CHAPTER – 1

Introduction
1. INTRODUCTION

1.1. DIABETES MELLITUS

Diabetes is defined as a state in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both at one or more points in the complex pathways of hormone action (Barcelo and Rajpathak, 2001). It is a hereditary or acquired incapability to transport sugar from the bloodstream into the cells. Devoid of sufficient insulin, the body cells are unable to absorb adequate glucose from the blood; thus blood glucose levels enhance, which is known as hyperglycemia. Hyperglycemia can damage some important body organs, for example the kidneys, liver, eyes, nerves, heart and blood vessels (Pari and Saravanan, 2004).

The word *diabetes* is Greek for a draw off, referring to the ejection of a more quantity of urine; and *mellitus* is Latin used for sugar. Consequently diabetes mellitus means the passage of huge amounts of sweet urine. This is derived from the information that excess glucose in the blood spills over into the urine, absorbing fluids with it. Diabetes mellitus is a clinically and hereditarily heterogeneous group of disorders characterized by abnormally elevated levels of glucose in the blood. The hyperglycemia is due to lack of insulin secretion or to resistance of the body's cells to the action of insulin, or to a combination of these. Frequently there are also disturbances of carbohydrate, fat, and protein metabolism. Glucose metabolism involves small intestine, pancreas, muscle cell and liver. If there are, some problem with any of this diabetes organ leads to defect in glucose metabolism and can develop diabetes (The expert committee on the diagnosis and classification of diabetes mellitus, 2002). The classical symptoms of diabetes are Polydipsia, Polyuria and Polyphagia; Polydipsia or excessive thirst is a method of restoring the water content of the tissues lost by Polyuria (Tiwari et al, 2002). The mechanism responsible for Polyuria or increased volume of urine output is based on the amount of glucose in circulating blood and accumulation of ketone bodies in blood acts as diuretics (Tierney et al, 2002). Symptoms may develop quite rapidly in insulin dependent diabetes, mainly in children; in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent (Tierney et al, 2002).

1.1.1. Epidemiology of Diabetes Mellitus

Demographic and epidemiological evidences suggest that in the absence of effective involvement of diabetes will persist to increase its incidence worldwide. Therefore prevention of diabetes and its consequences is not just a major challenge for future but essential, if health for all is to be an achievable target.
1.1.1.1. Diabetes Mellitus Scenario in World

The occurrence of diabetes is hastily rising all over the world at a frightening rate (Huizinga and Rothman, 2006). Over the past 30 years, the status of diabetes has changed from being measured as a kind disorder of the old to one of the main causes of morbidity and mortality disturbing the childhood and middle aged people. It is essential to note that the increase in prevalence is seen in all six populated continents of the globe. Diabetes is deadly disease in both developed and developing countries. In 2000, there were a probable 175 million people with diabetes universal and by 2030, the projected estimate of diabetes is 354 million (Wild et al., 2004). The worldwide increase in the popularity of diabetes is owed to population growth, aging, urbanization and an augment of obesity and physical inactivity. The prime determinants of the epidemic are the rapid epidemiological transition associated with alteration in dietetic patterns and reduced physical activity. Unlike in the West, wherever older populations are mainly affected, the burden of diabetes in Asian countries is excessively high in young to middle-aged adults (Chan et al, 2009; Ramachandran et al, 2010). Healthcare expenditures on diabetes are expected to account for 11.6% of the total healthcare expenditure in the world in 2010. Expected global healthcare expenditures to treat and avert diabetes and its complications are expected to total as a minimum 376 billion U.S. Dollars (USD) in 2010. By 2030, this number is expected to go above some USD490 billion (IDF Diabetes Atlas, 2009)

1.1.1.2. Diabetes Mellitus Scenario in India

India goes in front the world with leading number of diabetic patients earning the doubtful distinction of being termed the “diabetes capital of the earth”. In India simply, the occurrence of diabetes is expected to rise from 31.7 million in 2000 to 79.4 million in 2030 (Wild et al, 2004). The World Health Organization guess that death from diabetes as well as heart disease cost India about $210 billion each year and is likely to increase to $335 billion in the subsequently ten years (Bjork, 2003). In the national investigation 54.1% of diabetes developed it in the mainly productive years of their life specifically prior to the age of 50 years and they also had a high threat of developing chronic complications of diabetes (Ramaiya et al., 1990; Ramachandran et al., 1992). The incidence of non-insulin-dependent diabetes is 4-6 times higher in the urban areas as compared to rustic areas. The pervasiveness of impaired glucose tolerance (IGT) in the rural inhabitants is also high at 7-8%, which indicates existence of a genetic basis for Type 2 diabetes in tribal Indian population (Viswanathan et al, 1996).
Diabetes is a costly disease for the health care sector, at communal and at individual level. Expenditure of diabetes care is extremely high. The cost of concern increases a lot of folds when complications occur or when access to hospital, operation or insulin treatment is needed. A study by the authors has shown that the yearly median expenses by patients on diabetes care are Rs 10,000 in city and Rs 6,300 in rustic areas (Ramachandran et al, 2007). A low-income person spends nearly 25–35% of their yearly income on diabetes concern. Due to the high financial burden on the patients as well as their families, people are likely to neglect health care causing severe morbidities and early death.

1.1.2. The Pancreas (General anatomy)

For both digestion and glucose homeostasis the vertebrate pancreas is an essential organ. The pancreas is also the solitary source of insulin production in vertebrates, and mutilation leads to a major health problem, diabetes mellitus. The vertebrate pancreas is elongated, accessory digestive gland that lies retroperitoneally and transversely across the posterior abdominal wall. (pan-all kreas-flesh) is retro peritoneal exocrine and endocrine gland about 12 to 15 c.m. long and 2.5 c.m. in thickness with weighs 60 to 170 g and is connected usually by two ducts, to the duodenum, pancreas divided into Head, Body and Tail. Head is ‘C’ shape curve of duodenum, central body and tapering tail. In most people pancreas ducts join the common bile ducts from liver and gall bladder and enters the duodenum as a common duct called hepatopancretic ampulla (Gray, 1994).

1.1.2.1. Components of Pancreas on Basis of Secretions

**Exocrine gland:** It secretes pancreatic fluid that contains digestive enzymes that pass to the small intestine. These enzymes facilitate to more break down the carbohydrates, proteins and lipids (fats) in the chyme.

**Endocrine gland:** it exudes glucagon and insulin from the pancreatic islets of Langerhans to facilitate enters the blood (Sturmhofel and Bartke, 1998).

The exocrine pancreas has acini that produce pancreatic juice into the duodenum through the pancreatic ducts. Pancreatic juice contains many enzymes, some of which are primarily made in an inactive form. Just the once activated, these enzymes assist to digest food and put in order it for absorption in the intestine. Disorders intrusive with regular pancreatic enzyme activity (pancreatic insufficiency) cause maldigestion of fat and steatorrhea (fatty stools). Malfunction of the exocrine pancreas results from inflammation (acute pancreatitis, chronic pancreatitis), neoplasm (pancreatic carcinoma), or duct obstacle by stones or unusually viscid mucus (cystic fibrosis). The endocrine pancreas is composed of the islets of Langerhans. The islets are dispersed all over the pancreas and contain several different hormone-producing cells. The islet cells produce hormones such as insulin that are important in nutrient absorption, storage, and metabolism. Dysfunction of the endocrine
pancreas causes diabetes mellitus. Both exocrine and endocrine pancreatic dysfunction occurs collectively in several patients. Insulin and glucagon, the two main hormones that arrange fuel storage and consumption, are produced by the islet cells in the pancreas (Constanti et al, 1998).

1.1.2.2. Cells Categories in the Pancreatic Islets

Every pancreatic islet consists of four types of hormones, secreting cells.

1. Alpha- secretes glucagon and constitutes about 20% of pancreatic islets,
2. Beta cells (70%) of pancreatic islets cells and secrets insulin.
3. Delta constitutes around 5% of pancreatic islets cells and secretes somastostatin.
4. F cells constitute the remains of pancreatic islets cells and secretes pancreatic polypeptides. Glucagon elevates blood glucose level, insulin lower blood glucose level, somastotatin inhibits the insulin release and pancreatic polypeptide inhibits the secretion of somastotatin. (Tortora and Grabowski, 2000)

1.1.3. Pathophysiology

1.1.3.1. Pancreatic Islet Hormones

Islets of Langerhans are cell clusters (often hundreds of cells) that are spread throughout the pancreas and perform the endocrine function (Henderson, 1969). Islets constitute about 2% by weight of the adult human pancreas and are multicellular microorgans (Bonner-Weir, 2005; Klöppel and Veld, 1997). The islets consist of five major cell types which are characterized by different hormone secretion profiles (Fig- 1.01): β-cells secrete insulin (to decrease blood glucose); α-cells secrete glucagon (to increase hepatic glucose production and blood glucose); δ-cells secrete somatostatin (to regulate α and/or β-cell hormonal secretion); PP cells secrete pancreatic peptide (to inhibit gall bladder contraction, increase gastrointestinal motility and self regulate pancreatic exocrine and endocrine secretion), and Epsilon cells secrete ghrelin (to increase hunger before a meal, stimulate growth hormone secretion and inhibit fat utilization in adipose tissue) (Wierup et al, 2002).

![Cell components of the islet of Langerhans](image-url)
1.1.3.2. Insulin

Insulin, the most important hormonal regulator of glucose metabolism, was earliest isolated from pancreatic tissue and it is a quite small protein, with a molecular weight of around 6000 Daltons. (Banting and Best, 1922). Insulin is a 51 amino acid anabolic peptide-hormone that is secreted by the β-cells in the Islets of Langerhans. Insulin made up of two chains (A and B) linked by disulfide bonds. One of its principal functions is the stimulation of glucose uptake from the systemic circulation, in addition to the restraint of hepatic gluconeogenesis, thus serving a main role in glucose homeostasis and preventing the metabolic disorder diabetes mellitus (Scott, 1912; Duville et al, 1997).

1.1.3.3. Biosynthesis, Secretion, Storage, and Degradation of Insulin

Insulin biosynthesis occurs in rough endoplasmic reticulum from a single-chain precursor, preproinsulin, with a molecular weight of 11,500 with containing 109 amino acids. This molecule consists of proinsulin in addition a hydrophobic extension of 23 amino acids (preregion) on the N terminus of proinsulin. Proinsulin is cleaved to form equimolar amounts of C-peptide and insulin. Insulin is a protein composed of fifty one amino acids in two chains (A and B chains), linked by two disulfide bonds. Insulin is synthesized and accumulated in the β-cells of the islets of langerhans, which are situated in the pancreas. The normal pancreas contains 200 U insulin, and a basal amount of insulin is secreted always at a rate of roughly 0.5 to 1.0 U/h.

![Fig-1.02: Synthesis of Insulin](image)

The main metabolic sites that are responsive to insulin comprise the liver, where glycogen is synthesized, stored and wrecked down; skeletal muscle, where glucose decomposition produces energy; and adipose tissue, where glucose can be transformed to fatty acids, glycercyl phosphate and triglycerides. Insulin influences carbohydrate, protein and lipid
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metabolism (Kitabchi, 1982). Insulin secretion is excited by a number of nutrients and non-nutrient secretagogues, like glucose, mannose, some amino acids, GLP-1, fatty acids, and sulfonylureas (Chan et al, 1976). In person Glucose is the most important stimulus to insulin secretion and is a key factor for the actions of various other secretagogues (Matchinsky, 1996).

Glucose stimulation of insulin secretion starts with its transport into the beta cell by the GLUT2 glucose transporter (Fig.1.03). Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose-regulated insulin secretion. Additional metabolism of glucose-6-phosphate via glycolysis produces ATP, which reduces the activity of an ATP-sensitive K+ channel. This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g. sulfonylureas, meglitinides); the other is an inwardly rectifying K+ channel protein (Kir6.2). Inhibition of this K+ channel induces beta cell membrane depolarization, which unlock voltage-dependent calcium channels (leading to an influx of calcium), and stimulates insulin secretion. Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose-stimulated insulin secretion and suppress glucagon secretion. Glucagon-like peptide 1 (GLP-1) the mainly strong incretin is released from L cells in the small intestine and stimulates insulin secretion, merely when the blood glucose is more than the fasting level. Incretin analogues, such as exenatide, are individual used to increase endogenous insulin secretion (Unger RH, Foster, 1998).

Further insulin is also released in response to blood glucose levels of 100 mg/dL or more. The normal daily insulin secretes in the adult is 30 to 50 U/day. Insulin is cleared metabolically by the liver, peripheral tissues, and kidneys. Insulin pursues first-order elimination kinetics, and the serum half-life is approximately 4 to 5 minutes (Helms and Quen, 2006).

Insulin degradation is a regulated process that plays a role in controlling insulin action by removing and inactivating the hormone. Degradation of insulin occurs mainly in liver, kidney, and muscle (Duckworth, 1988). About 50 percent of the insulin that reaches the liver via the portal vein is destroyed and never reaches the general circulation. Renal glomerulus filtered the Insulin and is reabsorbed by the tubules, which also degrade it. Severe destruction of renal function appears to affect the rate of disappearance of circulating insulin to a greater extent than does in hepatic disease (Rabkin, 1984).
1.1.4. Metabolic effects of Insulin

1.1.4.1. Effects on Carbohydrate Metabolism:

The effects of insulin on glucose metabolism are most prominent in three tissues: liver, muscle and adipose tissue. Insulin is the main hormone that regulates uptake of glucose into most of the cells from the blood (primarily muscle and fat cells, but not central nervous system cells), lack of insulin or the inconsiderateness of its receptor plays a central role in all forms of diabetes mellitus (Czech, 1977).

Insulin suppresses hepatic glucose output by stimulating glycogen synthesis and inhibiting glycogenolysis and gluconeogenesis Hyperglycemia developed by increased rate of production of hepatic glucose especially fasting hyperglycemia, in patients with type 2 diabetes (DeFronzo et al, 1992). Insulin exerts direct effect on the liver (Michael et al, 2000) as well as influences the substrate availability and fluxes of free fatty acids (FFA) (Bergman and Ader, 2000). There are a number of important enzymatic checkpoints that act to control hepatic glycolysis and glycogen synthesis (glucokinase, glycogen synthase kinase-3), glycogenolysis (phosphorylase), gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase), or ladder that are common to the pathways (glucose 6-phosphatase). A number of them are directly controlled by insulin via phosphorylation and dephosphorylation (Zhang and Tan, 2002).

1.1.4.2. Effects of Insulin on Fat Metabolism

One main function of insulin is to stimulate the storage of food energy subsequent the utilization of a food. This energy storage is in the type of glycogen in hepatocytes and skeletal
muscle. Furthermore, insulin excites hepatocytes to synthesis triglycerides and storage of triglycerides in adipose tissue.

Insulin diminishes the discharge of fatty acids from adipose tissue through:

a) Decrease in triglycerol degradation: Insulin restrains the activity of hormone-susceptible lipase in adipose tissue.

b) Increase triglycerol synthesis: Insulin enhances the transport and metabolism of glucose into adipocytes, as long as glycerol 3-phosphate for triglycerol synthesis. Insulin in addition increases lipoprotein lipase activity of adipose tissue by escalating the enzyme synthesis; provide fatty acids for esterification (Rang et al, 2003).

1.1.4.3. Effects of Insulin on Protein Metabolism

Insulin controls appearance of many genes, whichever positively otherwise negatively that then influence overall metabolism. Insulin has a universal effect on protein metabolism-increasing the rate of protein synthesis and diminishing the rate of protein deprivation. Insulin stimulates the opening of amino acids into cells and increases protein synthesis in nearly all tissues (Rang et al, 2003).

1.1.5. Categorization of Diabetes Mellitus

Diabetes mellitus emerges in two diversities, everyone with its individual cause: diabetes mellitus type I (previously known as juvenile onset diabetes), caused by lack of the pancreatic hormone insulin (whose principal function is to encourage the entry of glucose into cells); and diabetes mellitus type II (previously known as maturity onset diabetes), in which insulin is accessible but cannot be appropriately utilized (The expert committee on the diagnosis and classification of diabetes mellitus, 2002). The third group consists of other less common types of diabetes that are caused or associated with certain specific conditions and/or syndromes. The very last group comprises diabetes diagnosed during pregnancy, called gestational diabetes (GDM) (Tuomilehto et al, 2001).

1.1.5.1. Type 1 Diabetes Mellitus (β-cell destruction, frequently leading to absolute insulin deficiency)

This usually leads to a type of diabetes in which insulin is required for survival. Individuals with type 1 diabetes are metabolically normal before the disease is clinically manifest, but the process of β-cell destruction can be detected earlier by the presence of certain autoantibodies. Type 1 is usually distinguished by the presence of anti–GAD, islet cell or insulin antibodies which discover the autoimmune processes that lead to beta-cell destruction. This form of diabetes previously encompassed by the terms Type I diabetes,
Insulin dependent diabetes or juvenile-onset diabetes results from autoimmune mediated destruction of beta cells of pancreas (Atkinson and Maclaren, 1994).

Individuals who have one of more of these antibodies can be sub-classified as having type 1A, immune-mediated type-1 diabetes. Particularly in nonwhites, type 1 diabetes can occur in the absence of autoimmune antibodies and without evidence of any autoimmune disorder. In this form of type 1 diabetes, the natural history also is one of progressive disease with marked hyperglycemia resulting in an insulin requirement for prevention of ketosis and survival. Such individuals are classified as having type 1B, or idiopathic, diabetes. Type 1A diabetes shows strong associations with specific haplotypes or alleles at the DQ-A and DQ-B loci of the human leukocyte antigen (HLA) complex. The rate of β-cell destruction is quite variable, being rapid in some individuals, especially in infants and children, and slower in adults. Some have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia or ketoacidosis, and others, particularly adults, may retain some residual β-cell function for many years and have sometimes been termed as having “latent autoimmune diabetes”. Such individuals may become dependent on insulin for survival only many years after the detection of diabetes. Individuals with type 1 diabetes have low or undetectable levels of insulin and plasma C-peptide. Patients with type 1A diabetes are also more likely to have other concomitant autoimmune disorders, such as Graves’s disease, Hashimoto thyroiditis, Addison disease, vitiligo, or pernicious anemia. Type 1B, or idiopathic, diabetes is characterized by low insulin and C-peptide levels similar to those in type 1A. Such patients are prone to ketoacidosis, although they have no clinical evidence of autoimmune antibodies. Numerous of these patients be of African or Asian basis. They may suffer from episodic ketoacidosis, but the pathogenetic basis for their insulinopenia remains obscure (Alberti and Zimmet, 1998).

1.1.5.2. Type 2 Diabetes Mellitus (ranging from primarily insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance)

This type of diabetes consists of heterogeneous conditions responsible for approximately 90% of all individuals with diabetes. It is often associated with central or visceral obesity, as well as other cardiovascular risk factors such as hypertension, and abnormalities of lipoprotein metabolism with the characteristic dyslipidemia of elevated triglycerides and low high-density lipoprotein cholesterol.

Type 2 diabetes (T2D) is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that diabetes becomes clinically manifest (Kolterman et al, 1985).
While the exact etiology of this form of diabetes is not known, autoimmune destruction of the β-cells does not occur. Patients with type 2 diabetes frequently contain insulin resistance and relative, rather than absolute, insulin deficiency. On the time of diagnosis of diabetes, and regularly throughout their lifetimes, these patients do not need insulin treatment to stay alive, though eventually many require it for glycemic control. This form of diabetes is associated with progressive β-cell failure with increasing duration of diabetes. Ketoacidosis rarely occurs spontaneously but can occur with stress associated with another illness such as infection (Abate and Chandalia, 2003).

Most patients with type 2 diabetes are obese when they develop diabetes, and obesity aggravates the insulin resistance. Type-2 diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and in the earlier stages is not severe enough to produce the classic symptoms of diabetes; however, such patients are at increased risk of developing macrovascular and microvascular complications. Their circulating insulin levels may be normal or elevated yet insufficient to control blood glucose levels within the normal range because of their insulin resistance. Thus, they have relative, rather than absolute, insulinopenia. Insulin resistance may improve with weight reduction or pharmacologic treatment and results in normalization of their glycemia. Type-2 diabetes is seen frequently in women who have a previous history of gestational diabetes and in individuals with other characteristics of the insulin resistance syndrome, such as hypertension or dyslipidemia. Patients who are not obese and who have relatives with type 1 diabetes, especially those of European origin, may present with a clinical picture consistent with type 2 diabetes but may have autoantibodies similar to those found in type 1 diabetes. Such patients have type 1A diabetes yet may appear to have type 2 diabetes unless antibody determinations are made. The threat of rising type 2 diabetes increases with age, fatness, and physical immobility. Type 2-diabetes shows strong familial aggregation, so that persons with a parent or sibling with the disease are at increased risk, as are individuals with obesity, hypertension, or dyslipidemia and women with a history of gestational diabetes. The disease can occur at any age and is now seen in children and adolescents (Ward et al, 1986).

This type of diabetes commonly goes undiagnosed for several years because the hyperglycemia develops steadily and at earlier stages is frequently not severe adequate for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased threat of developing macro-vascular and micro-vascular complications (Kuusisto et al, 1994).
1.1.5.3. Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is carbohydrate intolerance associated with hyperglycemia of uneven harshness with the onset or first detection during pregnancy (American Diabetes Association, 2004). The pervasiveness might range from 1 to 14% of pregnancies (Engelgau et al., 1988) and it characterizes nearly 90% of all pregnancies complicated by diabetes (Coustan, 1995). The prevalence of gestational diabetes mellitus in India ranging from 3.8 to 21% in diverse parts of the country, depending on the geographical locations and diagnostic methods used. GDM has been found to be more prevalent in urban areas than in rural areas (Seshiah et al, 2009).

Gestational diabetes can have harmful corollary for both the fetus and mother. Diabetes taking place before or recognized throughout pregnancy with elevated fasting blood glucose level is linked with an increased threat of intrauterine fetal death during the last 4 to 8 weeks of development and additional complications, including congenital abnormalities. Gestational diabetes mellitus with no severe fasting hyperglycemia has not been associated with increased perinatal death, but GDM of any harshness increases the risk of fetal macrosomia. Neonatal hypoglycemia, jaundice, polycytemia, and hypocalcaemia are other fetal complications of GDM. Progeny of women with GDM or with type 2 diabetes earlier pregnancy are at increased risk of obesity, glucose bigotry, and diabetes in teenage years or as young adults (American Diabetes Association, 2008).

1.1.5.4. Other Particular Types of Diabetes

Other particular types of diabetes are individuals in which the fundamental defect or disease development can be recognized in a relatively specific approach or those that have other unique, distinguishing features. This category includes a range of types of diabetes inferior to other specific conditions or connected with particular diseases or syndromes with a distinct etiology.

1.1.5.5. Genetic Imperfections of the β-Cell

A number of forms of diabetes are related with monogenetic faults in β-cell action. They are known as maturity onset diabetes of the young (MODY) and are differentiates by impaired insulin secretion with minimum or no defects in insulin action. It is a nonketotic diabetes mellitus and start generally prior to the age of 25 years (and commonly in childhood or teenage years). Genetic abnormalities that consequences in the inability to convert pro insulin to insulin have been identified in a few families and such characters are inherited in an autosomal dominant model (Gruppuso et al, 1984). MODY is not a solitary thing but represents genetic, metabolic, and clinical heterogeneity (Costa, et al, 2000). Linkage analysis
in tightly defined MODY pedigrees has been central to the rapid advances in the genetics of MODY. Beta cell dysfunction in different sub-types of MODY 7-10 is caused by mutations in genes coding for hepatic nuclear factor 4α (HNF4α) (MODY1), glucokinase (MODY2), HNF1α (MODY3), insulin-promoting factor 1 (IPF1) (MODY4), and HNF3β genes (MODY5), Neurogenic differentiation factor 1 (NeuroD1) or beta-cell E box transactivator 2 (BETA2) (MODY 6) (Yamagata et al, 1996; Vionnet et al, 1992).

1.1.5.6. Virus Induced Diabetes

Definite viruses have been associated with β-cell demolition. Diabetes occurs in patients with inborn cytomegalovirus and rubella which may stimulate autoimmunity, leading to type-1 diabetes (Forrest et al, 1971). In addition, cox sackievirus B, cytomegalovirus, adenovirus, and mumps have been occupied in inducing certain cases of the illness (Karjalainen et al, 1988).

1.1.5.7. Diabetes Owed to Genetic Deficiency of Insulin

Some forms of diabetes are associated with rare autosomal dominantly inherited genetic imperfection of insulin or insulin action. In one type affected persons are unable to change proinsulin to insulin. In common the glucose bigotry is gentle. Structurally abnormal insulins, from specific mutations in the insulin gene, with resultant impaired receptor binding, has been identified in some families. Affected individuals may have either mildly impaired or even normal glucose metabolism but have high circulating levels of insulin or C-peptide. Numerals of particular mutations of the insulin receptor gene have been identified that also result in impaired insulin action (Kahn et al, 1996).

1.1.5.8. Medication or Chemical-Induced Diabetes

Drug-induced diabetes occurs due to a variety of drugs and mechanisms (Comi, 2004). An underlying and often unsuspected abnormality in carbohydrate metabolism in the patient or a family history of diabetes greatly increases the risk for developing drug induced diabetes. A lot of drugs can damage insulin secretion. These drugs can not cause diabetes by themselves, except they may impulsive diabetes in individuals with insulin resistance.

These offending drugs are collected according to the mechanism by which they stimulate diabetes. The foremost group interferes with insulin production or secretion (e.g. Beta Blockers), the second group blocks insulin action (e.g. Steroids), the third group interferes with both insulin secretion and action (e.g. Thiazides), and the final group increases blood glucose using mechanisms independent of insulin’s actions (e.g. Nicotinic acid) (Pandit et al, 1993). Body builders who take huge doses of anabolic androgens be able to develop
impaired glucose tolerance. A number of drugs including Pentamidine, nicotinic acid, aspirin, glucocorticoids and nalidixic acid can cause transient hyperglycemia in overdoses (Ferner, 1992).

1.1.5.9. Environmental Toxins

The parallel increase in our environmental toxic burden and obesity must be addressed on a policy level and in the clinical treatment of diabesity. Environmental toxins interfere with glucose and cholesterol metabolism and induce insulin resistance (Jones et al, 2008). Toxins induce obesity and insulin resistance through multiple other mechanisms, including inflammation, oxidative stress, mitochondrial injury, altered thyroid metabolism, and impairment of central appetite regulation (Hyman, 2007).

Toxins interfere with and slow metabolism and contribute to weight gain and diabetes. Heavy metals for example mercury, lead, and arsenic also cause diabesity. A recent studies linked arsenic exposure to increases in the risk of type-2 diabetes (Navas et al, 2008). Toxins induce insulin resistance by interfering with the function of a class of nuclear receptors called PPARs (peroxisome proliferator-activated receptors) needed for optimal insulin function, glucose control, fatty oxidation, and regulation of inflammation (Remillard et, al, 2002). Cycasin, a toxin obtained from the cycad plant is of special interest since this agent might be implicated in diabetes mellitus in the western pacific region (Eizirik et al, 1996).

1.1.5.10. Diseases of the Exocrine Pancreas

Any process that diffusely injures the pancreas can cause diabetes. Diabetes due to underlying exocrine pancreatic disease is a specific sub-type. It includes conditions such as pancreatitis (acute, deterioration or chronic), shock, pancreatectomy and cystic fibrosis. On the other hand, a degree of overlie exists since long-standing Types 1 and 2 diabetes are associated with some degree of exocrine pancreatic malfunction (Hardt et al, 2000).

1.1.5.11. Endocrinopathies

Diabetes mellitus may result from several endocrinopathies. It may occur in association with Cushing disorder, acromegaly, pheochromocytoma, malignant glucagonoma, hyperthyroidism (overactive thyroid gland), and somatostatinoma. Somatostatinoma and aldosteronoma induced hypokalemia be able to cause diabetes by inhibiting insulin secretion (Berelowitz and Eugene, 1996).

1.1.5.12. New Genetic Conditions Associated with Diabetes

Though these are unusual reasons of diabetes, they must be considered if circulating insulin levels are remarkably high and if there are another clinical characteristic of insulin
resistance syndromes such as acanthosis nigricans, ovarian dysfunction, hyperandrogenism, Lawrence-Seip syndrome, or severe hypertriglyceridemia. The possibility of diabetes due to antibodies in the insulin receptor should be entertained if other autoimmune diseases such as systemic lupus erythematosus, Sjogren syndrome, or ataxiatelangiectasia are present. Defects of insulin action with a genetic basis are present in donohue syndrome, the Rabson Mendenhall syndrome, and lipoatrophic diabetes (Kakehi et al, 1998).

1.1.6. Etiology of Diabetes, the Predisposing Factors

1.1.6.1. General Factors

The general factors which are responsible for type 1 and type 2 diabetes mellitus are as follows:

a) Type 1 diabetes mellitus is primarily due to autoimmune-mediated destruction of pancreatic beta cell islets resulting in absolute insulin deficiency.

b) Type 2 diabetes is made up of different forms, each of which is characterized by a variable degree of insulin resistance a condition in which the body’s muscle, fat, and liver cells do not use insulin effectively and b-cell dysfunction.

c) Genetic defects, e.g., mutation of insulin gene.

d) Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes

e) Genetic defects in insulin processing or insulin action

f) Exocrine pancreatic defects

1.1.6.2. Overweight and Obesity

Obesity is an escalating threat to human health worldwide. There are numerous theories as to the exact cause and mechanism in type 2 diabetes. Abdominal obesity is known to predispose individuals to insulin resistance. Abdominal fat is mainly active hormonally, secreting a set of hormones called adipokines that can possibly damage glucose tolerance. Obesity is found in approximately 55% of patients diagnosed with type II diabetes.

Obesity means excessive storage of fat in the adipose tissue. Overweight and obesity are risk factors for cardiovascular diseases and metabolic diseases (Knypl, 2002).

The concurrent rise in weight and obesity, which accompanies Type II diabetes 80 percent of cases interferes with diabetes treatment and exacerbates the likelihood of high blood pressure, dyslipidemia, atherosclerosis, and polycystic ovarian syndrome (PCOS) (Hensrud, 2005). Thus obesity by itself is inadequate to account for all or even most cases of
NIDDM; physical inactivity and / or deficiencies of specific nutrients may also be involved. Obesity appears to play no role in IDDM pathogenesis

**1.1.6.3. Environmental Factors**

A number of environmental factors are well-known to be important to the development of diabetes mellitus

- **Life style pattern:** Sedentary behavior has recently been proposed as a key driver of the current diabetes pandemics, frequently independent of other related behaviors such as physical activity. Along with family history, the strongest predisposing factor for type-2 diabetes is obesity. However, physical inactivity increases the risk of diabetes independent of obesity (Owen et al, 2010). People living in cities and urbanized states have a higher risk of developing diabetes as they are less active, lead more stressful lives and have a more fatty diet (Pillai, 2006).

- **Dietary:** The composition of dietary fat intake is linked to diabetes risk; decreasing consumption of saturated fats and trans fatty acids while replacing them with unsaturated fats may decrease the risk (Riserus et al, 2009; Salmeron et al, 2001). Sugar sweetened drinks appear to increase the risk of type 2 diabetes both through their role in obesity and potentially through a direct effect (Malik et al, 2010). Diet alone or exercise alone or diet and exercise combined have all shown promise in reducing incidence of Type II diabetes mellitus (Jayakumar and Nisha, 2005).

- **Viruses and infections:** Individual virus cannot cause diabetes, but people are occasionally identified with type 1 diabetes during or after a viral contamination, suggesting a link between the two. Furthermore, the beginning of type 1 diabetes occurs more often during the wintry weather when viral infections are more common. Viruses maybe connected with type 1 diabetes comprise coxsackievirus B, cytomegalovirus, adenovirus, rubella, and mumps. However one theory suggested that type 1 diabetes is a virus-triggered autoimmune response in which the immune system attacks virus-infected cells with the beta cells in the pancreas (Fairweather and Rose, 2002)

- **Sleep:** A fall in sleep is associated with a significant increase in the incidence of type II diabetes. This possibly will account for the increased occurrence of diabetes in developed countries in the last decades, since "the causes of this pandemic are not fully explained by changes in traditional lifestyle factors such as diet and physical activity (Kristen et al, 2007)"
• **Exercise:** Lack of exercise, a deprived diet and smoking were associated with considerably increased threat of diabetes, even after alteration of the body mass index. The majority of cases of Type II diabetes could be prevented by the acceptance of healthier life style practices (Hu et al, 2002). Regular physical activity is a main lifestyle factor associated with a reduced incidence of both cardiovascular disease and Type II diabetes (Anderson et al, 2003).

• **Cigarette smoking:** Cigarette smoking causes specific consequence on people with diabetes and is even more intricate and macro vascular and micro vascular complications ensue more quickly in smokers with diabetes (Justin and Sherman, 2005). Diabetes risk factor can be reduced by non-smoking campaigns and low cholesterol diets (Janka and Michaelis, 2003). Cigarette smoking has been associated with development of determined micro-albuminuria as well as nephropathy in diabetic patients (Rossing et al, 2003).

• **Alcohol consumption:** Alcohol consumption is a life style factor that has been suggested to be relevant with respect to the risk of diabetes mellitus (Lando et al, 2005). Alcohol intake increases the risk of hyperglycemia and may induce ketoacidosis, lactic acidosis and may contribute to peripheral neuropathy (Sahay and Rakesh, 2002). Moderate to high alcohol consumption was positively associated with incidence of diabetes (Weiman, 2005)

• **Stress:** Stress has a major role to play in the causation and progression of diabetes, particularly in developing countries. With increasing affluence and mechanization of civilized societies, people are becoming increasingly sedentary (Sengupta and Maju, 2005). Studies from World Health Organization indicate that up to 80 percent of cardiovascular diseases and up to ninety percent of Type II diabetes mellitus and one third of cancers could be prevented through healthy life style changes (Patel, 2002).

Depressive symptoms impart subsequent physical symptoms of poor glucose control by influencing patient’s ability to hold to self-care treatment. Higher aggressive management of depression among patients with diabetes may recover their physical health in addition to mental health (Mc kellar et al, 2004).

**1.1.6.4. Host factors**

• **Age:** Diabetes is more likely to affect older people, although there are people of all ages with the disease. Approximately 27% of people of age 65 years and older have diabetes in 2010. Around 215,000 people younger than 20 years have diabetes (type 1 or type 2). This represents 0.26% of all people in this age group (The National Diabetes Fact Sheet,
The prevalence of diabetes increases markedly with age. Utmost incidence of non-insulin dependent type of diabetes occurs above the age of 35 years. High proportion of Indians develops NIDDM at much younger age and therefore the prevalence of maturity onset diabetes of young is higher in India (Bamji et al, 1999; Raghuram, 1999).

- **Sex:** In most of the countries in the world women are more prone to diabetes than men, especially married women who have had lots of children, as they tend to be fatter than the women who do not have had any children. Women who develop diabetes while pregnant (gestational diabetes) have a 35% to 60% chance of developing type-2 diabetes in the next 10 to 20 years (The National Diabetes Fact Sheet, 2011).

1.1.7. **Complications of Diabetes Mellitus**

Diabetes is the seventh leading cause of death and can lead to permanent disability and poor health. Diabetes mellitus can affect nearly every organ system of the body. People with diabetes can experience numerous serious and deadly complications; include coronary heart disease and stroke, loss of sight, acute renal failure, and amputations.

Diabetes represents group of metabolic disorders. Poorly managed diabetes can lead to a host of long term complications like myocardial infarction, stroke, poor visual perception, kidney failure, peripheral vascular disease, nerve damage and erectile dysfunction (Banga et al, 2005). Complications of diabetes can be generally divided into micro vascular disease such as diabetic retinopathy and diabetic nephropathy and macro vascular disease, for example accelerated atherosclerosis and they are the main cause for morbidity and early mortality among diabetic patients (Yu and Timothy, 2005). The risk for stroke is 2 to 4 times high among individuals with diabetes. Young persons with diabetes contain heart disease death rates about 2 to 4 times more than adults with no diabetes. Diabetes is the foremost cause of innovative cases of blindness among adults aged 20–74 years. Diabetes is also the most important cause of kidney failure, accounting for 44% of new cases in 2008. More than 60% of leg and foot amputations not related to accidents and injuries were performed on people through diabetes. This amounted to 65,700 amputations in 2006 (The National Diabetes Fact Sheet, 2011).

Approximately 80 percent of the diabetic patients are distress from hypertension while 5-25 percent of hypertensive persons are diabetic (Stamler et al, 1993). High blood pressure is more common with diabetics and is reported to exaggerate the cardiovascular symptoms of diabetes (Barrett Connor et al, 1981).

- **Microvascular Complications:** Micro vascular abnormality and dysfunction are systemic illness in diabetes. Clinically microangiopathy (small blood vessel disease)
results in retinopathy, nephropathy, neuropathy and embryopathy are the most common complication of diabetes which cannot investigate so frequently.

- **Macro Vascular Complications:** Cardiovascular disease is the main cause of early mortality in patients with type 2 diabetes. Coronary artery disease, high blood pressure, stroke and peripheral vascular disease occur with high frequency in diabetics due to altered lipid profile.

**1.1.7.1. Hyperglycemia**

A person with very high (usually considered to be above 300 mg/dl (16 mmol/L)) blood glucose levels it is called hyperglycemia (Joel et al, 1996). Hyperglycemia causes the auto oxidation of glucose, glycation of proteins and the activation of polymetabolism. These changes accelerate generation of reactive oxygen species and increase in oxidative chemical modification of lipids, DNA and proteins in different tissues (Osawa and Joji, 2005). The symptoms are; tiredness, Headaches, vomiting, severe thirst, frequent urination, blurred vision, Dry mouth and skin, unexplained weight loss.

**1.1.7.2. Diabetic Ketoacidosis**

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency and requires prompt medical attention. Diabetic ketoacidosis an acute metabolic complication of diabetes is characterised by hyperglycemia, acidosis and ketosis and is often as a result of lack of treatment (Charfen and Madonna, 2005).

Diabetic Ketoacidosis is secondary to increased serum levels of Keto-acids in an individual with type I diabetes mellitus.

- Serum glucose > 300
- Serum pH < 7.20
- Plasma Ketone bodies >2 nM/L
- Diagnosed before 25 years of age (Matthew Kane, 2002).

**1.1.7.3. Hyperglycemia Hyperosmolar State**

Hyperosmolar hyperglycemic state (HHS) is 1 of 2 serious metabolic derangements that occurs in patients with diabetes mellitus (DM) and can be a life-threatening emergency. Non-Ketotic hyperglycemia (NKH) is a clinical syndrome seen in patients with chronic diabetes mellitus. NKH and DKA are not entirely separated and many patients will present features of both. It is characterized by marked hyperglycemia, hyperosmolality and increased urinary losses of free water, excessive loss of Na and also mild elevations in serum ketone body levels.
and ketonuria. In general, the syndromes (NKH and DKA) are distinguished on the relative severity of hyperosmolarity and acidosis (Balasubramanyam et al., 1999).

- Serum glucose >610
- Serum osmolality >310 m osm/kg
- Serum pH > 7.3.
- Some change in consciousness
- Serum bicarbonate > 14 (Matthew Kane, 2002).

1.1.7.4. Lactic Acidosis

Lactic acidosis is occasionally responsible for metabolic acidosis in diabetics. It can take place in the presence of regular blood levels of the ketone bodies, and such cases are frequently explained as having “non-ketotic diabetic acidosis. Lactic acidosis is a physiological state described by low pH in body tissues and blood (acidosis) accompanied by the buildup of lactate, particularly L-lactate, and is considered a discrete form of metabolic acidosis. Lactic acidosis is characterized by lactate levels >5 mmol/L and serum pH <7.35 (Luft, 2001).

1.1.7.5. Diabetic Retinopathy

Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for ~ 10,000 new cases of blindness every year in the United States alone (Fong et al, 2004). Nearly all patients who have Type I diabetes for about 20 years are likely to have evidence of diabetic retinopathy. DR is the most common cause of blindness and characterized by increased proliferation of blood vessels, vascular occlusion, angiogenesis, micro aneurysms, hemorrhages and infarction affecting the retina of the eye (Nathan et al., 1986). The longer someone has diabetes, the more his or her probability of developing diabetic retinopathy. Other visual damage caused or facilitated by diabetes includes cataract, keratitis, and optic nerve damage.

1.1.7.6. Diabetic Neuropathy

Diabetic neuropathy is the mainly common complication of diabetes. This can lead to sensory loss and damage to the limbs and it is the leading cause of lower extremity amputations not related to injury. With reference to 67,000 inhabitants undergo diabetes related lower edge amputations every year. Fifty percent of diabetics have some form of neuropathy, and develop nerve problems at any time, but longer a person has diabetes, the greater the risk. The maximum rates of neuropathy are in the middle of people who have had the diabetes for at least 25 years. The prevalence of autonomic nervous system dysfunction is
not precisely known, however tests of autonomic function have shown impairment in nearly 20 to 40 percent of diabetic patients (Mehta et al., 2002).

1.1.7.7. Diabetic Nephropathy

Diabetic nephropathy is characterized by a thickening of the basement membrane, expansion of the mesangium, reduced filtration, albuminuria and ultimately renal failure (Mauer et al., 2001). Diabetic nephropathy is a very important cause and contributor for chronic renal failure in India (Agarwal, 2002).

The renal lesion fundamental renal dysfunction differs in type 1 and 2 diabetes; though the clinical sign of diabetic nephropathy, albuminuria, reduced glomerular filtration rate and increasing blood pressure are similar (Viberti and Keen, 1984). Dietary protein intake, salt restriction and restricted intake of saturated fatty acids may have an important role in the prevalence and treatment of diabetic nephropathy (Holler et al, 2000).

1.1.7.8. Diabetic Embryopathy

Diabetic embryopathy is described by inborn anomalies or foetal/neonatal complications in an infant that are connected to diabetes in the mother. In infants of women with established insulin-dependent diabetes mellitus, the danger of inborn malformations is 10 times higher than that in the common population, and the rate of stillbirths is five times higher than that in the general population. Diabetic mothers with deprived glycemic control are prone to embryopathy, wherever the infant has an improved frequency of congenital malformations. The exact mechanism of embryopathy in diabetes is mysterious, but a decrease in congenital malformations is seen in pregnancies where the hyperglycemia is well controlled (Mills et al, 1988).

1.1.7.9. Diabetic Foot Infections

Foot infections are a universal and severe problem in people with diabetes. Foot ulceration is a prominent cause of diabetes mellitus morbidity and mortality in developing countries. A problem in diabetic patient is the development of ulcers in the feet and lower extremities and is attributed primarily to abnormal pressure distribution, secondary to diabetic neuropathy. Diabetic motor neuropathy is articulated as the loss of purpose and the shortening of the intrinsic muscles of the paw, leading to the typical fingernail toe malformation. This deformity influence the foot to ulcerations on the tip of the toes (Kim et al, 2008).
1.1.7.10. Cardio-Vascular Diseases

Amongst diabetic complications cardio vascular complications for example coronary heart disease, heart attack, angina and hyperlipidemia turn into more possible with the longer duration of diabetes and are more probable to develop at an earlier age. Heart disease is usually analogous in patients with type 1 or type 2 diabetes and patients with no diabetes. Death from first or consequent myocardial infarctions is more in diabetic patient than non diabetic patients (Singer et al, 1989). The levels of chronic hyperglycemia, as resolute by measurements of glycosylated hemoglobin (HbA1C), might also be a self-determining risk factor for atherosclerotic heart disease (Singer et al, 1992).

People with diabetes have a four-fold-greater threat for having a cardiovascular diseases incident than people lacking diabetes subsequent to controlling for conventional risk factors for CVD, like age, fatness, tobacco use, dyslipidemia, and high blood pressure. These heart diseases risk factors are frequent in diabetes, but facts suggest that diabetes is an independent risk factor for CVD (Buyken et al, 2007; Bonora et al, 2002). Cardio vascular disease is the most important reason of morbidity and mortality. Diabetes mellitus and hypertension are together main health problems in India which coincide regularly resulting in considerable morbidity and mortality. The leading cause of morbidity and death in people with type 2 diabetes mellitus is heart disease (Rube and McDonald, 2002).

1.1.7.11. Hypertension and Stroke

Necessary hypertension accounts for the majority of hypertension in individuals with diabetes, particularly those with NIDDM (type II diabetes), who constitute more than 90% of people with a dual diagnosis of diabetes and hypertension (Epstein and Sowers, 1992). Hypertension is a major threat to diabetics and is caused due to increased peripheral vascular resistance, elevated insulin and insulin resistance. People with diabetes mellitus have higher risk for heart attacks (Manchanda, 2000). Clustering of cardiovascular disease factors like abnormal blood pressure, elevated cholesterol, obesity or overweight are the reason for higher incidence of heart attacks in diabetics they predisposes an individual to development of blocks in the blood vessels of the heart (Prasad, 2002). Impaired glucose metabolism is associated with an increased risk of cardiovascular events and cardiovascular associated mortality (Rodriguez, 2008).

Diabetes doubles the risk of stroke in the diabetics. If high blood pressure is prevailing the risk is even greater. World Health Organization, (2002) cautions that heart diseases accounts for approximately fifty percent of all deaths among people with diabetes in industrialized countries. The burden of cardiovascular diseases and disability among people
with diabetes is growing at an alarming rate and India will have 60 percent world's cardiovascular diseases below the age of 40 years by 2010.

1.1.7.12. Psychological Effects of Diabetes

Psychosocial issues play an essential role in the management of diabetes in both children and adults. Diabetes is a psychologically and behaviorally demanding disease; hence, psychosocial factors are significant to nearly all features of its management. The psychosocial impact of diabetes has been recognized as a stronger predictor of mortality in diabetic patients than lots of clinical and physiological variables (Davis et al, 1988) Person with diabetes mellitus experience feelings of sadness, anxiety, and anger, which affect their health and largely quality of life. By understanding and addressing the emotional health of person with diabetes, the relationships between the patient, family, and health care provider may improve, allowing for more successful diabetes management.

1.1.7.13. Early Loss of Teeth

Diabetes causes early loss of teeth. Hyperglycemia disturbs the delivery of nutrients and elimination of waste products from the tissue in the gums. Eventually, that leads to periodontal disease and, ultimately, to tooth loss. Although those who have poorly controlled diabetes are most liable to experiences tooth decay, even well-managed diabetics are more likely to suffer from periodontal disease.

1.1.7.14. Erectile Dysfunction

Erectile dysfunction is a common problem for men who have diabetes. It is been estimated that about 35%-75% of men with diabetes will experience at least some degree of erectile dysfunction also called ED or impotence throughout their lifetime. Men with diabetes are likely to develop impotence 10 to 15 years earlier than men lacking diabetes. As men with diabetes age, impotence becomes more common. More than the age of 50, the possibility of having difficulties with an erection occurs in about 50%-60% of men with diabetes. More than age 70, there is approximately a 95% possibility of having some difficulty with erectile function.

1.1.8. Diagnostic Criteria for Diabetes Mellitus

The criteria used to diagnose diabetes and impaired glucose tolerance was recommended by WHO in 1985. These criteria were originally based on longitudinal studies in the US and UK which demonstrated that subjects with 2-hour post-challenge values above 200mg/dl (11.1mol/l) were at risk of developing diabetes related complications (Heine, 1996). For decades, the diagnosis of diabetes was based on plasma glucose criteria, either the fasting
plasma glucose (FPG) or the 2-h value in the 75-g oral glucose tolerance test (OGTT) (American Diabetes Association, 2010).

1.1.8.1. Glycosylated Haemoglobin (HbA1c)

Glucose glycate to the hemoglobin to create a 'glycosylated haemoglobin' molecule, called haemoglobin A1c or HbA1c. The excess glucose in the blood, the more haemoglobin A1c or HbA1c will be there in the blood. Diabetes may be defined as having an HbA1c more than 6.5%; 'pre-diabetes' or 'at risk of diabetes' if between 6.0- 6.5% and not diabetic if less than 6.0% (Cowie et al, 2010).

Glycosylated haemoglobin reveals average plasma glucose over the previous eight to twelve weeks (Nathan et al, 2007). It can be performed at any time of the day and does not require any special preparation like fasting. These properties have created it the well liked test for evaluating glycaemic management in persons with diabetes. Recently, there has been significant attention in using it as a diagnostic check for diabetes and as a selection test for peoples at elevated risk of diabetes (International Expert Committee report, 2009).

1.1.8.2. Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance tests (OGTT), also known to as the glucose tolerance test, and measures the body’s ability to metabolizing glucose, or clear it out of the blood. Test can be used for the detection of diabetes, gestational diabetes (diabetes during pregnancy) or prediabetes (a condition characterized by higher-than-normal blood sugar levels that can lead to type 2 diabetes). In the most generally performed version of the test, an oral glucose tolerance test (OGTT), a usual dose of glucose is ingested by mouth and blood levels are checked two hours afterward. Fasting plasma glucose (measured before the OGTT begins) should be lower than 110 mg/dL. Fasting blood glucose levels between 110 and 125 mg/dL are borderline ("impaired fasting glucose") and fasting levels generally at otherwise above 126 mg/dL are diagnostic of diabetes. A 2 hour OGTT glucose level below 140 mg/dLis normal, while higher glucose levels indicate hyperglycemia. Blood plasma glucose between 140 mg/dL and 200 mg/dL indicate "impaired glucose tolerance", and levels above 200 mg/dL at 2 hours confirm of diabetes (Guyton and Hall, 2006).

1.1.8.3. Acetone Breath

Use of fatty acids by ketosis produces acetone which is excreted into the blood, prior to equilibrating by air in the lungs. Acetone (2-propanone) is the main ketone in human breath. It is supposed that the fruity smell in the breath enhanced considerably during periods of glucose deficiency and have long been known as a helpful biomarker of type I diabetes. The general understanding is that acetone in human breath is a metabolic product of acetoacetate
by the removal of carbon monoxide. Diabetes can consequently be characterised by increased acetone levels in breath (Guyton and Hall, 2006).

1.1.8.4. Urinary Glucose

Urinary glucose is a simple official test to detect the abnormally high levels of glucose in the urine. The excretion of glucose in the urine is referred as a glycosuria or glucosuria. Usually, urine has no glucose for the reason that the kidneys are able to recover all of the filtered glucose rear into the bloodstream. Glycosuria is always caused by increased blood glucose levels, mainly due to untreated diabetes mellitus. Infrequently, glycosuria is due to an intrinsic problem with glucose reabsorption within the kidneys themselves, a condition termed renal glycosuria (Burton and Helmut, 1994).

1.1.8.5. Postprandial Plasma Glucose

A postprandial glucose test measures the quantity of glucose, in the blood after a meal. A two hour postprandial blood glucose test measures blood glucose precisely two hours subsequent to eating a meal, timed from the start of the meal. Through this point blood sugar has usually gone back down in fit people, other than it might still be high in people with diabetes. Therefore, it serves as a test of whether a person may have diabetes, or a person who has diabetes is successfully controlling their blood sugar.

1.1.8.6. Lipids

Elevated level of plasma triglyceride, cholesterol and very low density lipoprotein cholesterol (VLDL) are commonly found in diabetics. On the other side, high density lipoprotein cholesterol (HDL) is usually low.

1.1.9. Mechanisms of Hyperglycemia Induced Cell Injury

Four major hypotheses regarding how hyperglycemia causes diabetic complications have produced a huge amount of data in addition to some clinical trials based on specific inhibitors of these mechanisms. Until recently, there was no consolidated hypothesis connecting these four mechanisms together, nor was there a clear association between any of these mechanisms, each of which responds quickly to normalization of hyperglycemia, and the phenomenon of hyperglycemic memory.

1.1.9.1. Polyol Pathway

The polyol pathway also called the sorbitol aldose reductase pathway is based on a family of aldo keto reductase enzymes which can use as substrates a broad diversity of carbonyl compounds, and decrease these by nicotinic acid adenine dinucleotide phosphate (NADPH) to their particular sugar alcohols (polyols). It was initial thought that glucose is
transformed to sorbitol through the enzyme aldose reductase, with sorbitol after that oxidized to fructose through the enzyme sorbitol dehydrogenase (SDH), with NAD⁺ as a cofactor. Aldose reductase is the first rate limiting enzyme of the polyol pathway (Bhatnagar and Srivastava, 1992).

Aldehyde reductase is present in tissues such as peripheral nerve, retina, ocular lens, renal glomerulus and vascular cells (Ramasamy and Goldberg, 2010). Under normal glucose level, aldose reductase performs a minute role in carbohydrate metabolism. Though, at the time of diabetes, its contribution is considerably improved (Kinoshita and Nishimura, 1988). In several of these tissues, glucose uptake is arbitrated by insulin independent GLUTs; intracellular glucose concentrations therefore rise in parallel with hyperglycemia. Numerous mechanisms have been projected to explain how hyperglycemia induced increases in polyol pathway change can harm the tissues engaged. The most mentioned is an increase in redox stress caused by the consumption of NADPH. While NADPH is a cofactor required to regenerate reduced glutathione (GSH), and GSH is a significant scavenger of reactive oxygen species (ROS), this can stimulate or exacerbate intracellular oxidative stress. Indeed, over expression of human aldose reductase enhanced atherosclerosis in diabetic mice and decreased the expression of genes that regulate regeneration of glutathione (Vikramadithyan et al, 2005).

1.1.9.2. Increased Hexosamine Pathway Flux

Hyperglycemia and insulin resistance induced excessive fatty acid oxidation also emerge to contribute to the pathogenesis of diabetic complications by rising the flux of fructose 6-phosphate into the hexosamine pathway (Kolm et al, 1998). In hexosamine pathway, fructose 6-phosphate is diverted into glycolysis to give substrate for the rate determining enzyme of such pathway, glutamine fructose 6 phosphate amidotransferase (GFAT). GFAT changes fructose 6-phosphate to Glucosamine 6 phosphate, which is then converted to uridine diphosphate N Acetylglucosamine. Specific O linked N acetylglucosamine (OGlcNAc) transferase (OGT) use this for post translational alteration of specific serine and threonine residues on cytoplasmic and nuclear proteins by O linked N Acetylglucosamine. Inhibition of glutamine fructose 6 phosphate amidotransferase obstructs hyperglycemia induced increases in the transcription of both TGF-α (Sayeski and Kudlow, 1996) and TGF-β1 (Kolm et al, 1998). The activation of the hexosamine pathway by hyperglycemia might consequence in several changes in both gene expression and protein function, which collectively contribute to the pathogenesis of diabetic complications (Brownlee, 2001).
1.1.9.3. Activation of Protein Kinase C (PKC)

Protein kinase is a group of, minimum eleven isoforms that are selectively distributed in mammal’s tissues. The protein kinase phosphorylates various target proteins in cells. The potentiality of the common isoforms is totally dependent on both calcium ions and phosphatidylserine, and is significantly improved by diacylglycerol (DAG) (Geraldes and king, 2010).

Constant and extreme activation of several protein kinase isoforms functions as a third common pathway mediating tissue damage, persuade by diabetes induced reactive oxygen species. These outcomes mainly derived from, increased synthesis of diacylglycerol from glucose using triose phosphate pathway, whose accessibility is increased because of increased reactive oxygen species that inhibit activity of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, raising intracellular levels of the diacylglycerol precursor triose phosphate (Inoguchi et al, 1992; Craven et al, 1990; Shiba et al, 1993). A fact recommends that the increased activity of protein kinase isoforms can also result from the interaction among advanced glycation end products and their cell surface receptors (Derubertis et al, 1994). High blood glucose level principally activates the β and δ isoforms of protein kinase in cultured vascular cells (Xia et al, 1994; Ayo et al, 1991). Activation of protein kinase contributes to increased microvascular matrix protein accumulation by inducing expression of tumor growth factor (TGF)-β 1, fibronectin and type IV collagen both in cultured mesangial cells (Studer et al, 1997) and in glomeruli of diabetic rats (Koya et al, 1997).

1.1.9.4. Increased Advanced Glycation End Product Formation

Advanced glycation end products (AGEs) are directly related to hyperglycemia. The formation of advanced glycation end products (AGEs) is arises by the non enzymatically by the reaction of glucose and other glycating agents obtained both from glucose and from elevated fatty acid oxidation in arterial endothelial cells and most possible heart with proteins (Wautier JL, Schmidt, 2004; Candido et al, 2003). During diabetes, advanced glycation end products are found in increased levels in extracellular matrix (Stitt et al, 1998; Stitt et al, 1997).

Intracellular formation of advanced glycation end products precursors can harm cells by three common mechanisms. The first mechanism, revealed at the peak of the endothelial cell, is the alteration of intracellular proteins including, proteins concerned in the regulation of gene transcription. Secondly, extracellular matrix elements manipulated by AGE precursors interact unusually with other matrix components and with matrix receptors (integrins) that are articulated on the surface of cells. Lastly, plasma proteins altered by advanced glycation end products precursors attach to AGE receptors on cells like macrophages, vascular endothelial
cells and vascular smooth muscle cells. Receptor for Advanced Glycation End products binding induces the production of reactive oxygen species, which in turn activates the pleiotropic transcription factor, nuclear factor kappa B (NFκB), resulting in multiple pathological modification in gene expression (Goldin, 2006).

Advanced glycation end products change the proteins in the circulation can influence a range of cells and tissues. A specific receptor for AGEs (RAGE) has been shown to mediate signal transduction by production of reactive oxygen species, activation of nuclear factor kappa B (Lander et al, 1997; Li and Schmidt, 1997; Yamagishi et al, 1998). The signaling of advanced glycation end products in cells can be blocked by expression of RAGE antisense cDNA (Yamagishi et al, 1998) or anti RAGE ribozyme (Tsuji et al, 1998) additionally to circulation in the blood; advanced glycation end products build up in tissues and therefore play a part in the development of diabetic complications. They cause damage to biological membranes and endothelium cells. Furthermore, they change low density lipoprotein particles and collectively with vascular damage, they are involved in the increasing rate of atherosclerosis (Vlassara, 1997).

Fig-1.04: Mechanisms of hyperglycemia-induced damage

1.1.10. Mechanism of Alloxan Induced Pancreatic β-cell Damage

Alloxan is an oxygenated pyrimidine derivative which is available as alloxan hydrate in water solution. Brugnatelli at first isolated alloxan in 1818 and the name was given by Wohler and Liebig in 1838 (Wohler and Liebig, 1838). Additionally, the compound was discovered by von Liebig and Wohler in 1828 and has been considered as one of the oldest named organic compounds that exist. The name Alloxan emerged from the merging of two words, i.e., Allantoin and Oxaluric acid. Additionally, the alloxan model of diabetes induction was first described in rabbits by Dunn, Sheehan and McLetchie in 1943 (Dunn et al, 1983).
Alloxan was originally prepared by the oxidation of uric acid by nitric acid. Since then, alloxan diabetes has been commonly utilized as an animal model of insulin dependent diabetes mellitus (IDDM). The monohydrate is simultaneously prepared by oxidation of barbituric acid by chromium trioxide (Viswanathaswamy et al, 2011).

The alloxan has been well known to exert its diabetogenic action while administering parenterally or subcutaneously. Furthermore, the dose of alloxan mandatory for inducing diabetes depends on the animal species, route of administration and dietary status (Federiuk et al, 2004). Furthermore, alloxan has been established to be safe to the human beta cells, even in extremely high amounts, because of the conflicting glucose uptake mechanisms in humans and rodents (Eizirik et al, 1994, Tyrberg et al, 2001).

Alloxan has been employed to induce investigational diabetes because of the selective damage of the insulin generating pancreatic β cells. Alloxan when injected into to an experimental animal it produces a multiphasic blood glucose response, which is escorted by consequent opposite changes in the plasma insulin concentration trailed by sequential ultra structural beta cell modifies eventually leading to cell death by necrosis. The initial phase that comes forwards within the first minutes after alloxan injection is temporary hypoglycemic phase that lasting maximally for 30 minutes (Lenzen, 2008, Wrenshall et al, 1950).The second phase emerging one hour subsequent to administration of alloxan leads to rise in blood glucose quantity. Moreover, the plasma insulin concentration has been noted to decrease at the same time. This is the primary hyperglycemic phase following the first contact of the pancreatic beta cells with the toxin (Lenzen, 2008, Goldner and Gomori, 1994, Tasaka et al, 1988, West et al, 1996). This hyperglycemic phase remains for 2-4 hours which is accompanied by reduced plasma insulin concentrations. The third phase is again a hypoglycemic phase that is remains 4-8 hours after the alloxan injection, which lasts for several hours (Jacobs, 1937).

Alloxan induced diabetes has been usually used as an investigational model of insulin dependent diabetes mellitus. The diabetogenic action of alloxan has been systematically studied which at present can be characterized fairly well. A number of investigational studies have established that alloxan elicits an unexpected rise in insulin secretion in the presence or absence of glucose which emerged just subsequent to alloxan treatment (Szkudelski et al, 1998, Lachin and Reza, 2012). This special alloxan induced insulin release arises for small time followed by the full suppression of the islet response to glucose even when high concentrations of glucose were used (Kliber et al, 1996). Additional, the alloxan action in the pancreas is preceded by its quick uptake by pancreatic beta cells that have been recommended
to be one of the vital features determining alloxan diabetogenicity. Likewise, in pancreatic beta cells, the decrease process occurs in the existence of special reducing agents such as reduced glutathione (GSH), cysteine, ascorbate and protein bound sulfhydryl groups (Lenzen and Munday, 1991, Zhang et al, 1992). Alloxan reacts with two sulfhydryl groups in the sugar binding site of glucokinase resulting in the development of the disulfide bond and inactivation of the enzyme. Consequently reduction of alloxan to dialuric acid which is then re oxidized reverse to alloxan establishing a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals (Munday, 1988; Das et al, 2012). The superoxide radicals release ferric ions from ferritin and condense them to ferrous and ferric ions (Sakurai and Ogiso, 1995). Additionally, superoxide radicals undergo dismutation to yield hydrogen peroxide in the existence of superoxide dismutase. Consequently, highly reactive hydroxyl radicals are produced according to the Fenton reaction in the presence of ferrous and hydrogen peroxide.

Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. The breakup of DNA takes place in the beta cells exposed to alloxan that causes DNA damage, which excites poly ADP ribosylation, a process taking part in DNA repair. Enzymatic antioxidants and the non enzymatic scavengers of hydroxyl radicals have been establish to protect against alloxan toxicity (Ebelt et al, 2000). In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an important step in the diabetogenic action of alloxan. It has been noted that alloxan elevates cytosolic free calcium ion concentration in the beta cells of pancreatic islets (Park et al, 1995). The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of calcium ion further contributes to supraphysiological insulin release that along with reactive oxygen species has been noted to finally cause damage of beta cells of pancreatic islets (Etuk et al, 2010, Lenzen, 2008, Szkudelski, 2001).

Therefore, using alloxan to induce diabetes, animals should be examined after appropriate period of time to reduce side effects of alloxan. It should also be give emphasis on the range of the diabetogenic dose of alloxan is quite low and even small overdosing might be usually toxic causing the death of several animals. This loss is mainly possible due to kidney tubular cell necrotic toxicity, in exacting when too elevated doses of alloxan are administered (Lenzen et al. 1996).
1.1.11. Treatment and Management of Diabetes Mellitus

The aim of treatment for diabetes is to reduce and control blood glucose levels, decrease the signs of the disease, and avoid complications. Exhaustive treatment and cautious control of blood glucose levels can reduce the risk of complications from diabetes. Diabetes is most excellent treated and managed also by diet alone and exercise (non pharmacological), otherwise diet with herbal or oral hypoglycaemic agents or insulin (pharmacological).

1.1.11.1 Non Pharmacological Involvements in the Treatment of Diabetes Mellitus

It has been shown that weight reduction and an increase in daily energy expenditure decrease insulin resistance and increase glucose tolerance. In fact, advice on diet and exercise are an important part of the treatment of type 2 DM (Stoffers et al, 1997).

1.1.11.1.1. Diet and Life Style Changes

Primary prevention is the main aim at preventing diabetes from occurring in susceptible individuals or in general population. Regular physical activity is an important component of the prevention and management of type 2 diabetes mellitus. Prospective cohort studies have shown that increased physical activity, independently of other risk factors, has a protective effect against the development of type 2 diabetes. Type 2 diabetes individuals with moderate or high aerobic fitness have long-term mortality 50-60% lower than diabetic individuals with low cardiorespiratory fitness (Ross et al, 2000, Helmrich et al, 1991, Manson et al,1992).

In type 1 diabetics, most studies have not found any benefit from exercise because of the likelihood of type 1 diabetics to consume additional carbohydrates in an effort to prevent hypoglycaemia. Again in type 1 diabetics, hypoglycaemia often develops during light to moderate exercise unless the insulin dose is reduced or extra carbohydrate consumed (Felig et al, 1982). Nevertheless, type 1 diabetics who exercises regularly have marked lower long-term morbidity and mortality compared to their sedentary counterparts. For both type 1 and type 2 diabetic patients’ physical activity is accompanied by gains as well as risks (Moy et al, 1993).

Dietary and lifestyle modifications are the mainstay of treatment and management for type 2 diabetes. The majorities of people with type 2 diabetes are overweight and usually have other metabolic disorders of the insulin resistance syndrome, so the major aims of dietary and lifestyle changes are to decrease weight, recover glycaemic control and reduce the risk of coronary heart disease (CHD), which accounts for 70% to 80% of deaths among those with diabetes (Bearse et al, 2004).
Fat is the most energy-rich of all nutrients and reduction of fat intake helps to reduce total energy intake, which is important for many people with type 2 diabetes and some with type 1 diabetes. By tradition most of the recommendations for people with diabetes were low carbohydrate diets.

Evidence suggests that reducing protein intake to the levels recommended by WHO (0.6 g/kg/day as a safe intake) can reduce albuminuria and improve renal hemodynamics in type 1 diabetes patients with incipient and established nephropathy (Jones et al, 1992, Pomerleau et al, 1993). It is generally agreed that adequate folate intake is important in diabetic patients. The recommended dietary allowance (RDA) for folic acid was doubled in 1998 to 400 µg per day compared to the previous RDA in 1989. Micronutrients have at one time or the other been the subject of interest in diabetes, in particular chromium, zinc, and magnesium, there is little evidence that those with diabetes have different requirements for vitamins and minerals than those who do not have diabetes (Walter et al, 1991).

1.1.11.1.2. Alcohol

There is evidence, in people who do not have diabetes, that modest intake of alcohol, especially wine, reduces CV risk because of a beneficial effect on HDL-cholesterol. In diabetics, alcohol consumption should be eliminated in those suffering from hypertriglyceridaemia, in those who are overweight and in those with hypertension (Christiansen et al, 1993). One of the major risks with alcohol consumption among individuals with diabetes is the potential danger of hypoglycaemia, especially among those who use sulphonylureas. However, in many clinical studies, no alterations in glucose homeostasis were observed when moderate alcohol is consumed with meals (Burge et al, 1993).

1.1.11.1.3. Cigarette smoking

Cigarette smoking markedly increases the risk of coronary heart dieses (CHD) in diabetes. Smoking cessation can have an important effect on CHD risk reduction in diabetic patients, and clinical trials in diabetics who lower their cholesterol levels achieved a 25-55% reduction in risk of major CHD events (Pyorala et al, 1997), tight blood pressure control achieved 21% reduction in CHD (UK Prospective Diabetes Study 38, 1998) and intensive blood glucose control achieved 16% risk reduction (UK Prospective Diabetes Study 33, 1998).

1.1.11.2 Pharmacological Involvements in the Treatment of Diabetes Mellitus

1.1.11.2.1. Insulin Treatment in Diabetes Mellitus

The introduction of insulin to treat diabetes has saved an estimated 5 million years of life for patients with type 1 diabetes during the year 2000 (Owens, 2001). Considerable progress has been made, in recent years, in the production, formulation and delivery of insulin.
preparations, as well as the development of insulin treatment regimens which maintains long-term-normoglycaemia, with a low risk of hypoglycaemia (Bolli, 1997, Bolli, 1992). It is an accepted fact that insulin is the most potent glucose-lowering agent, with hypoglycaemia being the only major dose-limiting factor.

Insulin therapy should aim to mimic nature, which is remarkably successful both in limiting postprandial hyperglycaemia and preventing hypoglycaemia between meals (Ciofeta et al, 1999), but unfortunately pharmacological problems complicate insulin therapy. Insulin analogues have an alteration in the amino acid sequence of human insulin, which change the rate of insulin absorption, or some other structural change like being linked to a fatty acid chain, that alters the insulin time action curve (Bethel and Feinglos, 2002). Insulin is prepared either from human, or porcine, or bovine or a mixture of bovine and porcine. Human insulin (Humulin, Novolin) is now widely available prepared by recombinant DNA techniques. The physicochemical properties of human, porcine and bovine insulins differ owing to their different amino acid sequences. Insulin is the mainstay for treatment of virtually all type 1 DM and many type 2 DM patients. Insulin may be administered intravenously (IV), or intramuscularly (IM); however for long-term treatment, subcutaneous (SC) route is preferred.

- **Complications of Insulin Therapy:** The most common adverse reactions to insulin are weight gain and hypoglycaemia (Henry et al, 1993; Kudlacek et al, 1992). Weight gain after starting insulin therapy for uncontrolled diabetes is an inevitable consequence and is the result of increased truncal fat and muscle bulk (Diabetes Control and Complications Trial Research Group, 1993; Yki et al, 1999). This is also due to reduced energy losses through glycosuria. Hypoglycaemia may result from an inappropriately large dose, from mismatch between the peak delivery of insulin and food intake or from superimposition of additional factors that increase sensitivity to insulin (adrenal insufficiency, pituitary insufficiency) or that increase insulin-independent glucose uptake (exercise). Use of physiological insulin regimens combined with education can actually decrease the frequency of hypoglycaemia (Pampanelli et al, 2002; Bott et al, 1997) and reduce the risk of hypoglycaemia (Lalli et al, 1999; Cryer, 2002).

### 1.1.1.1.2. Oral Hypoglycemic Agents

- **Sulfonylureas:** Sulphonylureas are structurally related to sulphenamides and were discovered accidentally, in 1942 when it was noted that some sulphonamides caused hypoglycaemia in experimental animals. These observations were extended, and 1-butyl-3-sulfonylurea (carbutamide) became the first clinically useful sulfonylurea for
the treatment of diabetes. Sulfonylureas cause hypoglycemia by stimulating insulin release from pancreatic β-cells. They bind to sulfonylurea (SUR) receptors on the β-cell plasma membrane, causing closure of adenosine triphosphate (ATP) sensitive potassium channels, leading to depolarization of the cell membrane. This in turn unlocks voltage gated channels, permitting entry of calcium ions and subsequent secretion of preformed insulin granules. Acute administration of sulfonylureas to type 2 DM patient’s increases insulin release from the pancreas and also may further increase insulin levels by reducing hepatic clearance of the hormone. Initial studies showed that a functional pancreas was necessary for the hypoglycaemic actions of sulfonylureas (Levine, 1984). Sulfonyl ureas augment insulin action in the cells in culture and stimulate the synthesis of glucose transporters. Sulfonyl ureas also have been shown to suppress hepatic gluconeogenesis (Bluementhal, 1977). However it is not clear if this is a direct effect of the drug or a reflection of increased sensitivity of Insulin. Glibenclamide is probably one of the most widely used oral hypoglycemic agents in the treatment of diabetes mellitus today. This agent can effectively control a significant proportion of patients developing secondary failure to first generation compounds. The receptors of sulphonyl urea are present in cardiac muscle cells, smooth muscle cells, liver and adipose tissue. There are several side effects mediated by the use of sulphonyl urreas. They are chronic renal failure, hepatic and cardiovascular diseases. Patients who are using the sulphonyl urea therapy tend to gain weight from 1-5 kg (Zimmerman, 1997).

- **Biguanides**: They have been used in NIDDM patients as adjuncts in insulin therapy and they are active only in patients with some endogenous insulin secretion. Biguanides are derivatives of guanide especially phenformin, metformin and buformin. Recently metformin gained popularity in diabetic patients because of its variations in the chemical structure. Metformin given alone or in combination with a sulfonylurea improves glycaemic control and lipid concentrations in patients who respond poorly to diet or to a sulfonylurea alone. Studies have revealed that metformin recovers insulin resistance in the skeletal muscle, liver, and adipose tissue, a most important pathogenic component of type 2 diabetes (Bailey, 1992). The mechanism of action of metformin is not fully understood. Metformin is antihyperglycaemic, not hypoglycaemic (Bailey, 1992). It does not cause insulin release from the pancreas and does not cause hypoglycaemia, even in large doses (Clarke and Duncan, 1979). Metformin has no considerable effects on the release of glucagon, cortisol, growth hormone, or somatostatin. Metformin has also been shown
to decrease serum triglycerides and fatty acid concentrations and slows the rate of lipid oxidation (Perriello et al, 1994; Wu et al, 1990), actions that indirectly inhibit gluconeogenesis.

- **Thiazolidinediones:** Thiazolidinediones (TZDs) are chemically and functionally unrelated to the other classes of oral antidiabetic agents. A thiazolidine-2, 4-dione structure is common to all agents. They contain a thiazole ring which has the antidiabetic effect. Two compounds in this class are currently in use. Rosiglitazone (Avandia) and pioglitazone (Actos) are the two thiazolidinediones in use (National Diabetes Data Group, 1997). The thiazolidinediones (TZDs), or glitazones, are the only class of drugs that directly target insulin resistance, a fundamental defect in the pathophysiology of type 2 diabetes. The mechanism of action of the TZDs they are selective agonists for nuclear peroxisome proliferator-activated receptor-gamma (PPARγ) (Lemberger et al, 1996). PPARγ receptors are found in key target tissues for insulin action such as adipose tissue, skeletal muscle and liver. There is strong evidence to indicate that these receptors may be important regulators of adipose differentiation, lipid homeostasis, insulin action, and Thiazolidinediones exert their principal action by lowering insulin resistance in peripheral tissue, but an effect to lower glucose production by the liver has also been reported (Matsuda et al, 1998; Park et al, 1998). Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter proteins (Lemberger et al, 1996). The thiazolidinediones can also activate genes that regulate free fatty acid (FFA) metabolism in peripheral tissue, hence decreasing triglycerides and non esterified fatty acid levels and inducing separation of adipocytes (Miyazaki et al, 2001).

- **Meglitinide Analogues:** The meglitinide analogues are a new class of drugs developed to get better early phase insulin secretion, which is one of the initial pathophysiological symptoms of type 2 diabetes mellitus. Meglitinide analogues are derivative of the meglitinide portion of sulfonylureas. Repaglinide and nateglinide are the main examples of this group. The meglitinides are rapid acting insulin secretagogues (also known as prandial glucose regulators) that have a fast onset and short duration of action resulting in more physiological secretion of insulin from the β-cell with no causing persistent increase of insulin in the post absorptive stage, therefore reducing glycaemia without raising the risk of hypoglycaemia. The meglitinide analogues act on β-cell receptors to stimulate insulin secretion by binding to the sulfonylurea receptor subunit and closing the K+ ATP channel (Hu et al, 2000),
but probably at a site distinct from that of the sulfonylurea receptor (Fuhlendorff et al, 1998). Closure of the potassium channel leads to depolarization of β-cell plasma membrane, which promotes influx of calcium ions through voltage gated calcium channels, consequential in exocytosis of insulin particles.

- **α- Glucosidase Inhibitors:** α- Glucosidase inhibitors have been developed specifically to delay the digestion of complex carbohydrates and decrease the postprandial rise in plasma glucose, thus reproducing the effect of a low glycaemic index/high fiber diet. These actions significantly reduce postprandial glycaemic and insulinaemic increase whether they are used as mono therapy or combined in the treatment of type 1 and type-2 diabetes. These drugs have an excellent safety profile. α- Glucosidase inhibitors competitively block small intestine brush border enzymes that are necessary to hydrolyze oligo and polysaccharides to monosaccharides (Bischoff, 1995). Inhibition of α- glucosidase reduce the absorption of carbohydrates; the postprandial increase in plasma glucose is weaken in both normal and diabetic persons (Reabasa and Chiasson, 1998). Three α -glucosidase inhibitors have been developed: acarbose, miglitol, and voglibose and all have similar pharmacological profiles. α- Glucosidase inhibitors reduce postprandial plasma glucose levels in type 1 diabetes mellitus and type 2 diabetes mellitus subjects. α- Glucosidase inhibitors do not stimulate insulin release and therefore hypoglycaemia does not occur.

1.1.11.2.3. Recent Agents for the Treatment of Diabetes Mellitus

Potential new antidiabetic agents and compounds are undergoing clinical trials:

- α- Glucosidase inhibitors such as voglibose have already been mentioned earlier. It reduces the postprandial increase in glycaemia similar to acarbose and miglitol.
- α- Amylase inhibitors like acarbose are weak inhibitors of a-amylase activity, but attempts to specifically inhibit a-amylase activity have not been successful.
- Novel insulin: Thyroxyl-insulin, insulin linked to thyroxine, is highly bound to plasma proteins via its thyroxyl moiety, ensuring a prolonged plasma half-life and limited transport across the endothelium, but has free access to hepatocytes. Others like insulin initiators and potentiators act by enhancing the effect of glucose and other nutrient initiators for example agents that increase cellular concentration of cyclic adenosine mono phosphate (cAMP).
- A lot of attention has recently been focused on the therapeutic potential of glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP). In the presence of stimulatory concentration of glucose, GLP-1 is a potent insulin
secretagogue in non-diabetic subjects, subjects with impaired glucose tolerance (IGT), and type 2 diabetic subjects. In acute and chronic studies, injection or infusion of GLP-1 before or during meals reduced postprandial hyperglycaemia and improved glycaemic control without causing clinical hypoglycaemia (Drucker, 1998).

- **Dipeptidyl peptidase 4 (DPP-4)** degrades a variety of circulating peptides (e.g. PYY and NPY) including glucagon, which might contribute to its glucose-lowering effect, but it is not yet evident whether the effects of DPP-4 inhibitors on other peptides could limit their use.

- Inhibition of **phosphodiesterase** (PDE) activity in islet β-cells offers a potentially therapeutic approach to raise intracellular cAMP and thereby increase insulin biosynthesis and secretion. Several PDEs are expressed by pancreatic islet cells (PDE-1 to −5), and PDE-1, -3 and -5 appear to be the main types in β-cells.

- **Minerals:** Magnesium, chromium and zinc are often reduced in diabetics, and supplementation may improve glycaemic control in cases of mineral deficiency. Hypomagnesium is not uncommon in insulin resistant states, and magnesium supplementation have improved insulin action and β-cell function, and possibly reduce cardiovascular mortality in magnesium deficient diabetic patients (Bailey, 1999). Adequate chromium is necessary for normal insulin sensitivity, but the site of action of chromium is unresolved. Zinc has been reported to protect against β-cell damage and to improve glycaemic control in diabetic patients with liver disease (Ohly et al, 2000).

- **Vanadium salts** exert insulin-like effects on glucose metabolism in vitro and lower blood glucose levels in animal models of hyperinsulinaemic and hypoinsulinaemic animal models of diabetes (Tsiani and Fantus, 1997). Vanadium can improve glycaemic control in type 1 and type 2 diabetic patients, reduce insulin requirements and increase peripheral glucose utilization in type 2 diabetes. Additionally it enhances glucose transport in skeletal muscle suggesting an effect at a more distal step in the control of glucose transport.

- **Aldose Reductase** inhibitors include Epalrestat and Sorbinil. These drugs inhibit the enzyme aldose reductase which catalyzes the conversion of glucose to sorbitol. Aldose reductase inhibitors have no influence on blood-glucose concentrations. They are given in diabetic complications including neuropathy.
1.1.12. Drawbacks of Presently Available Antidiabetic Therapy

- Up to 2.5% and 17.5% of sulfonylurea (SU) treated patient’s incident major and minor hypoglycemia.
- Additional side effects of sulfonylureas incorporate nausea and vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, and dermatological reactions.
- Body weight gains of 2.2 to 11.0 lb (1 to 5 kg) are general with sulfonylurea (Bolen et al, 2007).
- Unfortunately, conventional human insulin is associated with several characteristics that limit its prospective. Original human insulin injected intravenously has a 17-minute half-life and a short duration of action (Tong et al, 2008).
- Insulin remedy is frequently accompanied by weight increase that may go beyond 10 kg is associated with increasingly intensive insulin treatment.
- Patients with renal injury should not receive biguanides. Other contraindications include hepatic disease, a past history of lactic acid overproduction (of several causes), heart malfunction requiring pharmacological therapy, or chronic hypoxic lung ailment.
- Severe side effects of biguanides include diarrhoea, abdominal discomfort, nausea, metallic taste, and anorexia.
- Peripheral edema is observed in up to 26% of thiazolidinedione treated patients. Thiazolidinediones are very prone to cause hepatotoxicity and can cause weight gain and oedema.

1.2 IMPORTANCE OF DIETARY ANTIOXIDANTS IN DIABETES MELLITUS

Antioxidant affluent foods not only help to recover the serum antioxidant profile but also help to control blood sugar and serum lipid profile. There is a significant positive relationship with antioxidants and existence diseases (Preethi and Nandini, 2005). Antioxidant defense has been recently recognized to originate from the chain – breaking antioxidant activity of natural polyphenol (Roginsky and Eduard, 2005).

Many studies have shown that supplementation of antioxidant wealthy foods have positive impact on the diabetic subjects. Aliyu et al, 2005 reviews that using up fruits and vegetables is associated with reduced risk of free radical production. The potential protective effects of these foods may be due to their antioxidant vitamin contents. Beta Carotene one of the dietary sources of vitamin A, ascorbic acid and vitamin E are free radical scavengers and have been shown to quench singlet oxygen, superoxide, hydroxyl radical and peroxy radicals.
The intake of total antioxidants was significantly correlated with plasma lutenin, zeaxanthin and lycopene. Among individual food groups, coffee, tea, cocoa, wine, vegetables, fruits were significantly correlated. It supports that, dietary antioxidants other than well-known anti oxidants contribute to our antioxidant defense. The single greatest contributor to the total antioxidant intake was coffee (Svilaas et al, 2004).

Drinking green and black teas infusion can significantly lower the glucose level in blood by reducing the absorption of glucose. The polyphenols in tea inhibit the activity of alpha amylase in saliva and reduce the activity of intestinal alpha amylase, which in turn lowers the hydrolysis of starch to glucose and reduces glucose assimilation. Polyphenols can also decrease the activity of other digestive enzymes and reduce glucose absorption (Rana, 2005).

Diets prosperous in fruits and vegetables that are rich sources of antioxidant vitamins (Sablani et al, 2006) like beta - carotene, ascorbic acid and tocopherols, (Easwaran et al., 2002). It lowers the occurrence of a number of diseases including Diabetes (Yochum, 2000).

The consumption of dry common beans (Phaseolus vulgaris) has been associated with a decrease risk for a wide variety of chronic and degenerative diseases for example tumors, obesity, diabetes mellitus and heart diseases. Beans are a good resource of high worth protein, complex carbohydrates, dietary fiber, some vitamins and minerals. Beans also contain phytochemicals often considered as antinutritional factors, which include poly-phenols (condensed tannins and anthocyanins), protease inhibitors, lectins and phytic acid (Gonzalez et al, 2005).

Oranges are rich in phytochemicals which play a vital role in preventing various diseases and maintaining good health. Though they are popular for their vitamin C content, they also contain about 170 elements, like flavonoids, phenolics, tannins and carotenes all of which play a crucial role in maintaining health in diabetes. They are rich source of dietary fibers (Dobriyal, 2005). Orange flavonoids significantly reduce the risk of diabetes and cardiovascular diseases. They also play a key role in reducing the oxidative stress by their potent antioxidant properties. Oranges also help to repair the body tissues and pace up the healing process due to its rich vitamin C content (Dobriyal, 2005).

Vitamin E and ascorbic acid have confirmed to be potent antioxidants protecting lipids in plasma against oxidation. A combined intake of utilization of vitamin E and C together brought better results in blood lipid levels than consuming vitamin E or C alone. Hyperlipidemics and overweight people should consume moderate amounts of fruits and
vegetables that are rich in antioxidant vitamins to uphold blood lipid levels (Easwaran et al, 2002).

The significance of natural antioxidants mainly of plant origin has greatly increased in recent years (Chidamabaramurthy et al, 2002). Synthetic antioxidants degrade cells over time and cause adverse health effect is proved by studies. Utilization of synthetic beta carotene increased risk of cancer. Synthetic vitamins have been shown to be treated like foreign substance in the body just as the drugs are. This means the body has to work hard to detoxify the body from them (Karen, 2007). Therefore dietary antioxidants are at present gaining high importance especially in controlling diseases like diabetes mellitus.

Alpha lipoic acid (ALA) also known as thiocitic acid is a worldwide antioxidant, currently in Germany using for the treatment of diabetic neuropathy. It is possible that lipoic acid could be more effectual as an enduring dietary supplement aimed at the prevention of diabetics from complications (Coleman et al, 2001).

### 1.2.1. Antioxidant Status in Diabetes Mellitus

Antioxidants are compounds in foods that neutralize chemicals called free radicals (unstable molecules), generated by oxidation in the human body. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can construct free radicals and these radicals can start chain reactions that damage cells. Antioxidants break these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Srinivas, 2005).

Antioxidants are substances that exertion in different ways to shield cells from biochemical damage (Preethi and Nandini, 2005). The most common antioxidant present in plant foods are carotenoids, phenolics, anthocyanins, flavonoids, flavones, ascorbic acid and vitamin E (Kaur et al, 2004). Plasma antioxidants can be decreased as compared to recognized normal values in abnormal or subnormal conditions, for example as a consequence of disease related free radical production. Plasma antioxidants may be below the normal range due to insufficient dietary supply. Therefore the antioxidant profile can be used in conjunction with other parameters of oxidative stress status in case of stress linked conditions like diabetes (Patel, 2002).

Antioxidants break off the signaling mechanism triggered by oxidants, thus playing an important role in inter and intracellular signaling. Such antioxidants in large number exist as nutritive and non-nutritive form in food and aid in withdrawing the reactive oxygen species effect (Srinivas, 2005).
According to Sesikaran, 2006 antioxidant systems help protect the body against reactive oxygen species but may be overwhelmed during periods of oxidative stress, which can cause lipid peroxidation, damage to DNA and cell death. Total Antioxidant Capacity (TAC) is the overall activity of antioxidants and antioxidant enzymes; and is used for monitoring and optimization antioxidant therapy.

1.2.2. Antioxidants in Diabetes Mellitus

Antioxidants have a major role in the prevention and control of diseases like diabetes mellitus. Free radicals having at least one unpaired electron that consume electron from other strong molecules cause damage/oxidative stress to β-cells in pancreas. Antioxidants, having one or more extra electrons donate an electron to a free radical; neutralize the free radical and oxidative stress (damage) in the cells (http://www.amazingglutathione.com).

Oxygen being a reactive compound is accomplished of becoming damaging molecules termed as free radicals or Reactive Oxygen Species fundamentally possessing unpaired electrons. Oxidative demolition is caused by reactive oxygen species called oxidants (e.g.: lipid peroxides such as hydrogen peroxide) and free radicals (e.g.: superoxide, hydroxyl radical) (Veach, 2004).

The level of oxidative stress in diabetes mellitus is determined by the balance between the rate at which pro-oxidants are produced and the rate at which they are removed by antioxidant defense mechanism. Component of oxygen metabolism or by UV radiation, nutritional deficiencies, bacterial, viral infections, noxious chemicals, xenobiotic metabolism, endocrine disorders like diabetes mellitus, cigarette or organic smoke contact and some genetic diseases are the causes by which Reactive oxygen species are generally produced in the body (Srinivas, 2005).

The excess free radicals circulating in the body oxidize the LDL, making them potentially lethal. The Reactive oxygen species can accelerate ageing process and have been linked to serious pathologies such as brain stroke, diabetes mellitus, cardio vascular diseases, hypertension, obesity (Panchnadikar et al, 2003) rheumatoid arthritis, Parkinson’s disease, Alzheimer’s disease and cancer (Campanella et al, 2006).

A deleterious effect of radiation is the production of reactive oxygen species (ROS), which consequence in alter to biomolecules. Free radicals and other reactive species are produced in the body primarily as a result of aerobic metabolism. Antioxidants and antioxidant enzymes exert synergistic actions in scavenging free radicals. Thus diabetics are much benefited (Fang et al, 2002). Oxidative phosphorylation, Nicotinamide Adenine
Dinucleotide Phosphate Oxidase (NADPH), Xanthine oxidase, the uncoupling of lipoxygenases and glucose auto oxidation are main factors to produced Reactive Oxygen Species (ROS), reactive oxygen species Once formed they deplete antioxidant defenses, rendering the affected cells (β cells) and tissues more susceptible to oxidative damage. Reactive oxygen species are also important as second messengers in the regulation of intracellular signaling pathways and ultimately gene expression (Niedowicz and David, 2005).

For the protection of cells against damage Human body possesses defense mechanisms. Neutralization of free radicals, Metabolism of free radicals by enzyme and Repair of macromolecular damage are three defense strategies are used to protect cells from oxidative damage. This is achieved by water soluble antioxidants e.g.: ascorbic acid, cysteine, lipid soluble antioxidants (e.g.; tocopherol and retinols), specific enzymes and flavonoids (Veach, 2004).

1.2.3. Free Radicals and Oxidative Stress in Diabetes Mellitus

Free radical is defined as any species able to independent existence containing one or more unpaired electrons. They are unstable and highly reactive molecules that have unpaired electrons in their outermost orbit (Halliwell, 1994). Under physiological and pathophysiological conditions Reactive oxygen species (ROS) and reactive intermediates are produced. (Halliwell and Gutteridge, 1986). Reactive oxygen species are chemically extremely reactive molecules, which can be free radicals for example superoxide (•O₂⁻), hydroxyl (•OH), peroxyl (•RO₂⁻), hydroperoxyl (•HRO₂⁻), nitric oxide (•NO) and nitro-gen dioxide (•NO₂⁻), or nonradicals such as hydrogen peroxide (H₂O₂), hydrochlorous acid(HOCl), peroxynitrite (ONOO⁻), nitrous oxide (HNO₂), and alkyl peroxynitrates (RONOO). Most of the studies regarding diabetes and its complications have addressed the role of superoxide (•O₂⁻), nitric oxide (•NO), and peroxynitrite (ONOO⁻) in this disease (McDermott, 2000).

1.2.4. Sources of Free Radicals

Free radicals come from a many different sources like natural by-products of ongoing biochemical reactions occurring in normal metabolic functions, in the removing impurities from the blood in the liver and in the immune system defense. Free radicals can be found in the food we eat, in our water supplies (particularly when chemicals and pollutants have gone into them), drugs and medicine we consume and the air we breathe. Our environment contributes immensely to the spread of free radicals, as act processes like medicines, radiation, insect killers, air contaminants, organic solvents, fried foods, alcohol, tobacco
smoke, and so on. the entire things most of us are exposed to every the time, airborne emissions, extreme sunlight, chlorination, chemical resources and toxic waste may create free radical harm to our body's cells causing oxidation.

Oxidative stress may be defined as assess the steady state level of reactive oxygen species or oxygen radicals in a biological system (Baynes, 1991). Reactive oxygen species (ROS) induced Oxidative stress and concerned in the pathogenesis of diabetes mellitus and a variety of disease (Kataja-Tuomola et al, 2011). The redox state is finely tuned to preserve cellular homeostasis through the expression of antioxidant enzymes and hence regulation of oxidants (Ziyatdinova et al, 2006). Oxidative stress is involved in the origin of type 1 diabetes (Chistiakov, 2004; Haskins et al, 2006). Diabetic patients are claimed to be under oxidative stress because of high blood glucose level. The impact of free radical production by this hyperglycemic induction may involve cardiovascular complications in diabetes (Likitlilid et al, 2007).

To scavenge reactive oxygen species mammalian cells have a complex network of antioxidants like catalase, superoxide dismutase (SOD), reduced glutathione etc. Oxidative stress ensues when ROS avoid or overwhelm antioxidants. Due to their vastly reactive and distracted nature, reactive oxygen species is able to attack about all biomolecules including lipid membranes and β cells (Kaur et al, 2008).

Lipid peroxidation is a process where pro-oxidant compounds such as reactive oxygen species react with poly unsaturated fatty acid of biological membranes (Veach, 2004). Lipid peroxides derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction (Kaur et al., 2008). In diabetes many factors raise blood lipid level, this is because carbohydrates and lipid metabolism are interrelated to each other; if there is any disorder in carbohydrate metabolism it also leads to disorder in lipid metabolism so there is high concentration of cholesterol and triglycerides and due to this there is reduction in HDL cholesterol levels (Smith, and Lall, 2008). Lipid peroxidation has been associated with several types of diseases including atherosclerosis, cancer and diabetes (Kurimura et al, 1988). Increased oxidative stress may contribute to the development of complications of diabetes mellitus.

Oxidative stress guide to damage of all major molecules including DNA, proteins and lipids, and is often the precursor for many diseases such as diabetes, cancer, arteriosclerosis and the aging process (Kaur et al, 2008). If there is marked unevenness between the production and removal of reactive oxygen, then oxidative stress take place which aggravates diabetic complications (Teksen et al, 2007).
Oxidative stress could stop from endogenous sources through common physiological processes such as mitochondrial respiration or haemoglobin oxidation; and from exogenous sources such as exposure to pollutants, ionizing irradiation or other excessive factors (Ziyatdinova et al, 2006).

Oxidative stress leads to the generation of reactive oxygen species (ROS) including free radicals. Their amount depends on generation rate and the antioxidant defense system of human body particularly of blood. ROS are strongly implicated in the pathophysiology of diseases such as diabetes mellitus, cancer, heart diseases and atherosclerosis, aging, renal, inflammatory, infectious and neurological diseases (Kaur et al, 2008).

High blood glucose level in diabetes can enhance the oxidative stress by numerous mechanisms, including glucose auto-oxidation, nonenzymatic protein glycation and activation polyol pathway (Tian et al, 2005).

Fig-1.05: Possible link between hyperglycemia – induced oxidative stress

High blood sugar (glucose) may lead to an increased production of free radicals through multiple mechanisms. While the sources of this oxidative stress stay unclear, it has been recommended that the chronic hyperglycemia in diabetes enhances the production of reactive oxygen species from glucose autooxidation (inter and intra molecular hydrogen abstraction by peroxy radicals), non-enzymatic protein glycation and advanced glycoxidation end products (AGEs) which leads to tissue damage. Also, increasing episodes of acute hyperglycemia can be source of acute oxidative stress (Dominique, 2002; Jakus 2000).
Recent studies have precise that a hyperglycemia induced more production of superoxide free radicals appears to be the main incident in the development of complications of diabetes. Superoxide free radicals overproduction is allied with increased production of nitric oxide and, as a result, development of the tough oxidant peroxynitrite and by poly (adenosine diphosphate ribose) polymerase activation, which initiates the pathways concerned in the development of diabetes related complications.

Hyperglycemia direct to various significant complications during oxidative stress in many cells. Free radicals create an important contribution in the development of diabetes complications (Hsu et al, 2006).

One of the serious pathogenic consequences of hyperglycemia in diabetes is insufficiency in detoxification of reactive carbonyl compounds. The raise in reactive carbonyls derived from both oxidative and non oxidative reactions increased chemical modification of proteins and then, oxidative stress and tissue damage (John et al, 1999).

Diabetes mellitus can be better controlled if free radical reactions are inhibited by decreasing the level of active products from oxygen reduction or by removing the transition metals group (Fe, Cu) by their bounding with proteins. Free radicals are also eliminated from the body by their interaction with antioxidants. At a later phase of oxidative stress, the Total Antioxidant Capacity (TAC) falls due to reduction of antioxidants, this in turn exacerbates diabetes mellitus. LMW antioxidants penetrate specific location in the cell where oxidative stress may occur and protect against ROS (Ziyatdinova et al, 2006). A significant increase of TAC occurs after supplementation with vitamins C, E, β carotene and phenolics of green and black tea (Larini et al, 2004), and is found beneficial in diabetes mellitus (Anuradha and Vidhya, 2001; Jasim et al, 2011).
1.2.5. Classification of Antioxidants

Antioxidants are classified on the basis of various factors like function, molecular weight, mechanism of action and reactions, ability to reduce ROS, conversion of other oxidants.

Ziyatdinova et al., (2006) classified antioxidant in to two categories they are mainly:

a) The low-molecular weight (LMW) compounds like tocopherols, ascorbate, betacarotene, glutathione, uric acid, bilirubin etc.

b) The proteins like albumin, transferrin, caeruloplasmin, ferritin, superoxide dismutase, catalase, glutathione peroxidase etc.

Low molecular weight antioxidants mechanism of action is to penetrate specific location in the cell where oxidative stress may occur and protect against ROS. After supplementation of vitamins C, E, and beta carotene and phenolics of green and black tea significant increase of Total antioxidant capacity (TAC) really occurs (Ghiselli et al, 2000).

According to Sesikaran, (2006) antioxidants are classified in two classes:

1) Endogenous antioxidants: Antioxidants which provide a buffer against the oxidative pressure of reactive oxygen species on other molecules by their own amenability to oxidation and

2) Exogenous antioxidants: These are mainly derived from food and other dietary sources.

Several herbs, spices, vitamins, foods, vegetables etc exhibits antioxidant activities

1.2.5.1. Endogenous Enzymatic Antioxidants

Endogenous enzymatic antioxidants are produced by the body and are not obtained from food sources; they are far more powerful than exogenous antioxidants. Endogenous antioxidants restore all free radicals damage by initiating cell regeneration from inside to outside, whereas, exogenous antioxidants only repair some of the free radical damage from the outside to inside by stimulating (not initiating) cell regeneration. There are five tremendously potent endogenous antioxidants. They are: Glutathione (GSH), Superoxide Dismutase (SOD), Catalase, Alpha Lipoic Acid (ALA) and Coenzyme Q10 (CoQ10). The production of endogenous antioxidants declines with age; glutathione levels decline about 10-15% per decade as we grow older. This decrease in endogenous antioxidants is found to be a strong factor in contributing to premature aging and degenerative diseases (http://www.amazing-glutathione.com).

Diabetes, Alzheimer's, Cancer, Heart Disease, many other health concerns and Age-related health issues can be prevented by escalating the endogenous antioxidants at cellular
levels. SOD, CAT and GPx constitute an equally supportive team of defense against Reactive Oxygen Species (Nirmala et al, 2011).

1.2.5.1.1. Glutathione Peroxidase

Gordon C. Mills discovered Glutathione peroxidase was in 1957 (Muller et al, 2007). The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione S transferases (GSTs). Glutathione peroxidase is the common name of an enzyme family with peroxidase activity whose key biological role is to protect the organism from oxidative damage. Glutathione peroxidase reduces lipid hydroperoxides to their corresponding alcohols and to diminish free hydrogen peroxide to water (Ran et al, 2007).

Glutathione is the most important endogenous antioxidant formed by the cells; contributes openly in the neutralization of free radicals and reactive oxygen species, in addition to maintaining exogenous antioxidants such as ascorbic acid and vitamin E in their reduced (active) forms (Scholz et al, 1964). Glutathione is the Master Antioxidant as it does not need any other antioxidants to do its job. Glutathione is about 5,000 times stronger than any other antioxidant and has more "extra" electrons to share. For example: Vitamin E has 3 extra electrons to share, Vitamin C has 5 extra electrons to share, Oligomeric Proanthocyanidin (OPC) has 250 extra electrons to share, Superoxide Dismutase (SOD) has 10,000 extra electrons to share, Glutathione (GSH) has 1 Million extra electrons to share (http://www.amazing-glutathione.com).

So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans. Glutathione peroxidase1 (GPx1) is the most rich version, found in the cytoplasm of nearly all mammalian tissues; with a substrate is hydrogen peroxide. Glutathione peroxidase2 is an intestinal and extracellular enzyme, while glutathione peroxidase3 is extracellular, especially abundant in plasma. Glutathione peroxidase4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels (Muller et al, 2007). Mammalian GPx1, GPx2, GPx3, and GPx4 have been shown to be selenium-containing enzymes, whereas GPx6 is a seleno protein in humans and cysteine-containing homologues in rodents. GPx1, GPx2, and GPx3 are homo tetrameric proteins, whereas GPx4 has a monomeric structure. As the reliability of the cellular and sub cellular membranes depend heavily on glutathione peroxidase, the antioxidative defensive system of glutathione peroxidase itself depends greatly on the presence of selenium (Ran et al, 2007).
The biochemical property of glutathione peroxidase is to decrease lipid hydroperoxides to their analogous alcohols and to reduce free hydrogen peroxide to water. The main reaction that glutathione peroxidase catalyzes is:

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS–SG} + 2\text{H}_2\text{O}
\]

Wherever GSH stands for reduced monomeric glutathione and GS–SG correspond to glutathione disulfide, \( \text{H}_2\text{O}_2 \) stand for hydrogen peroxide and \( \text{H}_2\text{O} \) shows water. Glutathione reductase then reduces the oxidized glutathione to complete the cycle (Muller et al, 2007)

\[
\text{GS–SG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

Lack of Glutathione peroxidase1 (GPx1) develop atherosclerosis among diabetics. GPx1 play a key role in antioxidant defense and the development of Diabetes Mellitus and associated atherosclerosis. Glutathione peroxidase is a comparatively steady enzyme, except it could be inactivated in conditions of severe oxidative stress. Inactivation of the enzyme might occur throughout glycation directed by established glucose concentration (Lewis et al, 2007).

**1.2.5.1.2. Superoxide-Dismutase**

Superoxide dismutases (SODs) are enzymes that function to catalytically convert \( \text{O}_2^- \) to oxygen (\( \text{O}_2 \)) and hydrogen peroxide (\( \text{H}_2\text{O}_2 \)). It is an important antioxidant defense in nearly all cells exposed to oxygen (Fridovich, 1995; Archibald and Fridovich, 1982). SOD was discovered by Irwin Fridovich and Joe McCord in 1968, which were known earlier as several metalloproteins with unknown function. A number of common types of SOD be present they are proteins co-factored with copper and zinc, or manganese, iron, or nickel (Muller, et al, 2006). Superoxide is one of the most important reactive oxygen species in the cell that serves as a key antioxidant. In humans, three types of superoxide dismutase are present. SOD1 is situated in the cytoplasm, SOD2 is in the mitochondria and SOD3 is extracellular has recently been explained contains copper (Cu-SOD). Superoxide dismutase protects the cell from superoxide toxicity (Groner, 1994).

A study shows that significant decrease in Cu, Zn- SOD activity in type 2 diabetes mellitus (Gawloski et al, 2009). Sundaram et al recognized that excessive lipid peroxidation is associated with reduced Cu, Zn- SOD in type 2 diabetes patients (Sundaram et al, 1996).

The rapid weaken of the endothelium-dependent responses in diabetic vessels was radically suppressed by pretreatment with superoxide dismutase. Pretreatment with dimethyl sulfoxide, deferoxamine mesylate, allopurinol, or indomethacin did not prevent the rapid fade of the endothelium dependent relaxation. The endothelium independent relaxation induced by nitric oxide also faded more quickly in diabetic vessels; this injury was less prominent in the
presence of superoxide dismutase. The provisional nature of the endothelium dependent relaxation is more noticeable in diabetic rat aorta as a consequence of an improved accretion of superoxide anion (Hatori et al, 1991).

Diabetes associated decrease in antioxidant enzymes activity can be improved by insulin and antioxidant therapy. Insulin therapy generally prevents weight reducing and regularized the activities and protein expression of all antioxidant enzymes. Antioxidant remedy in the diabetic rats increased the expression Cu Zn SOD and GPX protein. Collective therapy with insulin and antioxidants normalized every considered antioxidant enzyme protein expression and activities (Ram et al, 2004). Manganese superoxide dismutase can be implicated in the pathogenesis of retinopathy by protecting the retina from increased oxidative damage experienced in diabetic situations (Renu et al, 2006).

Higher circulating Cu/Zn superoxide dismutase might defend insulin dependent diabetes mellitus in children and young person’s against endothelial dysfunction (Bert et al, 2007).

1.2.5.1.3. Catalase

Catalase is an enzyme which is present in almost all living things that are exposed to oxygen, wherever it performs functions to catalyze the breakdown of hydrogen peroxide to water and oxygen. Catalase can transfer thousands of molecules of hydrogen peroxide to water and oxygen per second (Chelikani et al, 2004).

In 1818 Louis Jacques Thenard was first noticed catalase as a ‘substance’ who discovered H$_2$O$_2$, suggested that the breakdown of H$_2$O$_2$ is caused by a catalase. In 1900, Oscar Loew was the first to provide it the name catalase, and establish its presence in many plants and animals (Loew, 1900). Hydrogen peroxide has been reported to damage pancreatic β-cells and reduce insulin signaling (Pomytkin and Kolesova, 2003). Catalase is commonly used by cells to decompose hydrogen peroxide into less reactive gaseous oxygen and water molecules (Chelikani et al, 2004).

Several works demonstrated that, an increased level of hydrogen peroxide, because of decreased catalase activity, can contribute to oxidative damage of pancreatic β cells, to reduced insulin secretion and insulin efficiency, and to the beginning of diabetes. Alteration of catalase synthesis might be responsible for reduced blood catalase in gestational diabetes and its change in the second and third trimester (Góth et al, 2005; Chistiakov, 2004).

1.2.5.2. Exogenous Non Enzymatic Antioxidants

Exogenous non enzymatic antioxidants are gained from diet by consumption antioxidant rich foods and by taking supplements. Several renowned examples of exogenous antioxidants
are vitamins A, ascorbic acid and vitamins E. although exogenous antioxidants can be obtained from food sources, in our contemporary day world it is almost impossible to get adequate exogenous antioxidants from our diet to deactivate all of the free radicals produced; hence antioxidant supplementation is so essential.

1.2.5.2.1. Vitamin C

Vitamin C or ascorbic acid is a water soluble antioxidant that can diminish radicals from a range of sources. It is a monosaccharide redox catalyst present in both animals and all higher plants. This redox catalyst is able to decrease, and neutralize, reactive oxygen species such as hydrogen peroxide. Mainly other animals are capable to produce ascorbic acid in their bodies and do not need it in their diets (Linster et al, 2007). Besides to its straightforward antioxidant effects, it is also a substrate for the redox enzyme ascorbate peroxidase, a function that is mainly significant in stress resistance in plants (Padayatty et al, 2003). Water soluble antioxidants like ascorbic acid and glutathione scavenge reactive oxygen species in fluid outer the cell and inside the cell. In eye, ascorbic acid has been exposed to decrease lipid peroxide damage. (Veach, 2004). Vitamin C is structurally analogous to glucose and can replace it in several chemical reactions, and therefore is efficient in protection of non-enzymatic glycosylation of proteins (Afkhami et al, 2003)

Ascorbic acid was found to account for 65-100% of the antioxidant capacity of liquor obtained from citrus fruits but fewer than 5% in apple and pineapple fruit drink (Gardner et al., 2000). High doses of ascorbic acid (2 g/day) have been shown to recover blood glucose regulation and decrease serum cholesterol and triglyceride in type 2 diabetes patients (Errikson and Kahvakka, 1997).

There is an opposite relationship between plasma ascorbic acid and glycated haemoglobin levels, in such a way that mean plasma vitamin C was significantly higher in individuals with glycated haemoglobin less than 7 percent than in individuals with self reported or prevalent undiagnosed hyperglycaemia (HbA1c > 7%) (Sargeant et al, 2000).

1.2.5.2.2. Tocopherol (Vitamin E)

Vitamin E is the collective name for a group of eight allied tocopherols and tocotrienols, this are fat soluble vitamins with antioxidant potential (Herrera and Barbas, 2001). Amoung these, α tocopherol have been most considered as it has the maximum bioavailability, with the body advantageous absorbing and metabolizing this variety (Brigelius and Traber, 1999). Vitamin E, a lipid dissolved antioxidant is widely distributed in wheat germ, sunflower seed, safflower seed, corn and soyabean. It has been claimed that the α-tocopherol form is the most significant chain breaking lipid dissolved antioxidant, and that it protects membranes from
oxidation by reacting with lipid free radicals formed in the lipid peroxidation chain reaction (Herrera and Barbas, 2001).

The free radicals are frequently removed or inhibited in vivo by a team of antioxidants Vitamins. The most excellent improvements to the epidemiology of the effects of dietary supplementation with vitamin E have been studied broadly, which demonstrated that a 40% decrease in coronary heart disease is seen in those persons who supplemented their diet with vitamin E ingestion (Bansilal et al, 2007).

This is aligned with conclusion showing that α tocopherol conveniently protects glutathione peroxidase 4(GPX4) poor cells from cell decease. Glutathione peroxidase 4 is the only known enzyme that efficiently decreases lipid hydroperoxides inside biological membranes (Seiler et al, 2008). Vitamin E, ascorbic acid and Glutathione are the three prime antioxidants in metabolism relevant to glaucoma (Veach, 2004). Vitamin E intake was extensively associated with a reduced risk of type 2 diabetes. Intake of alpha tocopherol, gamma tocopherol, and delta tocopherol and beta tocotrienol was inversely related to a risk of type 2 diabetes. It shows that progress of type 2 diabetes may be decreased by the intake of antioxidants in the diet (Montonen et al, 2004).

1.2.5.2.3. Vitamin A

Vitamin A is a group of unsaturated dietary organic complexes, that contains retinol, retinal, retinoic acid (RA), and many provitamin A carotenoids, among which beta carotene is the most significant (Fennema, 2008). It is a fat soluble antioxidant, which is necessary for growth, maintenance of visual function, reproduction and segregation of epithelial tissue (Tanumihardjo, 2011).

High levels of vitamin A may control the onset of type 1 diabetes by defensive against the attack of insulin-producing beta cells. Therefore dietary involvement with foods or food constituents may prove to be advantageous in the prevention and/or management of insulin-dependent diabetes. Rising polyphenol or vitamin A quantities in the diet might have deep effects on suppressing inflammatory immune cells and decreasing the oxidative destruction in the islets that contributes to loss of beta cells (Zunino et al, 2007). Recent studies have shown that plasma concentrations of vitamin A (retinol) and its carrier proteins, retinol-binding protein (RBP), and transthyretin (TTR), are decreased in human subjects with insulin-dependent (IDDM) but not with noninsulin dependent diabetes mellitus (NIDDM) (Basu and Basualdo 1997).
1.2.5.2.4. Carotenoids

Carotenoids are widely distributed fat soluble natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. There are numerous carotenoids in the food that we consume, and nearly all of these carotenoids have antioxidant properties. Beta carotene is the most widespread carotenoid in fruits and vegetables (Paiva and Russell, 1999). These precursors to vitamin A are occasionally called provitamin A. Beta carotene is the most important carotenoid for adequate vitamin A intake because it yields more vitamin A than alpha or gamma carotene. People taking diets rich in carotenoids from natural foods, for example fruits and vegetables, are healthier and have lower death from many chronic diseases (Diplock et al, 1998). Partially, the advantageous effects of carotenoids are thought to be due to their function as antioxidants. Beta carotene might have additional benefits due its capability to be converted to vitamin A.

1.3 HERBS

Herbs are defined in several ways depending on the context, which the world is used. In the field of medicine, they are most accurately defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health. Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluidextracts, extracts, or the like, are known as phytomedicinals. Herbs are used as medicine by about 80% of the world population, mainly in the developing countries, for primary healthcare because of better cultural acceptability, better compatibility with the human body and lesser side effects. India is one of the countries in the world today where ancient system of medicine, such as Ayurveda, Siddha, Unani, Tribal medicine and Naturopathy have been in practice for several years (Pushpangadan, 1995, Ahmedullah and Nayar, 1999).

Medicinal plants are not only used for primary health care and not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used (Bent and Ko, 2004). In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses in 2004 (Kamboj, 2000). Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine (Dubey and Tripathi, 2004).
Medicinal Plants in India

Medicinal plants are a source of great economic value in the Indian subcontinent (Parekh et al, 2005) and India has one of the richest plant medical traditions in the world (Shankar, 2012). India has a tradition that is of remarkable contemporary relevance for ensuring health security to the millions. Nature has bestowed on India a rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity (Parekh et al, 2005). India has 2.4% of world’s area with 8% of global biodiversity. It is one of the 12 mega-diversity hot-spot regions of the world. There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. It is difficult to estimate the number of medicinal and aromatic plants present worldwide; the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient and 20-44% of all the plants found in India are used for medicinal purpose (Parekh et al, 2005).

1.3.2. Herbal Medicine and Their Potential to Treat Diabetes Mellitus

Long before the birth of orthodox Western medicine, medicinal herbs were applied to treat a wide range of disease categories (Basch et al, 2003). Due to emphasis on scientism and other complicated reasons, Western medicine now prevails over “traditional” forms of medicine including herbal medicine systems. The use of a medicinal herb, alone or in combination with other herbs, can be thought of as a type of combination therapy because of the complexity of the phytochemicals and bioactivities in the plant. Thus, a single antidiabetic herb with thousands of phytochemicals may have multiple benefits by targeting several metabolic pathways and essentially “killing several birds with one stone.” One study supported this principle by demonstrating that a combination therapy of orthodox medicine and herbal medicine exhibited a better (synergistic) effect than either medicine alone (Kaur et al, 2012). Therefore, herbal medicine can complement orthodox therapy in diabetes mellitus and provides hope for a cure.

Medicinal herbs have never become obsolete and still play a prominent role in human health care. Among them, over 1200 plants have been claimed to be remedies for diabetes (Marles and Farnsworth, 1995; Habeck, 2003). Over 400 plants as well as 700 recipes and compounds have been scientifically evaluated for T2D treatment (Singh et al, 2011). Metformin was developed based on a biguanide compound from the antidiabetic herb, French lilac, and is now a first-line drug for T2D (Oubré et al, 1997). Medicinal herbs contain diverse bioactive compounds and can have multiple actions on insulin action, insulin production, or
both. Ayurvedic preparations remarkably effective in controlling blood glucose levels. This was particularly true of case 2, for which the HbA1c decreased from 8.3 to 6.9% (Kulambil et al, 2009).

A huge number of plants have been used for the treatment of diabetes all over the world. In fact, in many parts of the world particularly in poor countries, this may be the only form of therapy available for treating diabetic patients. There are several literature reviews by different authors about anti-diabetic herbal agents, but the most informative is the one on anti-diabetic plants by Rahaman and Zaman, 1989. This review documented more than 300 plant species accepted for their hypoglycemic properties and classified according to their botanical names, country of origin, parts used and nature of active agents. Some of the plants available in India that are reported to have antidiabetic properties are *Achyranthes aspera*, *Aegle marmelos*, *Anacardium occidentale*, *Areca catechu*, * Artemesia pallens*, * Bauhinia forficata*, * Beta vulgaris*, * Calmellia sinensis*, *Cassia auriculata*, *Cassia fistula*, *Ceiba pentandra*, * Enicostemma littorale*, *Euphorbia prostrata*, *Ficus hispida*, *Ganoderma lucidum*, *Gum arabic*, *Gymnema sylvestre*, *Lepechinia caulescens*, *Memecylon umbellatum*, *Momordica charantia*, *Musa sapientum*, *Nigella sativa*, *Ocimum sanctum*, *Opuntia fukiginosa*, *Pterocarpus marsupium*, *Rhizoma polygonati*, *Salacia reticulata*, *Smallantus sonchifolius*, *Terminalia catappa*, *Tinospora cordifolia* and * Vinca rosea* (Oliver-Bever, 1986; Rahman and Zaman, 1989; Bailey and Day, 1989; Ivorra et al, 1989; Nagaraju and Rao, 1990; Swanston-Flatt et al, 1990; Handa, 1991; Rastogi and Mehrotra, 1993; Marles and Farnsworth, 1995; Rai, 1995; Rajathi and Daisy, 2003; Jasmine and Daisy, 2004; Daisy et al, 2004a, b, 2007; Chen et al, 2005; Narendhirakannan et al, 2006; Al-Fatimi et al, 2007). Though many of the plants are reputed in the indigenous systems of medicine for their hypoglycemic activities, several are unknown to the medical community, since these remain to be scientifically established along with their active compounds (Kameswara Rao et al, 2001a; Jayakar and Suresh, 2003; Subash Babu et al, 2007).

However, the market situation is complicated as typical herbal medicinal products are replaced by dieting supplements. The issue of safety of their use is evenly stressed since dietary supplements including herbals, such as sports nutrition supplements, weight management products, special supplements etc. All these preparations are also the combination of potentially biologically active compounds that exist in these marketed products, containing structurally diverse chemicals and several of them possess inherent pharmacological activity (Dietary Suppl, 1994). Moreover, since the herbal products are coming from biological origin, they can subside the limitations arising from the use of
conventional hypoglycemics. Therefore, the present research work was emphasized on the extensive evaluation of the drugs/products from herbal source.

1.3.3. Mechanism of Action of Herbal Antidiabetics

The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal anti-diabetic could be grouped as-

- Stimulation of insulin secretion (*Teucrium polium, Allium sativum, Allium cepa, Panax ginseng*) (Pulok et al, 2006).
- Inhibition in renal glucose reabsorption (*Fraxinus excelsior*) (Eddouks and Maghrani, 2004).
- Stimulation of glycogenesis and hepatic glycolysis (*Momordica charantia*) (Miura et al., 2001).
- Protective effect on the destruction of the beta-cells (*Thea sinensis*) (Kim et al, 2003).
- Improvement of digestion and reduction of blood sugar and urea (*Aegle marmelos*) (Krishnan, 1968).
- Prevents pathological conversion of starch to glucose (*Eugenia jambolina, Pterocarpus marsupium*) (Sepha and Bose, 1956).
- Increasing the use of glucose by tissues and effect on adrenergic receptors (*Panax ginseng, Allium sativum, Allium cepa*)
- Potentiates the action of exogenously injected insulin
- Cortisol lowering activities (*Boerhaavia diffusa, Ocimum sanctum*) (Gholap and Kar, 2004).
- Inhibition of alpha-amylase (Heidari et al, 2005).
- Inhibition of β-galactocidase and α-glucocidase (Sharma and Mujumdar, 1990).
- Preventing oxidative stress that is possibly involved in pancreatic β-cell dysfunction found in diabetes (Kaneto et al, 2005).
- Regenerating and/or repairing pancreatic beta cells (Mohamed et al, 2006).

1.4. ROLE OF MEDICINAL PLANTS IN DRUG DISCOVERY

The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs, but in recent years its use has been deemphasized by many pharmaceutical companies in favor of approaches based on combinatorial chemistry and genomics, among others. However over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug substance. Natural products have played an important role throughout the world
in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Newman et al, 2000) and its importance in modern medicine has been discussed in different reviews and reports (Newman et al, 2000; Newman et al, 2003; Koehn and Carter, 2005; Paterson and Anderson, 2005; Balunas and Kinghorn, 2005; Jones et al, 2006;). In recent times, there have been increased waves of interest in the field of Research in Natural Products Chemistry. This level of interest can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural products as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products (Clark, 1996).

The R & D thrust in the pharmaceutical sector is focused on development of new innovative indigenous plant based drugs through investigation of leads from the traditional system of medicine (Patwardhan et al, 2004). The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines (Akerele, 1993).

The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more “drug-likeness and biological friendliness than totally synthetic molecules,” (Koehn and Carter, 2005) making them good candidates for drug development (Balunas and Kinghorn, 2005; Drahl et al, 2005). Analysis of the sources of new and approved drugs during the period 1981 to 2002 reveals that natural products play a highly significant role in the drug discovery and development process (Jones et al, 2006). Despite competition from other drug discovery methods, Natural Products are still providing their fair share of new clinical candidates and drugs and there is rapidly evolving recognition that a significant number of natural product drugs/leads are actually produced by microbes and/or microbial interactions with the “host from where it was isolated”, and therefore this area of natural product research should be expanded significantly (Newman and Cragg, 2007).

It is often noted that 25% of all drugs prescribed today come from plants (Farnsworth and Morris, 1976; Raskin and Ripoll, 2004). This estimate suggests that plant-derived drugs
make up a significant segment of natural product–based pharmaceuticals. Out of many families of secondary metabolites, or compounds on which the growth of a plant is not dependent, nitrogen-containing alkaloids have contributed the largest number of drugs to the modern pharmacopoeia, ranging in effects from anticholinergics (atropine) to analgesics (opium alkaloids) and from antiparasitics (quine) to anticholinesterases (galantamine) to antineoplastics (vinblastine/vincristine) (Raskin et al., 2002). Although not as plentiful as alkaloids in the modern pharmacopoeia, terpenoids (including steroids) have made an equally important contribution to human health. They range from Na⁺/K⁺ pump-inhibiting cardiac glycosides from Digitalis spp. (Dewick, 2001), to antineoplastic paclitaxel (Cragg, 1998) to antimalarial artemisinin (Abdin et al., 2003), to anti-inflammatory triptolide (Goldbach-Mansky et al., 2006; Kupchan et al., 1972).

Various plant originated compounds have been used as medicine, either in their basic form or semi-synthetic form. Plant secondary metabolites are able to serve as drug precursors, drug model, and pharmacological investigates. So, computer modeling can be used to predict the pharmacological profile, ADME and toxicological properties of natural products easily (Ntie-Kang et al., 2013). New molecular structures identified from natural sources can be suitably modified to get designer molecules for drug designing. Testing of pharmacological properties and derivation of newer compounds on basis of these natural products by use of combinatorial chemistry and in silico screening or docking is helpful in development of series of similar but homologous structural compounds. Uses of molecular docking in drug discovery are multi-dimensional;

(1) To get new lead compounds for potential agents from plant-derived natural products as, and/or modification of the compounds to find more potent agents,

(2) Being multi-target computational tool, the predicted bioactivity can be extrapolated to prioritize the targets for experimental studies (Singh and Sharma, 2011).

1.5. DOCKING STUDIES

In-silico approaches have gained enormous popularity and have become an essential part of the industrial and academic research, directing drug design and discovery (Colmenarejo, 2005; Jennings and Tennant, 2005; Kalyanaraman et al., 2005; Wang and Richards, 2005). The first and prime task in any of the rational approaches for a given disease is to assess the diverse metabolic pathways and select the potential biological target (Mitcheson et al., 2000; Walters and Namchuk, 2003). Transforming ligands into active compounds with non-promiscuous binding behavior, recognized as hits; and then refining them into a structure or series of structures with relevant biological and drug-like activity,
known as leads; are the key starting points for drug discovery programs (Pickett et al, 2000; Kenakin, 2003).

Computational tools, which delineate the strength of interaction between a variety of ligands and targets in combination with good graphic three-dimensional visualization, are growing into important technologies to pick up lead molecules from databases (Joseph-McCarthy, 1999; Alvarez and Shoichet, 2006)

Virtual screening has reached a status of a dynamic and lucrative technology in probing for novel drug-like compounds or so called hits in the pharmaceutical industry (Shoichet, 2004). Various physiochemical descriptors, e.g. PSA, predicted pharmacokinetic properties (ADME), passive transcellular permeability in the intestine or in the brain, are used to filter out these compounds that do not meet the user-defined criteria. Docking and scoring would then be applied only on these compounds that meet these filtering criteria (Tudor, 2002).

Based on the structure-permeability paradigm, the Lipinski rule of five have become a standard property filtering protocol. Using a simplified, yet efficient version of the 3D-QSAR (Quantitative Structure-Activity Relationship) paradigm for structure-permeability, as suggested by Van de Waterbeemde et al,1996, Chris Lipinski and co-workers have concluded that poor absorption or permeation are more likely to occur when

- The molecular weight (MW) is over 500;
- The calculated octanol-water partition coefficient (CLOGP) is over 5, and
- There are more than 5 H-bond (hydrogen bond) donors (HDO-expressed as the sum of O-H and N-H groups);
- There are more than 10 H-bond acceptors (HAC-expressed as the sum of N and O atoms)

The “rule of five” (RO5) probability scheme had a major impact in the pharmaceutical industry, as most managers realized that due to its simplicity, it could be implemented early on in drug discovery, and potentially increase the chance of success in drug discovery projects (Tudor, 2002).

Lipophilicity is a two-edged sword. Many other drug parameters are affected by lipophilicity. High lipophilicity frequently leads to compounds with high rapid metabolic turnover and low solubility and poor absorption. As lipophilicity (LogP) increases, there is an increased probability of binding to hydrophobic protein targets other than the desired one, and therefore, there is more potential for toxicity (Vande Waterbeemd et al, 2001).

The process, which brings and binds the two molecular structures together, is called docking. Docking has been acknowledged with significant attention among all the virtual screening methods.Docking has been a capable choice for the modelling of three-dimensional structure of the receptor-ligand complex and evaluating the stability of the complex that
determines the specific biological recognition (Koehler and Vilar, 2000; Kellenberger et al, 2004; Verdonk et al, 2004).

The docking problem can be subdivided into two steps:

- Exploring the conformational space of ligands that bind to target molecules.
- Scoring this set, i.e. ranking it in accordance to the estimated binding affinity.

That is a confirmation of ligand is typically generated, and with the help of scoring function compared to the earlier conformations. The current conformation is then accepted or rejected on the basis of the score for that respective conformation. Then again a new conformation is generated, and the search process comes to an endpoint. Thus, searching and scoring can be tightly coupled in docking (Shoichet et al, 2002).