inert plant
components the stability of the active component might be increased and the rate of side effects be minimized. If there are different active compounds present in a plant drug, they might have additive or potentiating effect.

The purpose of standardization of traditional remedies is obviously to ensure therapeutic efficacy. The quality assurance of traditional remedies relies upon good manufacturing practices with adequate batch analysis and standardized methods of preparation.

**Steps necessary for promoting herbal drugs**

Phytochemistry or natural product chemistry research is the backbone of herbal industry. For promoting use of herbals in modern medicine, phytochemistry should be envisaged for; isolation, purification and characterization of new phytocomponents, use of newly isolated phytocomponents as “lead” compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity and conservation of lead phytocompounds into medicinally important drugs (King *et al.*, 1998).

**Ethno-pharmacological approach to herbal drugs**

The term ethno-pharmacology refers the interdisciplinary scientific observation, description, and experimental investigation of indigenous drugs and biological activities. Recent interest in the use of ethno-pharmacological information of plant drugs has greatly increased for several reasons Scientists showed that 119 important plant derived drugs used in one or more countries, 88 were regarded as having been discovered as a result of being derived from a plant used in traditional medicine.

**Current status of herbal drugs**

Recent years newer and newer diseases are posing threat to humanity. In fact diseases are not new but are detected newly. Despite this, WHO had taken the vouch of providing “Health for all” by 2000 AD. In spite of stupendous advances made by modern medicine, the present century has many more health problems than earlier centuries. Drugs for viral diseases like AIDS, certain type of cancers, arthritis, Parkinsonism are yet to come. The newer concepts about herbal drugs have immunomodulators and are recognized for prophylactic and preventive therapy.
Surprisingly, a recent survey revealed that more than 50% of all prescription drugs issued by rational physicians are either directly derived from the natural sources or synthesized from the natural models as the sole ingredient or as one of the several ingredients. It seems certain that the continued scientific study of medicinal plants afford a plethora of novel, structurally diverse and bioactive compounds. Multidisciplinary research on plants has lead to many new drugs, as well as prototype active molecules and biological tools.

**Future prospects in herbal medicines**

At the moment, scientific research on medicinal plants is continuing most intensely in research institutes, universities and pharmaceutical laboratories as well as in the clinics of many developed countries. This research is oriented mainly in two directions. Firstly the active ingredients of plants that have long been known for their healing properties are been investigated.

Each and every traditional medicine are tested and validated scientifically. CSIR, New Delhi, already involved in this filed, validated about 350 formulations for different activities. The WHO has emphasized the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several countries have herbal pharmacopoeias and lay down monographs to maintain their quality (Patel, 2009). The Ayurvedic pharmacopoeia of India which was recommends basic quality parameters for eighty common Ayurvedic herbal drugs. Problems with modern (Allopathic) drugs which includes (Mukherjee, 1998). High cost and long time were taken in development of new drug. Most synthetic drug utilizes fossil resources like petrochemicals which leads to depletion of natural resources. Advantages of plant-based drugs have long history of use and better patient tolerance as well as public acceptance, use of renewable source, ecofriendly cultivation and processing techniques and local availability, especially in developing countries.

Herbal medications have been used for the treatment of variety of ailments; a huge number of population in the world is entirely dependent on traditional medicines. A number of medicinal plants and their formulations are used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices. In India, indigenous remedies have been used in the treatment of diabetes mellitus since
the time of Charaka and Shusrutha. From the ethnobotanical information, about 800 plants which may possess anti-diabetic potential have been found. Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. This practice may be due to its fewer side effects compare to the synthetic hypoglycemic agents and because of their safety, effectiveness, and availability (Patel et al., 2009). Although various synthetic drugs were developed to treat diabetes but still very less number of drugs is available for the treatment of diabetes (Bhandari et al., 2002). There are about 200 pure compounds from plant sources reported to show blood glucose lowering effect. The compounds may be alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, peptides and amino acids, lipids, phenolics, glycopeptides and iridoids. Many antidiabetic products of herbal origin are now available in the market. More than 1200 species of plants have been screened for activity on the basis of ethnomedicinal uses. The ethnobotanical information reports a huge number of plants that may possess anti-diabetic potential, of which Momordica charantia, Pterocarpus marsupium, and Trigonella foenum greacum have been reported to be beneficial for treatment of Type 2 diabetes. Herbal treatments for diabetes have been used in patients with insulin dependent and noninsulin dependent diabetes, diabetic retinopathy, diabetic neuropathy etc. The families of plants with the most potent hyperglycaemic effects include Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae (Kohli, 2008).

Type 2 diabetes prevalence increases with age, with about 50% of all cases occurring in 55 years of age. The international Diabetes Federation and WHO estimate that, worldwide over 100 million people suffer from Type 2 diabetes and 50% of those cases are undiagnosed. Type 2 diabetes mellitus has been shown to be a state of increased free radical formation. The increased production of reactive oxygen species has been attributed to protein glycation and (or) glucose auto-oxidation due to a hyperglycemic environment. Lipid peroxidation of cellular structures; a free radical-induced activity is thought to play an important role in ageing, atherosclerosis and late complications of diabetes mellitus. An impaired radical scavenger function has been linked to altered activity of enzymatic and non enzymatic free radical scavengers. Type 2 diabetes is also associated with characteristic histological changes of organs like pancreas, liver etc., resulting in the alterations of their functions (Bhandari,
2002). Factors such as age, obesity, malnutrition, and macrovascular and microvascular disease may contribute to wound infection and delayed wound healing especially in the type 2 diabetic patient. In addition, hyperglycemia caused by decreased insulin availability and increased resistance to insulin can affect the cellular response to tissue injury (Rosenberg, 1990).

Four main classes of antidiabetic compounds are available to individuals with Type 2 diabetes such as sulfonylureas, biguanides and disaccharidase inhibitors etc. which cause adverse reactions such as gastrointestinal intolerance, liver toxicity and allergies. There may also be unavoidable and unwanted alterations in body metabolism such as hyperinsulinemia (abnormally high insulin levels that may damage cardiovascular system, hypoglycaemia (cause unconsciousness by lowering the blood glucose levels in elders). Finally there is an emerging need of alternate therapeutics for Type 2 diabetes.

Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs on Type 2 diabetes. Scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. *Chromolaena odorata* (L.) belongs to family Asteraceae is one of such plants that are being investigated for diverse health benefits. *Chromolaena odorata* is a rapidly growing perennial herb, a multi -stemmed shrub up to 2.5 m tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas, it becomes etiolated and behaves as a creeper, growing on other vegetation. It can then become up to 10 m tall. The plant is hairy and glandular and the leaves give off a pungent aromatic odour when crushed. The leaves are opposite, triangular to elliptical with serrated edges, 4-10 cm long by 1-5 cm wide. The plant can regenerate from the roots. In favorable conditions, the plant can grow more than 3 cm a day (Vital, 2009). *Chromolaena odorata* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties (Suksamrarn et al., 2004). The phytochemical studies have revealed the presence of a
wide range of chemical entities in the plant. In the present research work, it was designed to evaluate the hyperglycemic, antioxidant and diabetic wound healing potentials of *Chromolaena odorata* leaves extracts with a view to making these common weed more useful to man.
AIM AND OBJECTIVES

AIM

The present study is aimed to evaluate antioxidant, antidiabetic and wound healing potential of *Chromolaena odorata* (L.) King and Robinson using *in vitro* and *in vivo* models with the following objectives:

OBJECTIVES

1. To perform preliminary phytochemical screening of *Chromolaena odorata* leaves using various organic solvents.
2. To screen *in vitro* antioxidant activity using different plant extracts.
3. To screen *in vitro* antidiabetic activity using different plant extracts.
4. To fingerprint the bioactive compounds using chromatographic techniques (TLC and HPTLC).
5. To carryout acute oral toxicity on using methanolic extract.
6. To evaluate antidiabetic activity using streptozotocin induced *in vivo* model.
7. To evaluate antidiabetic activity using *in vitro* glucose uptake assay (skeletal muscle cell line) and
8. To evaluate diabetic induced wound healing activity using excision model.
Evaluation of Antioxidant, Antidiabetic and Wound Healing Potential of Chromolaena Odorata (L) King and Robinson Using In Vitro and In Vivo Models