2.1 DIABETES MELLITUS

The diabetes was first time described by Asian Egyptian by all most 3000 years ago. The term was first coined by Araetus of Cappodocia in 81-133AD. Later on in the history, Thomas Wills added the word mellitus (honey sweet) in 1675 after rediscovering the sweetness of blood and urine. Similarly, 1776 Dobson first confirmed the presence of excess sugar in blood and urine coincides with the emergence of experimental diabetes. The biggest achievement in the history of diabetes was the role of liver in glycogenesis and its relation with the excess production of glucose.

Diabetes is the major globally threatened metabolic disorder characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. The estimated numbers of people with diabetes are 246 million across the globe of which 80 % reside in developing countries. Although diabetes is often not recorded as the cause of death globally, but by 2005 it became the 4th leading cause of death after communicable diseases, cancer, CVD and injury.

Currently, there are 40 million people with diabetes in India and estimated to rise to almost 70 million by 2025 (IDF, 2006). According to Wild et al. (2004), the ‘top’ three countries in terms of the number of T2DM individuals are India (31.7 million in 2000; 79.4 million in 2030), China (20.8 million in 2000; 42.3 million in 2030) and the US (17.7 million in 2000; 30.3 million in 2030). Clearly, T2DM has become an epidemic in the 21st century where India leads the world with largest number of diabetic subjects (Singh, 2011). Until recent time T2DM was typically regarded as a metabolic disorder of middle age and elderly stage. Perhaps, this age group has higher risk compared to the younger adults. T2DM has also been reported in children in
number of developing countries including Japan, USA, India, Australia and UK (Bloomgarden, 2004).

Diabetes mellitus is a metabolic disorder characterized by resistance to the action of insulin, insufficient insulin secretion, or both. The clinical manifestation of DM is hyperglycaemia i.e., high blood sugar (Davidson et al., 1996). It is further classified as

- Type 1
- Type 2
- Gestational diabetes
- LADA
- Other types of diabetes

2.1.1 **Type 1 diabetes Mellitus** is characterized by inadequate or absolute absence of insulin. It is brought about as a result of organ specific autoimmune destruction of β-cells of the pancreas by cytotoxic T-cells (Ahmed and Goldstein, 2006). It is one of the idiopathic disorders, where there is no evidence of immune-mediated β-cells destruction and characterized by inadequate insulin progression and β-cells loss. Destruction of β-cells cells compromises the production of insulin and the function associated with it (Harris, 2000). Maturity onset diabetes of young (MODY) is also associated with autosomal inheritance and is characterized by onset of hyperglycemia to an individual younger than 25 year.

2.1.2 **Type 2 diabetes mellitus** is the most recent concerned and characterized by progressive deterioration of normal β-cells function (Ahmed and Goldstein, 2006). It could be managed by a combination of exercise, diet, oral hypoglycemic drugs and at times insulin injections (Bailey, 1989). In T2DM, β-cells become exhausted and eventually undergo apoptosis due to elevated glucose level with insufficient insulin in the blood that leads to over burden to β-cells (Buttler et al., 2004). Symptoms of hyperglycemia include,
polydipsia, polyuria, weight loss, blurred vision and sometimes with polyphagia. Growth impairment and susceptibility to infection may also accompany long term hyperglycemia.

2.1.3 **Gestational diabetes mellitus** (GDM) identifies woman who develop glucose intolerance during their last half of pregnancy. Hyperglycemia during last trimester of gestational period is associated with number of maternal and paternal adverse outcomes (Landon *et al*., 2009) as well as it also increase lifelong risk of glucose intolerance, obesity and metabolic syndrome to the offspring. On the other hand mother will have higher risk of metabolic syndrome and diabetes in future (ADA, 2012). The range of prevalence of GDM is approximately 1 % to 4 % all across the globe. Higher prevalence of GDM was recorded in African, Indian and Hispanic woman. It has been estimated that 70-90 % woman are diagnosed with GDM and can achieve targeted glycemic goal with life style modification and nutritional therapy alone (Lee-Parritz, 2011). Studies have revealed that more than 80 % of patients presenting with DM suffer from T2DM (Mycek *et al*., 2000; Maiti *et al*., 2004; Ahmed and Goldstein, 2006). About 15-20 % of patients present with T1DM. It has also been reported, though uncommon, that 2-5 % of pregnant woman suffer from gestational diabetes (Urger and Foster, 1998; Maiti *et al*., 2004).

2.1.4 **Latent autoimmune diabetes in adults** (LADA) is a disorder in which despite the presence of islet antibodies the progression of autoimmune β-cell failure is slow. Initially, up to almost six month there is no need of insulin to regulate the glucose level after the diagnosis of diabetes. The frequency of individual affected by LADA is 10 % among the phenotypic of T2DM.

2.1.5 **Other types of diabetes** include several rare causes of DM that do not fit into T1DM, T2DM, or gestational diabetes. These are as follows; diseases of the exocrine pancreas, genetic defects in beta cells, insulin resistance with
or without lipodystrophy (abnormal body fat deposition), endocrinopathies, drug or chemical-induced and other genetic syndromes sometimes associated with diabetes.

2.1.6 Risk Factors

Several factors are responsible to accelerate diabetic epidemic in Asians including normal weight metabolically obese, heavy alcohol use, smoking, high intake of carbohydrate and sedentary life style.

2.1.6.1 Age

Total prevalence of NIDDM increases with age, from 2.0 % at age 20-44 years to 18.7 % at age 65-74 years (Harris et al., 2000). In both male and female this percentage is similar across all age groups. The number of cases of DM increases with the increase in age.

2.1.6.2 Family history

Among the major risk factors family history leads to the prevalence of diabetes. Individual with family history is 50 % more susceptible to diabetes as compared to the individual with clear background (Abate and Chandila, 2001).

2.1.6.3 Diet

Poor nutrition in early life is followed by over nutrition in later life which plays an important role in Asia's diabetes epidemic.

2.1.6.4 Obesity

Obesity is one of the major risk factor and precursor for T2DM followed by insulin resistance (Frayn et al., 1996). The hypothesis that relates obesity with DM leads to change in the profile of the hormone level secreted by adipose tissue (adipokines). In the state of obesity adipose tissues secreted more adipokines that causes insulin resistance and fewer that promote insulin sensitivity.
2.1.6.5 Smoking

Cigarette smoking is a well documented risk factor in diabetes. Although diabetes and coronary heart disease also share a close relationship that leads to insulin resistance.

2.1.6.6 Alcohol

Alcohol consumption by diabetics can worsen the blood glucose level. Conversely long term alcohol consumption in none adequately nourished diabetics can lead to dangerous low blood sugar level. Heavy drinking causes accumulation of certain harmful acids in the blood that lead to adverse effects (Ben et al., 1991).

2.1.6.7 Insulin resistance

In the condition of insulin resistance, the muscle, fat and adipose tissue do not respond properly to insulin and thus cannot easily absorbed blood glucose from the circulating blood. As a result the blood stream needs more level of insulin to keep up the normal level and proper glucose absorption into the cell.

2.1.6.8 Urbanization

The growing urbanization and industrialization of the world leads to the prevalence of diabetes which has increased dramatically in the past few decades (IDF, 2000). The developing world will suffer largely, affecting younger age group due to communicable to chronic diseases. The factors contributing to these epidemiological transitions has increased due to prevalence of obesity, decreased physical activity, changes in dietary habits, increased exposure to environmental triggers and increased virulence of viruses.

2.1.6.9 Stress

Relationship between diabetes and stress is little complex. Stress may have a role in the onset of diabetes, metabolic disorder and in quality of life.
2.1.7 Pathogenesis

2.1.7.1 Pathogenesis of Type 1 Diabetes

Type 1 diabetes results from autoimmune destruction of β-cell. Absence of insulin results into severe requirement of insulin in T1DM usually found in children and adolescents. On the other hand in adults this disease may manifest in milder, initially non-insulin requiring form and is also called LADA. The three major interlocking mechanisms that are mainly involved in the destruction of β-cell are genetic susceptibility, autoimmunity and environmental stress. Firstly in genetic susceptibility it has been found that the 20 chromosomal regions regulate susceptibility of T1DM and the best characterized loci associated with chromosome 6p21, where the major histocompatibility complex class II genes map. Further, this locus account to 45 % of genetic susceptibility of T1DM. Secondly, major interlocking mechanism is autoimmunity, the typical manifestation of this disease occurs after 90 % of the β-cell has been destroyed. In the early clinical manifestation of this disease a lymphocyte-rich inflammatory infiltrate is usually observed in the islets of patients and the infiltrate consist mainly of CD8+T lymphocytes along with variable number of CD4+T lymphocytes and macrophages. Cytotoxic CD8+T lymphocytes appear to kill islets either through release of cytotoxic granules or by inducing Fas-medicated apoptosis. T cells have the capability to directly kill β cells via cell to cell contact, through a cytotoxic process. On the other hand they can also influence their destruction through other factors, that involves the release of granzyme B, pro-inflammatory cytokines, possible signaling through pathways of programmed cell death. Another bunch of immune cell types including B cells, natural killer T cells, NK cell, γδT and macrophages have been implicated in T1DM progression. Finally environmental stress triggers autoimmunity by damaging the β-cell and it has been epidemiologically observed and suggested that
viruses may be the cause to trigger β-cell loss. Viruses associated with T1DM are, rubella mumps, coxsackievirus B, measles and infectious mononucleosis (Clare-Salzler, 2003).

2.1.7.2 Pathogenesis of Type 2 Diabetes

The two major metabolic defects that characterized T2DM are

a) Derangements in β cells

In the early phase of diabetes, insulin secretion seems to be normal and plasma insulin level is not reduced. However, in the later phase of the disease, mild deficiency of insulin is observed which is less severe than T1DM. Later in the course of this disease, there is 20 % to 50 % loss of beta cells, but this is not sufficient to account for a failure in glucose-stimulated insulin secretion. In fact there appears to be defect in glucose identification by β-cells. Further, failure of β-cells in T2DM is also related to the deposition of amyloid in the islets. Amylin, the major component of the amyloid deposited, produced by pancreatic beta cells and is co-secreted with insulin in response to a glucose load. Beta cells are surrounded with amylin that may render them somewhat refractory to receiving the glucose signal. More importantly, amyloid is toxic to beta cells and may thus contribute to the beta-cell loss as seen in advanced cases of type II diabetes (Clare-Salzler, 2003).

b) Insulin resistance and obesity

In pregnancy and obesity the insulin sensitivity of target tissue decreases that ultimately results in the enhanced insulin level to compensate for insulin resistance. Similarly, obesity is also one of the major risk factor in the pathogenesis of T2DM and it is mainly affecting children all across the globe (Clare-Salzler, 2003).

2.1.8 Morphology of diabetes mellitus and its late complications

In most of the cases after 10 to 15 year morphological changes are likely to be found in arteries (atherosclerosis), basement membrane of small
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vessels (microangiopathy), nephron (diabetic nephropathy), retina (retinopathy), nerves (neuropathy) and also in some other tissues as shown in Figure 2.1. These changes are prominent in both types of diabetes.

![Diagram of complications associated with diabetes]

Figure 2.1: Diabetic complication (Source: Kide, 2014).

2.1.8.1 Pancreas

Distinctive morphological changes are commonly related with T1DM and T2DM. Reduction in the number and size of islets of langerhan, leukocytic infiltration of the islets, beta cell degranulation, and degenerated cells appeared with nuclear pyknosis, fragmentation and others showed cytoplasmic vacuolation. Apart from these changes islet cell hormone are known to act differently in cases with DM as compared to the healthy individual. Few of them play important role in the regulation of digestive and metabolic functions and in turn they lead to dysregulation of exocrine pancreatic function (Hellestrom, 1977).
2.1.8.2 Vascular system

Myocardial infarction caused by atherosclerosis is the most common cause of death in cases with diabetes. Severe atherosclerosis is accelerated by change in the size of aorta. Similarly, large renal arteries are also subjected to severe atherosclerosis and the most severe damaging effects are seen in kidneys (Clare-salzler, 2003).

2.1.8.3 Diabetic microangiopathy

Electron microscopy revealed vigorous endothelial proliferation accompanied by thickening and reduplication of basal lamina in each instance. Fegerberg (1959) described thickening of neural endoneural blood vessels with accumulation of periodic acid shiffs.

2.1.8.4 Diabetic nephropathy

It is one of the major causes of end stage renal disease affecting worldwide. It is clinically defined as progressively increasing proteinuria that is accompanied by increasing blood pressure and impairment of glomerular filtration (Alsaad, 2007).

2.1.8.5 Diabetic ocular complications

Studies have suggested a strong relation between prevalence of retinopathy and hypertension. High blood pressure causes damage to retinal capillary endothelial cells thus results in development and progression of microvascular complication. Lession in the retina acquire two forms: nonproliferative retinopathy and proliferative retinopathy. Moderate and severe non-proliferative diabetic retinopathy lead to the increased sign of development of ischemia followed by arterial thinning and occlusion or more frequently venous beading and looping, and intra retinal microvascular abnormalities. Further, advancement of proliferative disease lead to fibrovascular progression and contraction, which causes vitreous haemorrhage and retinal detachment (Negi, 2003).
2.1.8.6 Diabetic Neuropathy

Diabetic neuropathies are the nerve disorder caused by diabetes. The major complications are diffused neuropathy of the distal symmetric sensorimotor type. Individual that suffers from mixed sensorimotor defect may experience pain, paresthesia, hyperesthesia, dysesthesia, proprioreactive defects, loss of sensation and muscle weakness (Brown and Asbury, 1984).

2.1.8.7 Amputation

People with diabetes can develop many type of foot problems. Even ordinary problem left unnoticed could turn into a serious problem. These problems are usually caused when there is nerve damage. This can cause pain, numbness, tingling and weakness in the foot. Poor blood flow is also one of the major causes of foot injury.

2.1.8.8 Nonketotic hyperosmolar syndrome (NKHS)

Metabolic complications that are most frequently occurring in T2DM, are characterized by extreme dehydration, hyperglycemia, hyperosmolar plasma and change in the consciousness. NKHS is diagnosed by uncontrolled hyperglycemia, plasma hyperosmolality and absence of significant ketosis. If left untreated leads to coma, seizures and death.

2.1.8.9 Acute diabetic ketoacidosis

Diabetic ketoacidosis is a complex metabolic disorder characterized by hyperglycemia, acidosis and ketonaemia. The consequences that lead to this metabolic state is absolute or relative insulin deficiency that is accompanied by an increase in regulatory hormones like glucagon, cortisol, growth hormones and epinephrine.

This hormonal imbalance enhances hepatic gluconeogenesis and glycogenolysis resulting in severe hyperglycemia that triggers lipolysis which leads to the accumulation of ketone bodies and subsequent metabolic acidosis.
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2.2 SIGN AND SYMPTOMS

Polydipsia, polyuria, polyphagia, fatigue, weight loss, blurred vision, slow healing, genital itching, dizziness, nausea are the symptoms that are similar in both type of diabetes but they vary in their intensity. Occurrences of symptoms are much more rapid in T1DM. Long term T1DM complications are microvascular and macrovascular. Uncontrolled hyperglycemia in both T1DM and T2DM lead to the development of acute and long term complications (Weiss and Sumpio, 2006). Acute complications of DM include ketoacidosis (T1DM) or nonketotic hyperosmolar coma (T2DM). Long term complications include hypertension, cardiovascular diseases, renal chronic failure, retinal damage, erectile dysfunction, nerve damage and macrovascular damage that in turn lead to poor healing of wood and gangrene (WHO, 1999). Chronically elevated blood glucose levels lead to increase in production of mitochondrial ROS, which activates number of metabolic pathways that include: the polyol pathway, formation of AGEs, hexosamine pathway and the protein kinase C (PKC) pathway and their end products contribute to the development of long term complication of diabetes (Weiss and Sumpio, 2006).

2.3 CAUSE OF DIABETES

2.3.1 Glucose metabolism and homeostasis

For every day to day activity like sitting, walking, and running or even for sleeping we require energy that depends on particular time and on the level of activity in which we are engaged. To fulfill our day to day requirements we require food. Ultimately, the fuel we burn in our cells to give energy for life is glucose, which is derived from our diet. Glucose levels in the blood are controlled within reasonably close limits by a complicated interaction of hormones. Glucose homeostasis is intricate interaction of metabolic pathways, regulated by a complex web of hormones acting on a
number of different tissues and cells. These processes coordinate together in order to maintain an optimal glucose level. Other hormones responsible to maintain the optimal glucose level are insulin, glucagon and adrenaline in which insulin is secreted from pancreatic beta cells into the portal circulation with a brisk increase in response to rise in blood glucose (after meals). A glucose sensor has been identified in the portal vein which modulates insulin secretion via neural mechanisms. Insulin lowers blood glucose by suppressing hepatic glucose production and stimulating peripheral glucose uptake in skeletal muscle and fat, mediated by the glucose transporter (Frier and Fisher, 2002) (Figure 2.2). Adipocytes and liver synthesize triglyceride from non-esterified fatty acids (NEFAs) and glycerol. Insulin stimulates lipogenesis and inhibits lipolysis, so preventing fat catabolism (Davidson, 1986). Lipolysis, mediated by triglyceride lipase, is stimulated by catecholamines and liberates NEFAs which can be oxidized by many tissues. Their partial oxidation in the liver provides energy to drive gluconeogenesis and also produces ketone bodies which are generated in hepatocyte mitochondria.

Ketone bodies are organic acids, which when formed in small amounts are oxidised and utilized as metabolic fuel. However, the rate of utilization of ketone bodies by peripheral tissues is limited (Figure 2.3). It is well established fact that liver glycogenolysis induce hyperglycemia in man and animal by the action of glucagon (Sutherland, 1950) and gluconeogenesis (Exton and Park, 1967). Ketogenesis is regulated by the supply of NEFAs reaching the liver and is therefore enhanced by insulin deficiency and release of the counter-regulatory hormones that stimulate lipolysis schematic representation shown in Figure 2.4.

It has been reported by several authors that when adrenaline was infused directly in to the portal vein, its effect on blood glucose and hepatic
Figure 2.2: A schematic representation of mechanism involved in the maintenance of glucose homeostasis (Source: Frier and Fisher, 2002).

Figure 2.3: Ketone body synthesis in liver and its use in peripheral tissues (Source: Harvey and Ferrier, 1987).
glycogen level which was not pronounced as when it was administered into systemic circulation (Sherlock, 1993). Further, in contrast to glucagon adrenaline had only small and transient effect on liver glycogen and it was concluded that glucagon is the only agent promoting glycogenolysis in the liver in physiological condition. Further, Sokal and Rohlf, (1995) demonstrated in their in vitro and in vivo study that doses of adrenaline within the physiological range had only small and transient effects on liver glycogen and phosphorylase activity as compared to the effect of glucagon (Sokal and Rohlf, 1995). Thus, they concluded that glucagon is the only agent promoting glycogenolysis in the liver in physiological conditions. In addition, tissue damage and pathophysiological complications is due to decreased uptake of glucose into muscle and adipose tissue that leads to chronic extracellular hyperglycemia resulting in heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy etc (Brownlee and Cerami, 1981).

Increased oxidative stress has been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. The
sources of ROS include oxidative phosphorylation, glucose auto oxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P₄₅₀ monooxygenases and nitric oxide synthase (NOS).

### 2.3.2 A brief overview of insulin signaling

Insulin metabolic action result from rapid interaction with insulin receptor (IR) found at the target tissue (liver, muscle and adipose tissue). Insulin binds to the alpha-subunit of IR composed of two extra-cellular α-subunits and two transmembrane β-subunits linked by double sulphur bonds and activate the intrinsic tyrosine kinase activity of the beta-subunit of the receptor. Activated IR results in the subsequent phosphorylation of intracellular substrates including insulin receptor substrates (IRSs) such as IRS-1 and 2, phosphatidylinositol (PI) 3-kinase and protein kinase B (PKB) (Figure 2.5). Under normal condition insulin action leads to increased glycogen synthesis, glucose transport and lipogenesis (Figure 2.5) (Postic, 2001).

![Insulin signaling mechanism](Image)

**Figure 2.5:** Insulin signaling mechanism (Source: Saltiel and Khan, 2001).
2.3.3 Diabetes and reactive oxygen species

Oxidative stress has been implicated to be an important etiological factor in the pathogenesis of diabetic complications. As discussed above, during diabetes mellitus, persistent hyperglycemia causes increased oxidative stress resulting in excessive production of free radicals. Diabetes mellitus therefore, is associated with increased oxidative damage of various tissues and organs due accumulation of lipid peroxides and AGEs (Lyons, 1991), which may lead to disruption of cellular functions and membrane damage. Free radicals also affect the cell components such as lipid, protein, DNA and carbohydrates of which lipid is the most sensitive part. The ROS includes free radicals as well as non radical species like superoxide (O$_2^•$), OH$^+$, peroxyl (RO$_2^•$), hydroperoxyl (HRO$_2^{2–}$) and hydrochlorous acid (HClO) and H$_2$O$_2$, respectively (Evans et al., 2003). Further nitric oxide (NO$^+$) and nitrogen dioxide (NO$_2^+$), are included as free RNS radicals such as nitrous oxide (HNO$_2$), peroxynitrite (ONOO) and alkyl peroxynitrates (RONOO) (Turko et al., 2003). The three major reactive molecules O$_2^•$, NO$^+$ and ONOO$^-$ are most extensively studied species and play significant roles in the diabetic-cardiovascular obstacles. Reactive oxygen species can excite oxidation of LDL, which is not identified by the LDL receptor, and are taken up by the macrophages which results in foam cell formation and atherosclerotic condition (Boullier et al., 2001).

Similarly, O$_2^•$, can initiate number of protein nitration and also causes lipid peroxidation (Evans et al., 2003). A review by Turko et al. (2003) illustrate that apoptosis of myocytes, endothelial cells and fibroblasts in diabetes are linked with increase levels of nitrotyrosine (Evans et al, 2003). Secondly, ONOO causes single-strand DNA breakage which in turn initiate nuclear enzyme poly (ADP-ribose) polymerase as well as it reduces NO$^+$ bioavailability causing improper inhibition and relaxation of the
antiproliferative effects of NO•. Membrane lipids peroxidation induced by ROS causes change in the structure and the fluidity of biological membranes which finally affects their function (Maritim et al., 2003). Multiple sources like non enzymatic, enzymatic and mitochondrial pathways initiate oxidative stress and vascular complications in diabetes. The non-enzymatic sources of oxidative stress initiated from the oxidative biochemistry of glucose. Excessive ROS generation can directly cause hyperglycemia where glucose undergoes autoxidation and OH• radicals are generated (Turko et al., 2003). Further, their interaction leads to the development of amadori products followed by AGEs formation when proteins react in non-enzymatic manner. ROS is consequently generated in multiple steps during this process. Moreover, there is increased metabolism of glucose through the polyol (sorbitol) pathway that also results in enhanced production of O₂•-. Another source of non-enzymatic generation of reactive species is the mitochondrial respiratory chain. Throughout the process of oxidative phosphorylation, NADH and FADH₂ transfer electron in four complexes of the inner mitochondrial membrane to oxygen, generating ATP (Green and Brand, 2004). In normal conditions, natural defense mechanisms immediately eliminates O₂•-. A study has demonstrated that hyperglycemia-induced generation of mitochondrial O₂•- radicals and is the initial trigger of vicious cycle of oxidative stress in diabetes (Nishikawa et al., 2000).

The various enzymatic sources of amplified generation of reactive species in diabetes include NAD(P)H oxidase, NOS, and xanthine oxidase (Guzik et al., 2002). Flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH₄ and Ca²⁺ calmodulin are the five cofactors/prosthetic groups required by all isoforms of NOS. The uncoupled state of NOS is referred when NOS lacks its substrate L-arginine or any of its cofactors, that further lead to the production of O₂•-, rather than NO•. The
levels $O_2^{\cdot -}$ in vascular specimens from diabetic patients was investigated by Guzik et al., (2002) using inhibitors of NAD(P)H oxidase, xanthine oxidase, NOS and mitochondrial electron transport chain. His study established that there is enhanced production of $O_2^{\cdot -}$ in diabetes and this is mainly mediated by NAD(P)H oxidase. ROS production is always accompanied by aerobic metabolism as shown in Figure 2.6.

Figure 2.6: Augmentation of ROS by various pathways under diabetic condition (Source: Kaneto et al., 2006).

Therefore, all aerobic organisms have antioxidant defense system with enzymatic and non enzymatic constituents and the amount and the quality of reactive species is determined by metabolic pathways within the organism, influenced by exogenous factors such as stress, radiation, food etc. In diabetes
alterations of metabolic processes also influence enzymatic defenses, and these changes may be associated with late micro and macro diabetes complications. Antioxidant enzymes primarily account for intracellular defense, while several nonenzyme molecules, small molecular weight antioxidants, protect various components against oxidation.

Plasma antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione peroxidase (Gpx), and glutathione reductase (Gred), convert ROS in to non-reactiveoxygen molecules. Intracellular antioxidant defense is primarily provided by antioxidant enzymes, which catalyze decomposition of ROS. The three major antioxidant enzymes, SOD, Gpx and catalase, differ from each other in structure, tissue distribution and cofactor requirement. Superoxide anion are catalyzed by SOD and converted to hydrogen peroxide and oxygen. McCord discovered SOD activity and later proved that the enzyme is required to sustain life in aerobic conditions (McCord et al., 1976). In human mitochondria, MnSOD and extra-and intracellular-CuZnSOD have been identified. Another enzyme Gpx is a selenium-dependent enzyme (selenoprotein). Mitochondrial forms also possess different antigenic structures and the extracellular form is a glycoprotein. Reduced glutathione (GSH) is the substrate of the enzyme and therefore it indirectly depends on the flavoprotein and cellular NADPH concentration of Gred. For the reduction of hydrogen peroxide, lipid and non-lipid hydroperoxides specific H donor is used by Gpx that is GSH. Similarly, active site of the enzyme contains seleno-cysteine in it, which is incorporated in to the polypeptide chain during translation. Deficiency of selenium, both in vitro and in vivo, leads to enzyme deficiency. Therefore, when assessing the function of Gpx, it is necessary to scan the status of selenium and free GSH concentration, at least when looking for the cause of altered activity as shown in Figure 2.7.
Figure 2.7: Diagrammatic representation of oxidative stress or increase ROS production in diabetes mellitus (Source: Son, 2012).

Similarly, peroxysomes is the site for catalase enzyme which is a heme-containing ubiquiter enzyme, in eukaryotes. The purpose of the enzyme is to degrade hydrogen peroxide produces by peroxisomal oxidases into water and oxygen. Few of the other enzymes such as Gred, is also involved in the prevention of oxidative damage or its repair. The glutathione-s-transferases (GSTs) are a family of multifunctional proteins that function both as important enzymes of detoxification and intracellular binding proteins. As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of compounds such as polycyclic aromatic hydrocarbons,
aromatic amines, azodyes, alkylating agents, carcinogens and neurotoxins. Additionally, a number of endogenous compounds, including prostaglandins, leucotrienes, organic hydroperoxides (including lipid hydroperoxides and products of lipid peroxidation) and steroids act as substrate for GST (Habig, 1974). Two types of products are produced by GST reactions. In one type of reaction, a stable glutathione conjugate is formed by nucleophilic attack of GSH. This type of reactions occurs with substrate such as epoxides (metabolites of benzo (a) pyrine and aflatoxin A) alkyl and amyl halides sulfobromophthalein). In the second type of reaction, a reduce substrate and glutathione disulfide (GSSG) are formed. In this second type of reaction an unstable intermediate is the enzymatic product, which is attached nonenzymatically by a second molecule of GSH, yielding the final product and GSSG. Examples of substrates for this second type of reaction are organic nitrates and organic hydroperoxides.

In diabetes disturbances in micronutrient status can influence antioxidant enzyme activity. Changes in iron metabolism leads to changes at the cofactor level. In a long-term experiment, Godin et al. (1998) observed a significant decrease in hepatic and renal SOD activity and in contrast an increase in SOD level in pancreas of diabetic rats. Loven et al. (1982) observed a decrease in Cu ZnSOD activity in liver kidney and erythrocytes after 10 days of STZ-induced diabetes. Tagami et al. (1992) reported a significant decrease of Cu ZnSOD activity in diabetic rabbit aorta endothelium although there was no significant change in aortic SOD activity among the diabetic and normal control rats (Pieper et al., 1992).

2.3.4 Diabetes linked hyperlipidemia and atherosclerosis

Coronary artery disease is the most morbid cardiovascular complication of DM with a two-to-four fold increased risk. Compared to CVD in non diabetic, diabetic patients have a greater overall coronary plaque burden and
higher rate of multivessel disease. The proportion of stenotic segments is directly proportional to the duration of disease. In combination, these factors are major concerned in diabetes that lead to greater risk for myocardial infarction (Giugliano et al., 1996).

The effects of diabetes on the vasculature are quite extensive as diabetes affects not only the endothelium and smooth muscle cells, but also platelets, lipoproteins, local vasoactive substance production and function, clotting factors, TG, as well as local arterial response to hypoxia and new collateral vessel formation (Ceriello, 2005) shown in Figure 2.8.

Figure 2.8: Diagrammatic representation of the generation of reactive species and downstream targets in diabetes (Source: Stocker and Keaney, 2004).

The pathogenesis of diabetic atherosclerosis involves not only the direct effects of chronic hyperglycemia, but also insulin resistance, non-esterified free fatty acid production, dyslipidemia, hypercoagulability and impaired response to injury. The development of diabetes-related
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Atherosclerosis follows the same histological course as atherosclerosis in non-diabetic. This includes endothelial injury; smooth muscles cell proliferation, foam cell development and increased infiltration. Sites of lesions are determined by altered hemodynamics forces and external sources of injury to the endothelial cells. Increased endothelial permeability leads to the retention of deleterious LDL that interacts with the underlying extracellular matrix (ECM).

This interaction retains the LDL in the vessels wall where it can undergo oxidation by ROS. This oxidized LDL can then stimulate the overlying endothelial cells to up regulate the overlying endothelial cells to up regulate cellular adhesion molecules, chemotactic proteins, growth factors, and inhibit NO production. These activities recruit monocytes and macrophages, which interact with highly oxidized aggregated LDL to form foam cells. ROS can activate several damaging pathways in diabetes including accelerated formation of AGEs, polyol pathway, hexosamine pathway and PKC, all such as NF-κB, p38-MAPK and STAT-JAK resulting in VSMC migration and proliferation.

Furthermore, chronic diabetes may enhance oxidative stress not only through the increased production of ROS but also through weakening the antioxidant defense system. In this context, antioxidant role of serum HDL-complexed paraoxonase (PON)/arylesterase enzyme in the protection of LDL as well as HDL from oxidative modification is noteworthy stenotic segments is directly proportional to the duration of disease. In combination, these factors are major concerned in diabetes that lead to greater risk for myocardial infraction (Giugliano et al., 1996).

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clotting factors, TG, as well as local arterial response to hypoxia and new collateral vessel formation (Ceriello, 2003). The pathogenesis of diabetic atherosclerosis involves not only the direct effects of chronic hyperglycemia, but also insulin resistance, non-esterified free fatty acid (NEFA) production, dyslipidemia and hypercoagulability. It is well known that lipid peroxidation end products are very commonly detected by the measurement of TBARS which is used as an index of lipid peroxidation measurements. Yagi, (1986) and his group also showed amplified plasma TBARS levels in diabetic individuals which were well justified by Nourooz-Zadeh et al., (1995). In DM oxidizability of plasma as measured by lipid hydroperoxides was greater, while baseline levels were similar in subjects with T2DM, impaired glucose tolerance and normal glucose tolerance (Haffner et al., 1998).

Glycosylated hemoglobin showed a significant correlation with MDA levels. Early events of lipid peroxidation are reflected by the formation of conjugated dienes. Further, next most important target for oxidative challenges are protein. Protein modification via changes in amino acid side chain residues (arginine, lysine, threonine, proline) is caused by ROS in order to form protein carbonyls. The content of protein carbonyl is the most extensively used marker of oxidative modification of proteins and suggested to be a reliable oxidative stress marker (Chevion et al., 2000).

2.4. DIAGNOSIS

2.4.1 Blood glucose

The level of blood glucose is kept in a very narrow range by hormonally and neurally controlled biochemical processes. This value in humans is 3.5-5.5 mM (80-100 mg %). If the blood glucose level is lower than the normal value we speak of hypoglycemia; when the level is higher than normal we call it hyperglycemia. In the past, blood glucose determinations were based on the redox property of glucose. The accuracy of
these methods is not good because there are other components in the blood having redox properties. In addition to monosacharides (fructose or galactose), other compounds (uric acid or creatinine) can disturb the accuracy of the assay.

2.4.2 Blood ketone

The estimation of blood "ketone bodies" (acetoacetate, 3-hydroxybutyrate) in addition to blood glucose is needed for the assessment of the severity of diabetic coma. This measurement is also essential for the early exclusion of hyperosmolar non-ketotic diabetic coma. The initial insulin requirements are often based on the extent of the existing hyperketonaemia.

2.4.3 Oral glucose tolerance test (OGTT)

In order to assess the efficiency of the glucose regulatory system it is crucial to measure insulin sensitivity, i.e., the ability of insulin to control glucose production and utilization. This index is useful not only for diagnostic purposes, but also to evaluate the efficacy of therapy. Insulin sensitivity is usually measured with methods entailing an intravenous administration of glucose and/or insulin, like the glucose clamp technique (DeFronzo et al., 1979) or the intravenous glucose tolerance test (IVGTT) interpreted with the minimal model.

2.4.4 HbA1c Testing

Due to the inconvenience of measuring day-to-day variability in fasting plasma glucose levels or performing an OGTT, an alternative to measure and diagnosis glucose of diabetics has long been sought. International Committee and ADA has recommended HbA1c as a means to diagnose diabetes. Even though it gives almost equal sensitivity and specificity to fasting or post-load glucose measurement as a prognostic of prevalent indicator of diabetes related micro and macro vascular complication, it is not performed in may part of world. However, it is interesting to know that many people which were
identified as diabetic based on HbA1c level will not show elevated blood glucose level by direct serum glucose measurement.

2.4.5 Urine albumin

The two key markers for chronic kidney disease (CKD) are urine albumin and estimated glomerular. Diabetic nephropathy (DN) is the most common single causes of end-stage renal disease in across the globe. Hyperfiltration and microalbuminuria characterize the clinical stages of DN. Alteration in the levels of urine albumin have been clearly established as significant determinant for renal complication of diabetes. Screening for increased albumin excretion has therefore been advocated to identify individual at risk for renal disease progression in a timely manner filtration rate (eGFR).

2.5 ANIMAL MODELS USED FOR INDUCING DIABETES

Table 2.1 List of various animal models in diabetes

<table>
<thead>
<tr>
<th>Diabetes inducing agent</th>
<th>Chemical Properties</th>
<th>Mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxin</td>
<td>Alloxan is 2,4,5,6 tetraoxypyrimidine; Alloxan was prepared by the oxidation of uric acid by nitric acid and monohydrate form is simultaneously prepared by oxidation of barbituric acid by chromium trioxide.</td>
<td>selective necrosis of the β- cells of pancreatic islets.</td>
<td>Etuk, 2010</td>
</tr>
</tbody>
</table>
2.6 TREATMENT AND MANAGEMENT OF DIABETES MELLITUS

The current medication to control and manage T2DM is performed by the combination of diet restriction, weight reduction and oral hypoglycemic drugs. The most commonly used oral hypoglycemic drugs e.g. sulfonylureas, repaglinide, metformin, alpha glucosidase inhibitors and thiazolidinediones (TZDs) are taken initially either alone or in combinations together with dietary control and weight reduction program (Tripathi, 2010). On uncontrolled hyperglycemic condition, the patient is switched to insulin injection with or without combination to improve insulin functioning (Tripathi, 2010). On the other hand, currently used antidiabetic drugs have toxic side effects including, nausea, diarrhea, hypoglycemia, liver problem, lactic acidosis and weight gain (Bastaki, 2005). Patients stop taking these anti-diabetic medications due to their side effects. Furthermore, in spite of the intensive use of current anti-diabetic agents, T2DM patients (>50 %) still demonstrate poor glycemic control and a number of subjects (18 %) within six years of diagnosis develops serious complications (Nathan et al, 2006). Thus, there is a clear need for new and safer anti-diabetic agents.

The major therapeutic approaches for decreasing post-prandial hyperglycaemia are to prevent absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes, such as α-glucosidase and α-amylase. Thus, the inhibition in the enzymatic activity of α-amylase and α-glucosidase by natural agents might be one of the most effective approaches to control **Streptozotocin**

Dithiozone, gold thioglucoce, mono sodium glutamate

| Streptozotocin Dithiozone, gold thioglucoce, monosodium glutamate | monofunctional nitrosourea derivative | prevents DNA synthesis, render special reaction with cystine and results in degeneration and degradation of DNA | Etuk, 2010 |
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T2DM. In 21st century medication has largely dependent on molecular science in which different macromolecules are specifically targeted by different drug molecules (Copeland et al., 2007).

Enzymes hold a prominent role among all the biological macromolecules because of their essential functions in the life process and pathophysiology. Furthermore, the structure of the enzyme active sites and their ligand binding pockets are ideally suited for affinity interactions with drug-like inhibitors (Rich, 2005; Copeland, 2007). For these reasons, most of the pharmacological drugs used today are enzyme inhibitors. Indeed, a survey conducted by Hopkins and Groom (2002) found that nearly half (47 %) of the therapeutic drugs used in modern clinical practice are enzyme inhibitors (Figure 2.9).

Figure 2.9: Action of orally administered hypoglycemic drugs in T2DM targeting major organ (Source: Cheng and Funtus, 2005).
2.6.1 Mechanism of action of conventional oral hypoglycemic drugs

Currently, there are five distinct oral hypoglycemic agents are available viz (1) Sulfonylureas, (2) meglitinides (3) Biguanide, (4) Thiazolidinediones, (5) Enzyme inhibitors. Each of the class displays unique pharmacological properties. Initially sulfonylureas mainly work by stimulating the release of insulin (secretagogue) from the remaining functional β-cells of the pancreas and by decreasing the potassium ion efflux at sulfonylurea receptors. Similarly, meglitinide also act like insulin secretagogues and bind to the ATP sensitive potassium ion channels on β-cells except the site occupied by sulfonylureas. Further, biguanides work to reduce blood glucose level by reducing hepatic glucose production (gluconeogenesis) and to some extent increase the peripheral glucose uptake. Thiazolidinediones appears to truly reduce insulin resistance also known as insulin sensitizers and are useful in cases mostly in T2DM and obese conditions. Enzyme inhibitor like α-amylase and α-glucosidase are typified by acarbose. These normally break oligosaccharides in to monomeric glucose for absorption (Tripathi, 2010).

Sulphonylureas is the most important class of oral hypoglycemic agent available for the treating hyperglycemia in NIDDM. They are the derivatives of sulfonamidine but do not posses antibacterial activity. They usually help to increase the productivity of insulin as well as its peripheral effectiveness (Tripathi, 2010).Traditionally sulphonylureas are divided into first and second generation agents.

**First-generation sulfonylureas**

1. Clorpropamide
2. Tolbutamide
3. Tolazamide
4. Acetohexamide
5. Carbutamid
Chapter 2: Review of literature

Second-generation sulfonylureas

1. Glibenclamide (Glyburide)
2. Glimipiride
3. Gliclazide
4. Glipizide
5. Glibunuride
6. Gliquidon
7. Glisentide
8. Glisomide
9. Glisoxepide
10. Glyclopyramide
11. Glycyclamide

Whereas third generation agents are also known like glimperide as antihyperglycemic agent as described in the list below in Table 2.2. Adverse effects of sulfonylyreas are infrequent, occurring in the range of 2-5 % for first generation and perhaps less in second generation agents (Paice et al., 1985). The most common side effect of sulphonyl urea is hypoglycemia fatal complication can be caused by mild to moderate level (Ferner and Neil, 1988)

Table 2.2 List of oral hypoglycemic agents (Source: Bastaki, 2005)

<table>
<thead>
<tr>
<th>Oral hypoglycemic agents</th>
<th>Group</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas</td>
<td>Thiazolidinediones</td>
<td>Stimulate insulin secretion from pancreatic β cells</td>
</tr>
<tr>
<td>Acetohexamide</td>
<td>Pioglitazone</td>
<td>Principal action is lowering insulin resistance in peripheral tissues</td>
</tr>
<tr>
<td>Carbutamide</td>
<td>Rosiglitazone</td>
<td>Lowering insulin resistance in peripheral tissues, is affective in lowering glucose production by liver</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chlorpropamide</td>
<td>Troglitazone</td>
<td>Hepatotoxic</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td></td>
<td>Inhibition of ATP dependent potassium channel</td>
</tr>
<tr>
<td>Glibornuride</td>
<td>Meglitinides</td>
<td>Rapid-acting insulin secretagogues Glucose dependent hence it lessen the risk of hypoglycemia</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>Nateglinide</td>
<td>Analogously act on β cell receptors stimulate insulin secretion by binding to sulphonylureas receptor and close K+ ATP channels</td>
</tr>
<tr>
<td>Glimepiride (3rd generation)</td>
<td>Repaglinide</td>
<td>Five time more potent at stimulating insulin secretion than glibenclamide Uses intermediate concentration of glucose this account for low risk of hypoglycemia</td>
</tr>
<tr>
<td>Gliquidone</td>
<td></td>
<td>Aldose Reductase</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Glisentide</th>
<th>Epalrestat</th>
<th>Aldose reductase inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glisolamide</td>
<td>Sorbinil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycophyramide</td>
<td></td>
<td></td>
<td>Alpha Glucosidase Inhibitors</td>
</tr>
<tr>
<td>Metformin</td>
<td>Glymidine</td>
<td></td>
<td>Increase peripheral glucose uptake and reduce hepatic glucose output</td>
</tr>
<tr>
<td>Glycyclamide</td>
<td>Acarbose</td>
<td></td>
<td>Inhibitor of intestinal α-glucosidase</td>
</tr>
<tr>
<td>Tolazamide</td>
<td>Miglitol</td>
<td></td>
<td>Inhibitor of intestinal α-glucosidase</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>Voglibose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanides</td>
<td>Miscellaneous</td>
<td></td>
<td>Inhibitors of gluconeogenesis</td>
</tr>
<tr>
<td>Buformin</td>
<td>Glybuzole</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 2.6.2 Plant with medical significance for diabetes

Green medication still continues to be one of the major markets among US pharmaceutical and consist of multibillion dollar business About 1500
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Botanicals are traded as dietary supplement and their safety and efficacy is assured by Food and Drug Administration (FDA). Similarly, Indian herbal drug market is approximately 1 billion dollar and the export of plant crude drug is about 100 million dollar. The current market status of herbal medicine is estimated about 80-250 billion dollar in both Europe and USA (Newman et al., 2003). While, about 650 million dollars in China, especially for the management of T2DM. Moreover, it also deals with an overview of historical and current use of traditional medicinal plants and/or their products.

2.6.3 History of herbal medicine

The oldest written evidence of usage of preparation of drugs has been found from the sumarian clay of Nagpur, India, approximately 5000 year old. There are number of archeological evidence indicating the employment of medicinal plants in the prehistoric era. Healing properties are recognized since the time of primates. A number of species of monkey and apes have been observed to consume particular botanical species contain secondary metabolites that act as analgesics, anti-microbials, anti-inflammatories, immunostimulants, anti-diarrheals, fertility regulators and digestive aids (Arvigo and Balick, 1998). Phytochemical constituents of plants were gradually discovered and exploited for specific medical and psychiatric applications. Studies have suggested that early healers were aware of the mind–body interconnection and the important role in medical treatments and in health restoration and rehabilitation (Balick, 1996).

In the ancient history, Dioscorides the father of pharmacognosy, one of the most prominent writers on plant drugs studied medicinal plants while travelling with the Roman army. Similarly, Circa 77AD wrote the work “De Materia Medic.” The classical work on ancient history offering plenty of data on the medical plants constituting the basic meteria medica until middle ages and Renaissance.
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There was an era of late 19\textsuperscript{th} and early 20\textsuperscript{th} centuries, a great danger ponders over, eliminating medicinal plants from therapy. Many researchers wrote that there were many shortcomings in drug obtained from them due to the destructive action of enzymes which in turn causes fundamental changes during the process of drying and their heeling property depends on the mode of drying medicinal plants. Soon it was assured that the action of pure alkaloids was much faster and was long lasting.

2.6.4 Current status of herbal medicine

The world health organization has realized that coordination with the health authorities in the relevant countries across the globe are responsible for providing leadership on global health matter, shaping it, setting norms, articulating evidence-based policy options and providing technical support to countries as well as monitoring and assessing health movement (Pal and Shukla, 2013). Ministry of health and family welfare, India, has been officially accorded department of ayurveda, yoga and naturopathy, unani, siddha and homoeopathy (AYUSH) with the responsibility of undertaking all activities related to growth, creation, standardization and value assurance of these medicinal products as well as to distribute the guidelines for the production of raw material used in ayurvedic, siddha and unani medicine.

Health systems around the globe are expected to experience increase level of chronic disease and escalating health care and costs. There is an increase demand of patients and health care that needs to be fulfilled. It has been reported that sufferers of chronic diseases in the developed countries are turning to herbal remedies as alternatives to modern synthetic medicines (Calixto, 2000). This reformed interest in herbal medicine and its use in developed countries are believed to be initiated by several factors that includes:
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- **Side effects of modern drugs:** In contrast to synthetic or chemical drugs that believed to have better or faster effects but associated with high degree of risks and side effects (Aronson, 2009), phytomedicines are believed to be devoid of side effects and therefore millions of people all around the world having been using phytomedicine for thousands of years.

- **The effectiveness of plant remedies:** Herbal medicine are said to be mild, effective and usually specific in function to organs or systems of the body (Iwu, 1993). Further it is believed that phytomedicine is used for the treatment of certain chronic diseases where conventional medicine fails.

- **High cost of synthetic drugs:** Green medicine are usually economical than synthetic drugs.

2.6.5 Role of Bioactive compounds isolated from plant extracts

2.6.5.1 **Flavonoids** (more than 8000) constitute the largest and most important group of polyphenolic compounds in plants. They are widely distributed in many frequently consumed beverages and food products of a plant origin, such as fruit, vegetables, wine, tea and cocoa (Ross and Kasum, 2002). The biological activity of food-borne polyphenolics has attracted interest since the work of Bentsáth et al. (1936) who proposed that the flavonols were an essential dietary factor contributing to the maintenance of capillary permeability.

Although this hypothesis was later abandoned, the growing interest in dietary antioxidants and metabolically active phytochemicals over the last decade has focused attention on other potentially beneficial effects of flavonoi. It is now widely accepted that dietary polyphenolics may play an important role in protecting the body against chronic diseases, such as cancer, cardiovascular diseases (Mojzisova et al., 1999) and DM. The potent antioxidant activity of flavonoids may be their most important function and underlies many of the above actions in the body. Flavonoids can exert their
antioxidant activity by various mechanisms, e.g., by scavenging or quenching
free radicals, by chelating metal ions, or by inhibiting enzymatic systems
responsible for free radical generation (Mojzisova et al., 1999). Flavonoids,
also referred to as bioflavonoids, are naturally occurring biological
compounds that are often found in plants. Quercetin, kaempferol, catechin
and EGCG are examples of flavonoids. They are actually a type of
antioxidant that acts as a secondary metabolite. Further, flavanoids are
categorized according to their chemical structure into flavones, flavanones,
flavonols, catechins, isoflavones, chalcones and anthocyanidins (Shanker et al.,
2011) (Figure 2.10). Main sources of natural flavonoids are citrus fruits,
berries, onions, parsley, legumes, green tea and red wine. More specifically,
anthocyanins are found in wine and bilberry flavans are found in apples and
tea, flavanones are found in citrus and isoflavones are found in soya products.

Since oxidative stress has been related to various diseases like cancer, aging,
atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases
(Parkinson’s and Alzheimer’s), flavanoids may help in combating diseases by
contributing, along with antioxidant vitamins and enzymes, to ameliorating
the total antioxidant defense system. The mortality of coronary heart disease
and the incidence of heart attacks are inversely related with the intake of
flavonoid. Flavonoids, like alpha-tocopherol, contain chemical structural
elements that may be responsible for their antioxidant property. Dr. van Acker
and his team suggest that flavonoids can be a substitute for vitamin E as chain
breaking anti-oxidants in liver microsomal membranes. The six major classes
of flavanoid are shown below. Flavonoids have been called "biological
response modifiers" due to their ability to modify the body's reactions to
various stressors such as allergens, carcinogens and viruses. Hence they have
been described as having anti-inflammatory, anti-allergic, anticarcinogenic,
antioxidant and antiviral properties (Cook, 1996)
2.6.5.2 Terpenoids

Among the terpenoids, isoprenoids are known to constitute the largest group of plant secondary metabolites (Bruneton, 1999). Terpenoids display major role in defense, wound scaling, thermo tolerance of plant and in the pollination of seed crops (Henrich et al., 2004). On the basis of the number of isoprene units, terpenoids are categorized as monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpene (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}). They are also responsible for flavor of fruits, fragrance and the quality of agriculture products.

2.6.5.3 Glycosides

Another important plant secondary metabolite is glycosidase made up of two components, a carbohydrate component known as glycone and a non carbohydrate component known as aglycone. The carbohydrate component consists of one or more glucose units whereas the aglycone consist of any secondary plant metabolites (Gurib-Fakim, 2006). Ethanomedicinal significance of glycosidase is not limited to anthraquinone glycosides, coumarin glycosides and steroidal (cardiac) glycosides though it is well known secondary metabolite of medicinal importance.

2.6.5.4 Phenols

Plants have ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. These substances serve as plant’s defense system. This class of the secondary plant metabolites is characterized by presence of one or more OH\(^{-}\) group linked to benzene ring. Phenols are the most widely occurring plant secondary metabolites that are responsible for the plant coloration, pollination, protection against ultra violet radiation and pathogens. They also provide color and astringency to some foods and are broadly classified into flavanoid and non flavanoid phenolic compounds (Heinrich et al., 2004). The non flavanoid compounds such as
catechol, euginol, hydroquinone, phloroglucinol and p-anisaldehyde, vannilic acid, gallic acid and protocatechic acid, cinnamic acid, caffeic acid, ferulic acid myristicin and synapyl alcohol etc are known for antibacterial, chloretic, antiseptic and antifungal activity. Further, flavonoid compounds such as flavones, flavonols, flavonones, catechins (flavanols) anthocyanidins and isoflavones are also known for their anti-inflammatory, anti-oxidant, antibacterial and antiviral properties (Cheynier, 2005) (Figure 2.10).

Figure 2.10: Structure of flavanoids (Source: Pal and Verma, 2001).
The herbal medicines have always been on the distinct position right from the primitive period up till today’s time. In the present scenario, herbal medicine continue to play an important role in the management and treatment of DM, especially in developing countries, where access to conventional antidiabetic therapies is not so common (Acharya and Shrivastava, 2008). Native remedies are believed to be more safe, effective and inexpensive and gain status among both rural and urban areas. Over the years many plant products and there derivatives from various plants part are used to treat diabetes traditionally (Table 2.3). Among antidiabetic plants, Panax ginseng, vegetable bitter melon, Gymnema sylvestre, Fenugreek and Tian Hua Fen (trichosanthes root) are popularly known to hold factors that regulate blood sugar levels in diabetic individual. It is interesting, to note that the parent compound biguanides was originally isolated from a plant source used in the treatment of insulin resistance.

Table 2.3 List of medicinal plants and their role in diabetes regulation

<table>
<thead>
<tr>
<th>Plant and Family</th>
<th>Plant part used</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abelmoschus moschatus (Malvaceae)</td>
<td>Ariel Part</td>
<td>Enhance glucose utilization to lower plasma glucose</td>
<td>Liu et al., 2007</td>
</tr>
<tr>
<td>Acacia Arabica (Leguminoseae)</td>
<td>Bark Seeds</td>
<td>Release of insulin from pancreatic β cells</td>
<td>Wadood et al., 1989</td>
</tr>
<tr>
<td>Azadirachta indica (Meliaceae)</td>
<td>Roots leaves</td>
<td>Lower blood sugar, Reduction in serum lipids, Decreases lipidperoxidation formation</td>
<td>Waheed et al., 2006</td>
</tr>
<tr>
<td>Achyranthes aspera L. (Amaranthaceae)</td>
<td>Whole plant</td>
<td>Providing trace elements (Cu, Zn, Mg, Mn) to β-cells</td>
<td>Akhtar et al., 1991</td>
</tr>
<tr>
<td>Achyrocline satureioides</td>
<td>Whole plant</td>
<td>Lower blood glucose level</td>
<td>Kadarian et al.,</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>(Asteraceae)</th>
<th>Bark</th>
<th>Lower blood glucose level</th>
<th>Andrade-Cetto and Wiedenfeld, 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acosmium panamense (Fabaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aegle marmelos (Rutaceae)</td>
<td>Leaf</td>
<td>Similar hypoglycemic action to that of insulin treatment Improved function state of $\beta$-cells</td>
<td>Ponnachan et al., 1993</td>
</tr>
</tbody>
</table>

### 2.7 SELECTION OF PLANT SPECIES

The genus Phyllanthus (Phyllanthaceae) is spread over American, African, Australian and Asian continents with approximately 1000 species, (Unander *et al*., 1995; Webster, 1994). Trees, shrubs and herbs are the three major bioactive parts seen amongst the Phyllanthus species. The majority of the herbs belonging to genus Phyllanthus are medicinally significant due to different combinations of secondary metabolites found in this genus. Among them alkaloids, flavonoids, lignans, phenols, tannins and terpenes are the major class of bioactive compounds that has been isolated from these herbs (Calixto *et al*., 1998; Nahar *et al*., 2011).

Several Phyllanthus herbs are used all across the globe as traditional herbal remedies while twelve important herbaceous species are discussed here. The species included are *P. ajmerianus* Rao and Choudhary, *P. amarus* Schum and Thonn, *P. debilis* Klein ex Wild, *P. fraternus* Webster, *P. kozhikodianus* Sivadasan and Manilal, *P. maderaspatensis* L., *P. rheedii* Wight, *P. virgatus* G. Forst, *P. scabrifolius* Hook.f., *P. tenellus* Roxb, *P. urinaria* L., and *P. rotundifolius* Klein ex Wild and *P. ajmerianus* (Vishwanatha *et al*., 2006). All the medicinal plants, except *P. ajmerianus*, *P. rotundifolius* and *P. scabrifolius* have been scientifically
studied and proven to be of pharmacological value. Since ancient times, Phyllanthus species are used as the herb by ethnic tribes of India and as traditional home remedies by other Asian countries. The different parts of the herbs are used for urinary, diabetes, sexually transmitted diseases, treating hepatic, cancer, hypertension and wounds. The contemporary society is now eager to resort to green medicines by taking signal from the ethnic medications and potential of herbal treatments which are without adverse side effects. In Ayurveda number of the Phyllanthus species form a fundamental part of Indian system of medicine. Considering the importance and potential of phyllanthus species most of the studies are directed towards the phytochemical and pharmacognostics analysis of this natural herb. While, the correct identification of these species is very important for appropriate utilization, ethnopharmacological investigations and preparation of herbal medicines. On the other hand, with the growing use of these herbs in pharmaceutical industries leads to the risk of loss of genetic diversity. There are comparatively a lesser number of reports focusing on molecular taxonomy for identification of species and interspecific/intraspecific genetic diversity studies.

2.7.1 Phyllanthus virgatus G. Forst.

Figure 2.11: Phyllanthus virgatus G. Forst.
2.7.1.1 Classification and description

Phyllanthus virgatus G. Forst. (Figure 2.11) also known as Phyllanthus simplex Retz (Euphorbiaceae) commonly known as “Bhui aonla” in Hindi and Shima-hime-mikan-so in Japanese. It is an annual monoecious herb. Stems usually branched at lower and middle part, glabrous, winged, 25-50 cm long. Leaves are arranged alternatively, blades elliptic or narrow elliptic, surface green, lower surface elliptic, apex is abtuse or acute, base abtuse or round, entire, glabrous, upper surface is green mid rib raised.

2.7.1.2 Distribution, habitat and phytochemical studies

Phyllanthus sp. is widely distributed in tropical and subtropical region of Asia and Pacific islands also grows in northan circars and carnatic coast from the Chilka Lake to Madras, Deccan and north Coimbatore on hot dry soil upto 3,000 feet in hilly area. P. virgatus is commonly seen along roadsides, in open fields and dry deciduous forests. It has been reported that Phyllanthus virgatus gave the tannius, flavonal sulfonates and a norlignan compounds (Huang et al., 1998). It has also found that P. virgatus is also rich in lignans (Shanker et al., 2011), hypophyllanthin, isointetralin, niranthin, nirtetralin, phyltetraline, virgatusui, lactone and acids like indole-3-carboxylic acid (Shanker et al., 2011).

2.7.1.3 Pharmacological Uses

Since long time P. virgatus is known for its antioxidant activity and is used in the treatment of intestinal, liver, kidney and bladder problem (Kirtikar and Basu, 1993). The extract of P. virgatus is useful to cure Chinese children suffering from malnutrition due to worm infestation. It is also used as an antiseptic and anti-inflammatory medicine in the ethnic Indian areas (Chouhan et al., 2011).

The phytochemical constituent like phyllanthin, hypophyllanthin, niranthin, lignans, nirtetralin, heliobupthalmin lactone and virgatusin are
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common to *P. virgatus* (Shanker *et al.*, 2011). The lignin virgatusin that inhibits growth of gram positive bacteria is also found in *P. virgatus*. The antiviral activity in *P. virgatus* is also attributed to niranthin, nirtetralin and hinokinin (Shanker *et al.*, 2011). Geraniin is the common compound found in *P. amarus*, *P. urinaria* and *P. virgatus* which shows antiviral property in the three herbs (Huang *et al.*, 2003).

One of the comparative study showed that the hydromethanolic extract of *P. virgatus* have more phenolic compounds than *P. amarus* extract and showed higher free radical scavenging activity as well as peroxidation inhibition in a linoleic acid system (Poompachee and Chudapongse, 2012). Treatment of *P. virgatus* extract clearly increased the oxygen consumption of HepG2 cells, on the other hand *P. amarus* extract had little stimulatory effect (Poompachee and Chudapongse, 2012). Shabeer *et al.* (2009) should the hypoglycemic activity of *P. virgatus* and proposed that the plant may have therapeutic value in diabetes and related complications

2.7.2 *Phyllanthus maderaspatensis* L.

Figure 2.12: *Phyllanthus maderaspatensis* L.
2.7.2.1 Classification and description

Another member of phyllanthus species is *Phyllanthus maderaspateniss* (Euphorbaicea) known as madras leaf flower in English, Nelausiri in Telugu; Bazaramani, Ranavali and Bhuamlaki in Hindi, Madara nellin in Kanada and Kanocha in urdu (Figure 2.12). It is an annual erect herbs of about 15-80 cm tall with similar erect and ascending branches and branchlets do not resemble pinnate leave. Scales leaves observed on main axis while foliar leaves shows alternate orientation. Leaf blades are thin, subcoriaceous, glabrous, oblong-ovate, obtuse, base cuneate, rounded at apic and main nerve visible. Seeds are long, triquetrous, brown with concentric minute lines are visible (Stone and Benjamin, 1970).

2.7.2.2 Distribution, habitat and phytochemical studies

*Phyllanthus maderaspatensis* is widely distributed in the tropics and presumably indigenous in Asia or Malaysia. Its introduction throughout the southern pacific was almost certain and unplanned (Bommu, 2008). The part of year for flowering and fruiting is from April–May to November. *P. maderaspatensis* has been reported to possess compound like lipid, linoleic acid 63, linolenic acid, myristic acid, oleic acid, palmitic acid and stearic acid (Unander et al., 1995).

2.7.2.3 Pharmacological Uses

*P. maderaspatensis* is widely used as an antioxidant, hepatoprotective, antihepatotoxic and choleretic activities in rats (Asha et al., 2007). The ethanol extract of *P. maderaspatensis* demonstrated chemoprotective effect in modulating cisplatin induced nephrotoxcity and genotoxicity, thus proving it to be a potent antioxidative agent (Chandrasekar et al., 2006). This extract is also popular among one of the dietary supplement in southern part of India. Investigational data have proved *P.maderaspatensis* as an ameliorative for adriamycin-induced toxicity and oxidative stress in mice (Bommu et al.,
The complete plant extract have shown antihepatotoxic, hepatoprotective and choleric agent.

Different extracts of plant showed a remarkable hepatoprotective activity for acetaminophen-induced hepatotoxicity illustrated via serum marker enzymes (Asha et al., 2007). *P. maderaspatensis* is rich in virgatusin, phyllanthin, hypophyllanthin, niranthin, lignans, nirtetralin and heliobupthalmin lactone. However, Khatoonm et al. (2006) confirms the absence of phyllanthin in *P. maderaspatensis*. Also, Sharma et al. (2011) confirms the non presence of phyllanthin and hypophyllanthin from *P. maderaspatensis*. On the other hand virgatusin is found in *P. maderaspatensis* confirming the antibacterial potential of this compound.