1.1. DIABETES MELLITUS

Diabetes Mellitus, one of the oldest and most prevalent chronic non-communicable disease, is a serious, costly and heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (Das et al, 1996) and is the fourth or fifth leading cause of death in most developed countries with the substantial evidence to be epidemic in many developing and newly industrialized countries, affecting about 25% of the population (Cline et al, 1991). This can also be characterized by a state of chronic hyperglycemia (peripheral insulin resistance), glucosuria, polyurea, polydipsia (excessive thirst), polyphagia (constant hunger), sudden weight loss, ketoacidosis, and ketonuria (urinary ketones) etc. (Joslin, 1928; Ronald, 1994). The name Diabetes Mellitus, corresponds to the group of disorders characterized by absent or absolute/relative deficient insulin secretion (Dhawan et al, 1996). The increased glucose level tends to glucose auto-oxidation and auto-oxidative glycosylation of proteins, which leads to oxidative stress, contributes to the development and progression of diabetes along with the various secondary complications (Baynes, 1991, Ceriello, 2003). Diabetes mellitus is an independent risk factor for the development of complications such as; retinopathy, nephropathy, neuropathy, amputation, coronary artery disease, myocardial infraction, stroke, hypertension and hyperlipidemia (UKPDS, 1998; ADA, 2000). The management of Diabetes Mellitus is considered as a global problem whose successful treatment is yet to be discovered. Insulin is widely accepted as an ideal choice for treatment of diabetes mellitus but the difficulty of repeated administration led to the search for the hypoglycemic agents. The modern drugs including insulin and oral hypoglycemic agents are known to control the blood sugar level as long as they are regularly administered (Upadhyay et al, 1996; Reynolds, 1997).

Indians get diabetes much earlier i.e. at an average of 35 years. About 25-30 million Indians suffer from diabetes, almost half of which are aware of the disease. However, urban population has a much higher incidence of diabetes than its rural counterparts. People leading sedentary lifestyle and consuming high cholesterol diet, who are generally obese or with a family history of diabetes are more prone to diabetes. Till date medical science cannot claim that it knows all that needs to be known about this disease, including the management. This is the main reason for the persistent interest all over the world to explore alternative remedies from the so called “Alternative systems” of medicine (Satyavati et al, 1989). Treatment of diabetes has been attempted with different indigenous plants and polyherbal formulations (Upadhyay et al, 1996; Chaurasia et al, 1994; Joy and Kottam, 1998). Many herbal products
have been described for the care of Diabetes Mellitus in ancient literature of Ayurveda in India (Gupta, 1994; Shukla et al, 2000).

1.1.1. Prevalence and incidence

Diabetes is generally accepted as a major challenging health problem all over the world (Rahimi et al, 2005) and especially in the developing countries. India has the dubious distinction of being home to one in five persons with diabetes world. The World Health Organization (WHO) predicts that the number of people with diabetes is to double in the next couple of decades and that the major burnt of this will be borne by the developing countries (Wild et al, 2004). In long run; it will appear as a pandemic from the stage of an epidemic in India. Globally, the burden of type-II diabetes is rising fast. Approximately 250 million people suffer from the condition worldwide (IDF, 2006). By the year 2015, the total number of people with DM is projected to reach 239 millions (McCarty, 1994). The global prevalence of diabetes among adults was estimated to be 150 million in 1995, and this is projected to increase up to 300 million by 2025 (Cooper et al 1997). Regions with greatest potential are Asia and Africa, where DM rates could rise to 2 to 3 folds than the present rates. Factors responsible for the rise in diabetes include unhealthy diet, overweight/obesity and physical inactivity (WHO, 2004). The World Health Organization (WHO) estimates that 41% of the global populations are insufficiently active, and up to 60% fail to achieve the recommended 30 minutes of moderate activity for most days of the week (WHO, 2004). The International Diabetes Federation has reported that the annual healthcare cost of diabetes globally for adults (20–79 years old) is 153 billion international dollars. This value is expected to increase to 396 billion international dollars by 2025 (Amoah, 2003).

1.1.2. Different forms of Diabetes Mellitus (Joel et al, 1996)

1.1.2.1. General – genetic and other factors not precisely defined

Two major types of clinical syndromes due to increased hyperglycemia can be mentioned here. The first one; Type–I Diabetes mellitus (formerly called insulin dependent diabetes mellitus, or IDDM) is characterized by insulin dependence and onset in early age, with weight loss and ketonuria (passing of ketone in urine). This is also recognized in two ways: Autoimmune type-I diabetes mellitus (type IA) and Non-autoimmune or idiopathic type-I diabetes mellitus (type IB). The type-I diabetes or insulin dependent diabetes mellitus is prevalent in south East Asia including India as in the west. Instead, young people in India have malnutrition related diabetes mellitus (MRDM), which can be efficiently controlled or reversed by proper and balanced diet.
The second; **Type–II diabetes mellitus** (formerly called non-insulin dependent diabetes mellitus or NIDDM) is characterized by late onset, insensitive to insulin and partial insulin deficiency. The prevalence of diabetes, especially NIDDM, is spiraling upwards, both in developed and developing countries.

1.1.2.1.1. **Type-I diabetes (IDDM) – Lack of Insulin Production by Beta Cells of the Pancreas** (Guyton and Hall, 2006)

Injury to the beta cells of the pancreas or diseases that impair insulin production can lead to Type-I diabetes. Viral infections or autoimmune disorders may be involved in the destruction of beta cells in many patients with Type-I diabetes, although heredity also plays a major role in determining the susceptibility of the beta cells to destruction by these insults. Type-I diabetes may develop very abruptly, over a period of a few days or weeks, with three principal sequels: (1) increased blood glucose, (2) Increased utilization of fats for energy and for formation of cholesterol by the liver and (3) depletion of the body’s proteins.

Blood Glucose Concentration rises to very high levels in diabetes mellitus due to the lack of peripheral glucose uptake. Increased blood glucose causes loss of glucose in urine and dehydration. In addition to the direct cellular dehydrating effect of excessive glucose, the loss of glucose in the urine causes osmotic diuresis. Thus polyurea (excessive urine excretion), intracellular and extracellular dehydration, and increased thirst are classic symptoms of diabetes. Chronic high glucose concentration causes tissue injury. This in turn leads to increased risk for heart attack, stroke, end-stage kidney disease, retinopathy and blindness and ischaemia and gangrene of the limbs. Chronic high glucose concentration also causes damage to many other tissues. For example, peripheral neuropathy, which is abnormal function of peripheral nerves and autonomic nervous system dysfunction are frequent complications of chronic, uncontrolled diabetes mellitus. These abnormalities can result in impaired cardiovascular reflexes, impaired bladder control, decreased sensation in the extremities and other symptoms or peripheral nerve damage. In addition hypertension, secondary to renal injury, and artherosclerosis, secondary to abnormal lipid metabolism, often develop in patients with diabetes and amplify the tissue damage caused by the elevated glucose.

Diabetes Mellitus causes increased utilization of Fats and Metabolic Acidosis. The shift from carbohydrate to fat metabolism in diabetes increases the release of keto acids, such as acetoacetic acid and β-hydroxybutyric acid, into the plasma tissue cells. As a result, the patient develops severe metabolic acidosis. This leads rapidly than they can be taken up and
oxidized by the tissue cells. As a result, the patient develops severe metabolic acidosis from the excess keto acids, which, in association with dehydration due to the excessive urine formation, can cause severe acidosis. This leads rapidly to diabetic coma and death. Diabetes causes depletion of the body’s proteins. Therefore a person with severe untreated diabetes mellitus suffers rapid weight loss and asthenia (lack of energy) despite eating large amounts of food (polyphagia).

1.1.2.1.2. NIDDM Type-II diabetes - Resistance to the metabolic effects of insulin (Guyton and Hall, 2006)

Type-II diabetes is caused by greatly diminished sensitivity of target tissues to the metabolic effects of insulin, a condition referred to as insulin resistance. This syndrome, like Type-I diabetes, is associated with multiple metabolic abnormalities, although high levels of keto acids are usually not present in Type-II diabetes. Type-II diabetes is far more common than Type-I, accounting for 80 to 90 per cent of all cases of diabetes. In most cases, the onset of Type-II diabetes occurs after age 40, often between the ages of 50 to 60 years, and the disease develops gradually. Therefore, this syndrome is often referred to as adult-onset diabetes. Plasma insulin is elevated in Type-II diabetes.

Type-II diabetes in contrast to Type-I, is associated with increased plasma insulin concentration. This occurs as a compensatory response by the pancreatic beta cells for the decrease in carbohydrate utilization and storage and the resultant increase in blood glucose. However, even the increased levels of insulin are not sufficient to maintain normal glucose regulation because of the greatly diminished insulin sensitivity of the peripheral tissues. As a result, mild hyperglycemia occurs after ingestion of carbohydrates in the early stages of the disease. In the later stages of Type-II diabetes, the pancreatic beta cells become exhausted and are unable to produce enough insulin to prevent more severe hyperglycemia, especially after the person ingests a carbohydrate rich meal.

1.1.2.2. Specific defined gene mutations

Maturity onset diabetes of youth (MODY): MODY 1-hepatic nuclear factor 4α gene mutations, MODY 2-glucokinase gene mutations, MODY 3-hepatic nuclear factors for gene mutations, MODY 4-pancreatic determining factor x gene mutations, MODY X-unidentified gene mutations.

Maternally Inherited Diabetes and Deafness (MIDD)
Mitochondrial Leucine tRNA Gene Mutations,
Insulin Gene Mutations and Insulin Receptor Gene Mutations.
1.1.2.3. Others

- Diabetes secondary to pancreatic diseases: Chronic pancreatitis, Surgery, Tropical diabetes (chronic pancreas associated with nutritional and/or factors).
- Diabetes secondary to other endocrinopathies: Cushing’s disease, Glucocorticoid administration, Acromegaly.
- Diabetes secondary to immune suppression.
- Diabetes associated with genetic syndromes: e.g. Prader – Willi syndrome.
- Diabetes associated with drug therapy.

1.1.3. Factors that induce Diabetes Mellitus

1.1.3.1. General Factors

The underlying cause of diabetes is insulin deficiency, which is absolute in IDDM and partial in NIDDM. This may be due to wide variety of mechanism.

a) Pancreatic disorders – Inflammatory, neoplastic and other disorders such as cystic fibrosis.

b) Defects in the formation of insulin, e.g., synthesis of abnormal biologically less active insulin molecule.

c) Destruction of beta cells, e.g., viral infection and chemical agents.

d) Decreased insulin sensitivity, due to adipocyte and monocyte insulin receptors.

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

f) Auto-immunity.

1.1.3.2. Host Factors

a) Age: Although diabetes may occur at any age, surveys indicate that prevalence rises steeply with age. NIDDM usually appears in the middle age of life. Malnutrition related diabetes affects large number of young people.

b) Sex: In some countries the overall male-female ratio is about equal but in Southeast Asia, an excess of male diabetic is observed (WHO, 1980).

1.1.3.3. Genetic factors

The genetic nature of diabetes is undisputed. Reports showed that in identical twins, WHO revealed NIDDM concordance was about 90 percent (WHO, 1985), thus demonstrating a strong genetic component and IDDM concordance was approximately 50 percent indicating that IDDM is not totally a genetic entity.
b) **Genetic markers:** IDDM is associated with specific human leukocyte antigen (HLA) B8 and B15, and more powerfully with HLA-DR3 and HLA-DR4. Individuals carry the highest risk with both DR3 and DR4. On the other hand, NIDDM is not HLA associated (Todd et al, 1987).

b) **Immune mechanisms:** There is some evidence of both cell-mediated and humoral activity against Islet cells. Some people appear to have defective immunological mechanisms and under the influence of some environmental trigger, attack their own insulin producing cells.

c) **Obesity:** Obesity has long been accepted as a risk factor for NIDDM and the risk is related with both the duration and degree of obesity (WHO, 1985). In some instances, obesity reduces the number of insulin receptors on target cells, but in most cases, it produces resistance to the action of insulin. However, many obese subjects are not diabetic. 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.

1.1.3.4. Environmental Risk Factors

Number of environmental factors act on genetically susceptible individuals. They include:

a) **Sedentary lifestyle:** Sedentary lifestyle appears to be an important risk factor for the development of NIDDM. Lack of exercise may alter the interaction between insulin and its receptors and subsequently leads to NIDDM.

b) **Diet:** Studies indicated that the diet of diabetics did not appear to differ in any marked way from that of non-diabetics except in quantity (ARC/MRC, 1974). There is no sound evidence that diabetes is specially associated with high intake of any of the major nutrients (WHO, 1985).

c) **Malnutrition:** Malnutrition in early infancy and childhood may result in partial failure of beta-cell function. Damage to beta cell may well explain the associated impaired carbohydrate tolerance in kwashiorkor. Excessive intake of alcohol may increase the risk of diabetes by damaging the pancreas and liver and by promoting the obesity.

d) **Viral Infection:** Rubella, mumps and human coxsackie virus B4 infection may trigger a sequence of events resulting in beta cell destruction in immuno-genetically susceptible people.
**e) Chemical agents:** A number of chemical agents are known to be toxic to beta cells e.g. alloxan, streptozocin, the rodenticide VALCOR etc, a high intake of cyanide producing foods e.g. Cassava and certain beans may also have toxic effects on beta cells (Arky, 1983).

**f) Stress:** Surgery, trauma and stress of situations, internal or external may “bring out” the disease.

**g) Other factors:** Number of social factors such as occupation, marital status, religion, economic status, changes in life style, etc. also indirectly related with the diabetes.

**1.1.4. Complications**

Complications resulting from diabetes mellitus are the third leading cause of death attributable to disease. Diabetes mellitus can affect nearly every organ system of the body. This is the most frequent cause of blindness among working age adults. The principal complications of Diabetes Mellitus are retinopathy, neuropathy, angiopathy, nephropathy and susceptibility to infect Hyperglycemic Hyperosmolar Nonketotic Coma (HHNC). With the single exception of HHNC, these diabetic complications occur more frequently for type I diabetes than for type II diabetes. The major factor behind these complications is the poor control of hyperglycemia in diabetes. Diabetes mellitus and hypertension are the common conditions that frequently coexist (Sowers and Zemel, 1990) or are multifactored (Reaven, 1988). Approximately 80 percent of the diabetic patients are hypertensive whereas 5-25 percent of hypertensive individuals are diabetic (Stamler et al, 1993). Hypertension is more prevalent among diabetics than non-diabetics and is reported to aggravate the cardiovascular symptoms of diabetes (Barrett Connor et al, 1981). It contributes to increase in morbidity and mortality due to coronary artery diseases and End Stage Renal Disease (ESRD) in diabetics.

Virtually every major organ system in the body is damaged by diabetes such as:

**1.1.4.1. Short term complications:**

Hyperglycemia, Diabetic ketoacidosis (DKA), Hyperosmolar Non-ketotic (HONK) state, Lactic Acidosis, Infections etc.

**1.1.4.2. Long Term Complications:**

Eye disorders, Diabetic retinopathy, Cataracts, Diabetic neuropathy, Macro vascular Disease, Heart disease, High blood pressure, Stroke, Kidney Disease (End stage renal disease), Feet problem and Amputation of extremities, Early loss of
teeth, Impotence, Pregnancy complication, Congenital malformations in babies, Death of the newborn etc.

1.1.4.3. Hyperglycemia:

When blood sugar level rises above the normal (>15 mmol/l) then it is called Hyperglycemia (Joel et al, 1996). The symptoms are; Fatigue, Headaches, Nausea, Extreme thirst, Frequent urination, Blurry vision, Dry mouth and skin, Unexplained weight loss.

1.1.4.4. Diabetic Ketoacidosis

Diabetes Ketoacidosis (Siprestein, 1992) is a metabolic abnormality characterized by hyperglycemia (blood glucose 14 mmol/L) and metabolic acidosis (arterial pH below 7.25 and/or plasma bicarbonate less than 16 mmol/L) due to hyperketonemia (plasma acetoacetate + β-hydroxybutyrate above 7mmol/L). It causes with depression of sensorium to at least a state of obtundation. The disorder is most common among juvenile or growth onset diabetic patients (type 1 diabetes). The major clinical manifestations of diabetic ketoacidosis are depression of sensorium and hyperventilation. Patients with diabetic ketoacidosis generally present with a history of polydipsia, polyuria, malaise, weakness and increasing lethargy.

With insulin lack, there is a shift to catabolism of fat, glycogen and muscle. The breakdown of these stores produces fuels that become substrates for the formation of glucose and ketones. The high blood levels of glucose, fatty acids and ketones (acetoacetic acid, β-hydroxybutyric acid and acetone), along with metabolic acidosis and ketonuria are the hallmarks of diabetic ketoacidosis. The hyperglycemia leads to an osmotic diuresis, with polyuria and loss in the urine of sodium, potassium and phosphate. Initially, there is a low plasma sodium and phosphate, with high potassium. The high potassium is largely due to breakdown of skeletal muscle and liberation of potassium from intracellular stores, with a small contribution from potassium / hydrogen exchange. The initial low plasma sodium is secondary to an osmotic shift of intracellular water due to the extracellular osmotic contribution of glucose. A number of potential mechanisms have been postulated to contribute to depression of sensorium in patient with diabetic ketoacidosis. These include a) ketonemia, b) metabolic acidosis, c) hyperosmolality, d) cerebral hypoxia, e) associated medical conditions such as sepsis, myocardial infraction and pneumonia.

1.1.4.5. Hyperosmolar Nonketotic State (Coma)

Diabetic ketoacidosis and Nonketotic coma actually represent continuous biochemical characteristics of both. However, in most patients, the predominant problem is either
hyperosmolality with dehydration or metabolic acidemia. Nonketotic hyperosmolar coma with hyperglycemia (Nonketotic coma) is characterized by hyperglycemia (plasma glucose above 34 mmol/L) and hyperosmolality (plasma osmolality above 350 mOsm/kg) without significant ketonemia (plasma acetate below 2+ at a 1:1 dilution) with depression of sensorium to at least a stupor. It causes aphasia, homonymous hemianopsia, hemisensory defects, hemiparesis, hyperreflexia and visual hallucinations. Transient hemiplegia has been observed in patients with nonketotic coma and this has usually reversed following correction of the metabolic abnormalities. A variety of autonomic nervous system changes, such as hyperpnoea and hypertension are also observed (Siprestein, 1992).

There is interplay between free fatty acid mobilization and metabolism, insulin secretion, growth hormone, dehydration and hyperosmolality. Patients with nonketotic hyperosmolar coma generally present with depression of sensorium and a history of polydipsia, polyuria, weakness and decreasing sensorium. The most prominent physical findings are related to volume depletion, such as postural hypotension and tachycardia. The mean plasma glucose is about 60 mmol/L with a range of about 33-90 mmol/L. The haematocrit, urea, creatinine and total proteins are generally increased while the plasma sodium tends to be normal.

1.1.4.6. Lactic Acidosis

Lactic acidosis is generally defined as metabolic acidosis due to the accumulation of lactic acid in the blood in excess of 5mM with an accompanying blood pH below 7.25. Unlike diabetic ketoacidosis, the plasma potassium in patients with lactic acidosis tends to be normal (Boyle, 1995).

1.1.4.7. Infections

Diabetes causes damage to the autonomic nerves that control sweat glands. This can cause overly dry skin that develops tiny cracks where bacteria and fungi can thrive, leading to infection.

1.1.4.8. Eye Disorders (Aiello et al, 1994; Beisswenger et al, 1994)

1.1.4.8.1. Diabetic Retinopathy - Diabetic retinopathy, degeneration of the retina (the layer of light sensitive cells lining the back of the eyeball), is potentially one of the more serious eye complications. More that 70 percent of diabetic patients have some degree of retinopathy after 10 years of having diabetes but most do not suffer serious loss of vision. Retinopathy most commonly affects the tiny capillaries nourishing the retina. The walls of these blood
vessels weaken and form tiny aneurysms (ballooned-out areas that may leak blood) resulting in dot or flame hemorrhages, which reduce the sharpness of vision. Eventually, some of these weakened blood vessels die, and if the retina does not get enough oxygen and other nutrients some of its tissue also dies.

In some eyes, new blood vessels over glow the retina, a condition called proliferative retinopathy. These vessels are often very fragile and can suddenly leak blood into the vitreous humor (the jelly like substance inside the eyeball), dimming vision or causing temporary blindness, which disappears when the blood is reabsorbed. Permanent loss of vision may occur, however, if scar tissue forms and damages the retina. The characteristic damaged red blood vessels or signs of leaking or blocked vessels were observed in the eye.

1.1.4.8.2. Cataracts - Diabetes can cause cataracts, loss of transparency of the eye lens or its capsule. In many substances, these cataracts are similar to those that commonly occur in older people, however, in diabetic patients; the cataracts form earlier and develop more rapidly. Cataracts result from a loss of function of the lens tissue. An eye exam reveals a gray-white film in the lens, behind the pupil.

1.1.4.9. Diabetic Neuropathy (Partanen et al, 1995)

This condition consists of nervous system complications associated with diabetes. It is not clear exactly why diabetes damages the nervous system, but high blood glucose is the likely cause.

Diabetic related disorders of the nervous system (diabetic neuropathy) cause a variety of symptoms including slowed reflexes, sexual impotence, loss of sensation, intermittent episodes of pain and exacerbation of circulatory disorders. The early signs of diabetic neuropathy include tingling sensation in the fingers and toes, feelings of muscular weakness and in a large percentage of men, impotence. The neuropathy may compound other diabetes related complications by causing a loss of sensation and diminished circulation of blood that reduces awareness of injury or infection until serious open ulcers ensue. Pain ranging from minor discomfort or tingling to severe aches is a common feature of diabetic neuropathy. Sharp stabbing pains may come and go, deep aches make sleep or normal activities difficult and very sensitive skin reacts to even a slight touch. In many instances the neuropathy expresses itself by affecting systems controlled by the nervous system. Sexual impotence, gastrointestinal problems, bladder disorders, irregular heartbeats or blood pressure abnormalities may be traced to autonomic nervous system problems.
1.1.4.10. **Macrovascular Disease** (Haffner, 1998; ADA, 1993; Haffner et al, 1998)

People with diabetes have a much higher rate of heart disease and circulatory disorders that the general population, cardiovascular complications cause about three-fourths of the deaths among diabetic patients. Risk factors for heart disease appear at an earlier age and advance more rapidly in people with diabetes. These factors include hardening of the arteries (arteriosclerosis), buildups of fatty deposits (atherosclerosis), low levels of HDL (“good”) cholesterol and elevated triglycerides and high blood pressure. As a result, people with diabetes are at increased risk of heart attack, stroke and impaired circulation involving both large and small blood vessels. Premenopausal women with diabetes, who would otherwise expect to have lower blood lipid levels and a lower incidence of heart disease than men, often have high levels of blood lipids.

The cause of cardiovascular and circulatory abnormalities is not clear, but research worker suggests that the cause is linked to abnormally high blood glucose. High blood glucose affects the chemical structure of several blood components, particularly red blood cells and perhaps the platelets, abnormalities that may play a role in the development of arteriosclerosis.

Insulin appears to increase lipid synthesis in the artery walls, which may help promote the build up of fatty deposits. Since many Type-II diabetics actually have high levels of insulin that their bodies cannot effectively utilize, some researchers believe their high insulin level may promote the arteriosclerosis by normalizing blood sugar.

Diabetes also damages the capillaries or microcirculation, which nourish the individual body cells and cause a thickening of the basement membranes, the substances that separate the epithelial cells lining the various body surfaces and the underlying structures. When the capillary basement membranes thicken, the vessels often are unable to carry adequate blood to the tissues they serve, resulting in poor circulation. The limbs are particularly vulnerable to these circulatory problems and poor circulation to the lower limbs is particularly common in diabetics, resulting in chronic skin ulcers.

**1.1.4.11. Kidney disease (Diabetic nephropathy)**

Kidney failure is the inability to adequately excrete waste products, which is another serious potential complication of long standing diabetes. Damage to the tiny blood vessels in the nephrons can eventually lead to progressive kidney failure, characterized by the excretion of protein and other nutrients in the urine. Diabetes also increases the kidney’s vulnerability
to infections. Symptoms of kidney or urinary tract infections (flank pain, difficult or burning urination, urgency to urinate, and passage of urine discolored by blood) should be promptly evaluated by a doctor. Examination of a urine specimen is necessary in most cases. (Krolewski et al, 1995; Sequist et al, 1989; Defronzo, 1995; Ohkubo et al, 1995)

1.1.4.12. Feet Problem and Amputation of Extremities (Rerber et al, 1995)

Careful attention to the feet is essential for those with diabetes. Ordinarily trivial ailments like corns; calluses, blisters, bunions, cuts or other injuries and ingrown toenails can become major problem possibly leading to serious foot infections, gangrene and amputation.

Persons those suffering from diabetes, may not perceive any pain and be unaware of the problem until it develops into an infected ulcer or other major disorder. Damage to the motor nerves, which in turn promotes a weakening and shrinking of muscles, also may promote the development of certain foot deformities, such as hammertoes or claw foot. These deformities also make the feet more vulnerable to infection that leads to amputations of lower extremities.

1.1.4.13. Psychological Effects of Diabetes

Any chronic disease particularly diabetes carries the potential for profound emotional effects on the patient, as well as family, lovers and friends. Anger, depression, anxiety and feelings of deep frustration are common reactions. Poorly controlled diabetes, with its abnormal swings from high to low blood sugar, also produces mood changes and feelings of irritability, anxiety, depression and euphoria.

1.1.4.14. Early Loss of Teeth

Diabetes causes early loss of teeth. Periodontal disease occurs with greater frequency and severity among people with diabetes. Periodontal disease has been reported to occur among 30 percent of people aged 19 years or order with Type-I diabetes.

1.1.4.15. Impotence

Impotence is a sexual dysfunction characterized by the inability to have or keep an erection. The nerve that cause an erection may be damaged by blocking of penile artery the prevalence of impotence in men with diabetes over the age of 50 years has been reported to be as high as 50-60 percent.
1.1.4.16. Pregnancy Complications

It is associated with an increase in the risk of intrauterine fetal death during the last 4-8 weeks of gestation. Although uncomplicated GDM has not been associated with increased perinatal mortality, GDM increases the risk of fetal macrosomia and neonatal morbidity including hypoglycemia, hypocalcaemia, polycythemia and jaundice. GDM is associated with an increased frequency of maternal hypertensive disorders and the need for caesarean delivery. (Metager et al, 1998; Franz et al, 1994)

1.1.5. Methods of Diagnosis:

The usual methods for diagnosis of diabetes are based on various chemical tests of the urine and the blood.

1.1.5.1. Fasting blood glucose and Insulin levels: The fasting blood glucose level in the early morning between 80 to 110mg/100ml is considered to be the normal. In Type-I diabetes plasma insulin levels are very low or undetectable during fasting and even after a meal. In Type-II diabetes, plasma insulin concentration may be several folds higher than normal and usually increases to a greater extent after ingestion of a standard glucose load during a glucose tolerance test (Guyton and Hall, 2006).

1.1.5.2. Glucose tolerance Test: When a normal fasting person ingests 500mg to 1 gram of glucose per kg of body weight, the blood glucose level rises from about 80 mg/dl to 120 to 140 mg/dl and falls back to below normal in about two hours. In a person with diabetes, the fasting blood glucose concentration is almost always above 110mg/100ml and often above 140mg/100ml. Also the glucose tolerance test is almost always abnormal. On ingestion of glucose, these people exhibit a much greater than normal rise in blood glucose level and the glucose level fails to fall below the control level demonstrate that either the normal increase in insulin secretion after glucose ingestion does not occur or there is decreased sensitivity to insulin. A diagnosis of diabetes mellitus can usually be established on the basis of this and Type-I and Type-II diabetes can be distinguished from each other by measurements of plasma insulin, with plasma insulin being low or undetectable in Type-I diabetes and increased in Type-II diabetes (Guyton and Hall, 2006).

1.1.5.3. Acetone Breath: Small quantities of aceto-acetic acid in the blood that increase greatly in severe diabetes are converted to acetone, which is volatile and vaporizes into the expired air. Consequently, one can frequently make a diagnosis of Type-I diabetes mellitus simply by smelling acetone in the breath of a patient. Also, keto-acids can be detected by
chemical means in the urine, and their quantification aids in determining the severity of the diabetes. In Type-II diabetes, however, keto-acids are usually not produced in excess amounts (Guyton and Hall, 2006).

1.1.5.4. Urinary Glucose: Simple official tests or more complicated quantitative laboratory tests may be used to determine the quantity of glucose lost in the urine. In general, a normal person losses undetectable amounts of glucose, whereas a person with diabetes losses glucose in small to large amounts, in proportion to the severity of disease and the intake of carbohydrates (Guyton and Hall, 2006).

1.1.6. Pathophysiology

1.1.6.1. Pancreatic Islet Hormones

The Islet of Langerhans is composed of four types of cells, each of which synthesizes β-cell, glucagon in the α-cell, somatostatin in the δ-cell, and pancreatic polypeptide in the PP in F-cell. The β-cells make up 60 percent to 80 percent of the Islet and from its central core. The α, β and F cells form a discontinuous mantle, one to three cells thick, around this core (Joel et al, 2001).

1.1.6.2. Insulin

Insulin was purified and crystallized by Abel within a few years of its discovery. The amino acid sequence of insulin was established by Sanger in 1960 and the elucidation of its 3-dimensional structure by Hodgkin and coworkers in 1972. Insulin was the first hormone for which a Radio Immuno-assay was developed (Yalow, 1978). The β-cells of pancreatic Islets synthesize insulin from a single chain precursor of 110 amino acids termed preproinsulin. After translocation through the membrane of the rough endoplasmic reticulum, the 24 amino acid N-terminal signal peptide of preproinsulin is rapidly cleaved off to form proinsulin, which is then converted to insulin in Golgi complex which consists two peptide chains (A and B) that contains one intra subunit and two inter subunits disulfide bonds. The A-chain consists of 21 and B-chain consists of 30 amino acid residues, thus the mol. mass is about 5734 daltons (De-Meyts, 1994).

1.1.6.2.1. Synthesis, Secretion, Distribution and Degradation

Glucose, amino acids, fatty acids and ketone bodies promote the secretion of insulin. The Islets of Langerhans are richly innervated by both adrenergic and cholinergic nerves. Stimulation of α2-adrenergic receptor agonists and vagal nerve stimulation enhance release. In general any condition that activates the autonomic nervous system suppresses the secretion
of insulin by stimulation of α2-adrenergic receptors (Porte and Halter, 1981). Glucose is the principal stimulus to insulin secretion in human beings and is an essential permissive factor for the actions of many other secretagogues (Matchinsky, 1996). The sugar is more effective in provoking insulin secretion when taken orally than when administered intravenously. This is true because the ingestion of glucose (or food) induces the release of gastrointestinal hormones and stimulates vagal activity (Malaisse, 1986; Brelje and Sorenson, 1998).

Glucose enters the beta cell by facilitated transport, which is mediated by GLUT-2, a specific subtype of glucose transporter. The sugar is then phosphorylated by glucokinase which gives it an important regulatory role at physiological concentration of glucose. The capacity of sugars to undergo phosphorylation and subsequent glycolysis correlates closely with their ability to stimulate insulin release. This fact has led to the hypothesis that one or more glycolytic intermediates or enzyme cofactors is the actual stimulator of insulin secretion (Matchinsky, 1996). The role of glucokinase as the glucose sensor was solidified by the recent association of mutations of the glucokinase gene with a form of MODY-2, a relatively uncommon form of diabetes. These mutations, which compromise the ability of glucokinase to phosphorylate glucose, raise the threshold for glucose stimulate insulin release (Gidh-Jain et al, 1993).

Insulin secretion ultimately depends on the intracellular concentration of Ca^{2+} (Wolf et al, 1988). Glucose metabolism initiated by glucokinase, results in change in the ATP/ADP ratio. This results in the inhibition of an ATP-sensitive K^+ channel and depolarization of the β - Cell. A compensatory activation of a voltage dependent Ca^{2+} channel results in the influx of Ca^{2+} ion into the β - Cell. Ca^{2+} activates phospholipase A_2 and phospholipase C, which result in the formation of arachidonic acid, inositol polyphosphates and diacylglycerol. IP_3 mobilizes Ca^{2+} from an endoplasmic reticulum like compartment, further elevating the cytosolic concentration of the cation. Intracellular Ca^{2+} acts as the insulin secretagogue. Elevation of free Ca^{2+} concentration also occurs in response to stimulation of phospholipase-C by acetylcholine and cholestokinin and by hormones that increase intracellular concentration of the cyclic AMP (Ebert and Creutzfeldt, 1987). β - Cell adenylyl cyclase synthesizes cyclic AMP activated by glucagon, gastrointestinal inhibitory peptide glucagon like peptide-1 and is inhibited by somatostatin and α2 adrenergic receptor agonists (Fleischer and Erlichman, 1989).

Degradation of insulin occurs primarily 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. Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules, which also degrade it. Severe impairment of renal function appears to affect the rate of disappearance of circulating insulin to a greater extent than does in hepatic disease (Rabkin, 1984). Proteolytic degradation of insulin in the liver occurs primarily after internalization of the hormone and its receptor and to a lesser extent, at the cell surface (Berman, 1980). The primary pathway for internalization is receptor-mediated endocytosis. The complex of insulin and its receptor is internalized into small vesicles termed endosomes, where degradation is initiated (Rabkin, 1984). Several enzymes have been implicated in insulin degradation. The primary insulin-degrading enzyme is a thiol-metalloproteinase.

1.1.6.2.2. Molecular Mechanism of Action

a) Cellular actions

The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle, and fat, but insulin exerts potent regulatory effect on other cell types as well. Insulin is the primary hormone responsible for controlling the uptake, utilization, and storage of cellular nutrient. Some effects of insulin occur within seconds or minutes, including the activation of glucose and ion transport systems, the covalent modification (i.e. phosphorylation or dephosphorylation) of enzyme and some effects on gene transcription (i.e. inhibition of the phosphoeneolpyruvate carboxykinase gene) (Granner, 1987; O’Brien and Granner, 1996). Other effects, such as those on protein synthesis and gene transcription may take a few hours effects of insulin on cell proliferation and differentiation may take days.

b) Effects of Insulin on Carbohydrate Metabolism

Insulin influences glucose metabolism in most tissues, especially the liver where it inhibits glycogenolysis and glucogenesis, while stimulating glycogen synthesis. It also increases glucose utilization (glycolysis), but the overall effect is to increase the hepatic glycogen stores (Rang et al, 2003). In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism. Glucose enters cells by facilitated diffusion through one of a family of glucose transporters (GLUT-1 through GLUT-5) which thought to be involved in Na⁺-independent facilitated diffusion of glucose into cell (Shepherd and Kahn, 1999). Insulin stimulates glucose transport at least in part by promoting the energy-dependent GLUT-4 and GLUT-1 glucose transporters to the plasma membrane (Suzuki and Kono, 1980; Simpson and Cushman, 1986). The facilitated diffusion of glucose into cells along a downhill
gradient is assured by glucose phosphorylation. This enzymatic reaction, the conversion of glucose to glucose-6-phosphates (G6P) is accomplished by one of a family of hexokinases. Hexokinase IV, a 50,000 dalton enzyme, more commonly known as glucokinase, is found in association with GLUT-2 in liver and pancreatic β-cells. Glucose-6-phosphatase, one of the key enzyme in the homeostatic regulation of blood glucose levels, catalyses the terminal step in both gluconeogenesis and glycogenolysis (Hers et al, 1991; Beaudet, 1991; Nordlie and Sukalski, 1985).

G6P is a branch-point substrate that can enter the glycolytic pathway and lead to the production of ATP through series of enzymatic reactions many of which are promoted by insulin. The effects of insulin on this pathway are exerted on gene transcription or through alteration of enzyme activity by phosphorylation or through alteration of enzyme activity by phosphorylation or dephosphorylation on serine and/or threonine residues. Alternatively, G6P can be incorporated into glycogen after isomerization to glucose-1 phosphate (G1P). Insulin promotes glycogen deposition by stimulating the activity of glycogen synthetase the rate limiting enzyme in glycogen synthesis, and by inhibiting phosphorylase, the rate-controlling enzyme in glycogen degradation. Covalent modification by phosphorylation/dephosphorylation is a major mechanism of action of insulin. [For example, phosphorylation increase the activity of acetyl-coA carboxylase and citrate-lyase and are activated by dephosphorylation (Froguel et al, 1993)]. Insulin increases glucose uptake by GLUT-4 adipose tissue, as well as muscle enhancing glucose metabolism. One of the main end products of glucose metabolism in adipose tissue is glycerol, which is esterified with fatty acids to form triglycerides, thereby affecting fat metabolism.

c) Effects of insulin on Fat Metabolism

Insulin increases fatty acid as triglyceride synthesis in adipose tissue and liver. It inhibits lipolysis, partly via dephosphorylation (and hence inactivation) of lipases. It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagons by opposing their actions on adenylate cyclase (Rang et al, 2003).

d) Effects of insulin on protein Metabolism

Insulin stimulates uptake of amino acids into muscle and increase protein synthesis. It also decreases protein catabolism and inhibits oxidation of amino acids in the liver (Rang et al, 2003).
1.1.6.3. Metabolic Processes in the Diabetes

In diabetes, both the production and metabolism of glucose are increased. Thus, in the fasting stage, hepatic glucose release is greatly elevated, causing the diagnostic fasting hyperglycemia of diabetics (Cryer and Gerich, 1985). Virtually all forms of diabetes mellitus are caused by a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance). These abnormalities lead to alterations in the metabolism of carbohydrates, lipids, ketones and amino acids; the central feature of the syndrome is hyperglycemia. In both types of diabetes glucagons (levels of which are elevated in untreated patients) opposes the effect of insulin on the liver by stimulating glycogenolysis and gluconeogenesis, but it has relatively little effect on peripheral utilization of glucose. Thus, in the diabetic patient with insulin deficiency or insulin resistance and hyperglucagonemia, there is an increase in hepatic glucose production, a decrease in peripheral glucose uptake and a decrease in the conversion of glucose to glycogen in the liver (De-Fronzo and Goodman, 1995). Alterations in secretion of insulin and glucagon also have profound effect on lipid, ketone and protein metabolism. At concentrations below those required to stimulate glucose uptake, insulin inhibits the hormone sensitive lipase in adipose tissue and thus inhibits the hydrolysis of triglycerides stored in the adipocyte. This counteracts the lipolytic action of catecholamines, cortisol and growth hormone and reduces the concentrations of glycerol (a substrate for gluconeogenesis) and free fatty acids (a substrate for production of ketone bodies and a necessary fuel for gluconeogenesis). These actions of insulin are deficient in the diabetic patient, leading in increased gluconeogenesis and ketogenesis. The liver produces ketone bodies by oxidation of free fatty acids to acetyl CoA, which is then converted to acetoacetate and β-hydroxybutyrate. The initial step in fatty acid oxidation involves transport of fatty acids into the mitochondria involving the interconversion of the CoA and carnitine esters of fatty acids by the enzyme acylcarnitine transferase. The activity of this enzyme is inhibited by intra-mitochondrial malonyl CoA, one of the products of fatty acid synthesis. In the diabetic patients, particularly the patient with Type-I DM, the consequences of insulin deficiency and glycogen in excess provide a hormonal milieu that favours ketogenesis and in the absence of appropriate treatment, may lead to ketonemia and acidosis (Foster, 1984).

Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. This enzyme hydrolyzes triglycerides present in very low density lipoproteins (VLDL) and chylomicrons, resulting in release of intermediate density lipoprotein (IDL)
particles. The IDL particles are converted by the liver to the more cholesterol-rich low-density lipoproteins (LDL). Thus, in the untreated or under treated diabetic patients, hyperglyceridemia and hypercholesterolemia often occur. In addition; deficiency of insulin may be associated with increased production of VLDL.

In protein metabolism insulin stimulates amino acid uptake and protein synthesis and inhibits protein degradation in muscle and other tissues; hence causing a decrease in the circulating concentration of many amino acids. Glutamine and alanine are the major amino acid precursors for gluconeogenesis. Insulin lowers alanine concentration during hyperinsulinemic euglycemia conditions. However, alanine utilization greatly exceeds production (owing to increased hepatic uptake and fractional extraction of the amino acid), and this result in a fall of peripheral alanine levels. In a poorly controlled by hyperglycemic diabetic subject, there is increased conversion of alanine to glucose, contributing to the enhanced rates of gluconeogenesis.

An almost pathognomonic feature of diabetes mellitus is thickening of the capillary basement membrane and other vascular changes that occur during the course of the disease. The cumulative effect is the progressive narrowing of the vessel lumina causing inadequate perfusion of critical regions of certain organs. The matrix is expanded in many vessel walls, in the basement membrane of the retina and in the mesangial cells of the renal glomerulus (McMillon, 1997). Cellular proliferation in many large vessels further contributes to luminal narrowing. These pathological changes contribute to some of the major complications of diabetes, including premature atherosclerosis, intercapillary glomeruloscleroisis, retinopathy, neuropathy and ulceration and gangrene of the extremities. The toxic effects of hyperglycemia may be the result of accumulation of non-enzymatically glycosylated products. Haemoglobin undergoes glycosylation on its amino-terminal valine residue to form the glucosyl valine adduct of haemoglobin termed HbA1c (Brown Lee, 1995). Since the amount of glycosylated protein formed is proportional to the glucose, the concentration of haemoglobin A1c in the circulation reflects the severity of the glycemic state over an extended period (4 to 12 weeks) prior to sampling. Thus, a rise in haemoglobin A1c from 5 percent to 10 percent suggests a prolonged doubling of the mean blood glucose concentration. Glycosylated products accumulate in tissues and may eventually form cross-linked proteins termed advanced glycosylation end products (Beisswenger, 1995).

Binding of such proteins to macrophages in these lesions may stimulate the production of cytokinesis such as tumor necrosis factor and interleukin-I, which in turn
induce degradative and proliferative cascades in mesenchymal and endothelial cells, respectively.

1.1.7. Function Tests

a) **Postprandial Plasma Glucose**: Measurement of the rate that the glucose load is cleared from the blood, as compared to the rate of glucose clearance in healthy persons detects impairment in glucose metabolism. A meal high in carbohydrates is often used as carbohydrate load, though a 75g glucose drink is usually preferred over a meal. It is called the Post-prandial test. Blood is drawn at 2 hours after ingestion of the meal or glucose drink. Glucose levels above 140 mg/dl are abnormal; levels of 120 to 140 mg/dl are ambiguous; and levels below 120 mg/dl are normal.

b) **Oral Glucose Tolerance Test (OGTT)**: The OGTT evaluates glucose clearance from the circulation after glucose loading under defined and controlled conditions. Standard conditions call for a minimum carbohydrate intake of 150 g/day for 3 days before the test. There should be an 8-hour to 16 hour fast before testing. Blood samples are drawn at fasting and 1, 2 and 3 hours after ingestion of glucose. Additional samples at ½, 1½ and 2½ hours after glucose ingestion are helpful and sometimes necessary for evaluation of the test.

c) **Fasting Plasma Glucose**: Fasting plasma glucose and urinary glucose are the most commonly used makers for diabetes mellitus. In general, repeated fasting plasma glucose levels exceeding 140 mg/dl are strongly suggestive diabetes. Repeated plasma glucose levels of 115 to 140mg/dl may indicate the presence of diabetes.

d) **Urinary Glucose**: Urinary glucose is a poor marker for diabetes mellitus. The normal renal threshold for glucose is 180 mg/dl. Blood glucose levels must exceed this value before excessive glucose is apparent in the urine.

e) **Glycosylated Haemoglobin and Plasma Albumin**: The minor haemoglobin derivative called HbA1c is produced by glycosylation. Since this reaction is non-enzymatic and since the red cell is completely permeable to glucose, the quantity of HbA1c formed is directly proportional to the average plasma glucose concentration that the red blood cell is exposed to during its 120-days life span, which is the 4 to 6 weeks before sampling. Thus, in long-term hyperglycemia, HbA1c constitutes a higher percentage of total haemoglobin than in normoglycemia. Transient elevations in plasma glucose only mildly affect HbA1c levels. The elevations are directly proportional to the long-term degree of hyperglycemia. Glycosylated haemoglobins are most useful for monitoring of diabetes as they are not sufficiently sensitive
to effectively detect borderline cases of diabetes mellitus (Larsen et al, 1990; Dods and Bolmey, 1979).

**f) Urinary Proteins:** One of the earliest signs of impending glomerular nephropathy is the increased excretion of albumin in the urine, also termed microalbuminuria.

**e) Electrolysis:** Uncontrolled diabetes can exhibit normal, low or high plasma sodium levels.

**f) Lipids:** Elevated plasma triglyceride, cholesterol and VLDL are commonly found in diabetics. On the other hand, HDL is usually low.

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**Pathophysiology of Diabetes Mellitus**

**Some Over-Simplifications**

- lack of insulin and/or insulin resistance
- increased hepatic gluconeogenesis
- hyperglycemia
- increased lipolysis
- increased burning of free fatty acids
- more acetate units produced
- increased ketones
- acidosis
- death from hyperosmolar nonketotic coma
- death from ketoacidosis
- late complications
- death (typically by myocardial infarct)
- interference with neutrophil function
- production of polyols
- increased glycosylation of body proteins
- decreased glucose uptake
1.1.8. Oxidative Free Radical and DM:

Oxidative free radical (OFR) induced degeneration of pancreatic β-Islet cells has been implicated in the etiopathogenesis of clinical diabetes mellitus. In keeping with this postulate, experimental diabetogenic agents, including alloxan and STZ, have been extensively investigated for their effects on pancreatic OFR activity (Halliwell and Gutteridge, 1989). After injection of alloxan in rats, it is selectively taken up by Islets and hepatocytes. The liver has very high concentration of OFR scavenging enzymes, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) which is, by comparison, are low in the Islet cells. Alloxan is converted into dialuric acid by a two electron reduction. Di-aluric acid is unstable and is oxidized back to Alloxan by a reaction accompanied with a reduction of oxygen to the OFR, O₂ and He. The latter, through a fentos type reaction in the presence of transition metals, generates the highly toxic OFR, OH. Increased production of OFR in the Islets, together with inadequate defence, makes the beta-islet cells susceptible to alloxan. Alloxan induces membrane lipid peroxidation and DNA strand breakage in these cells (Halliwell and Gutteridge, 1989). Administration of SOD, scavengers of OH like dimethylurea and other compounds, and iron chelators protect animals against alloxan induced diabetes mellitus (Halliwell and Gutteridge, 1989). Like alloxan, STZ also induces OFR induced lipid peroxidation and DNA strands breaking in pancreatic Islet cells. Both alloxan and STZ have been shown to significantly reduce Islet cell SOD activity in rats and mice (Papaccio et al, 1991; Bhattacharyra, 1995; Bhattacharyra, 1995). Administration of either SOD or agents which reverse alloxan or STZ induced reduction of Islet cell SOD activity attenuating the diabetogenic effect of these compounds.

The capacity of nutrients to stimulate insulin release from the pancreatic β-cell reflects their capacity to augment oxidative fluxes in the Islet cells (Malaisse, 1983). Also, oxidant stress associated with insulin resistance and non-insulin-dependent diabetes mellitus (Gopaul et al, 1995; Nourooz-Zadeh et al, 1995) contributes to poor insulin action (Paolisso et al, 1994; Faure et al, 1997; Rudich et al, 1997). Thus, the treatment aims to reduce insulin resistance (diet, exercise and drug therapy) and to stimulate insulin secretion. In DM, oxidative stress seems mainly to be due to an increased production of free radicals and / or a sharp reduction of antioxidant defenses (Cross et al, 1987; Oberley, 1988; Hunt et al, 1992; Young et al, 1992; Thompson and Lee, 1993; Giugliano et al, 1996; Low et al, 1997). Oxygen-derived free radicals have been implicated in the pathophysiology of various disease states, including diabetes mellitus (Giugliano et al, 1996). It is well known that superoxide
anion is the primary radical formed by the reduction of molecular oxygen that may lead to secondary radicals or reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical (Grisham and McCord, 1986; Katusic, 1996). Also, it is found that increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue damage (Jang et al, 2000). On the other hand, there is evidence that diabetes induces changes in the activities of antioxidant enzymes in various tissues (Oberley, 1988). Diabetes mellitus is characterized by increased generation of glycoxidation products is associated with the advanced oxidative stress (Mullarkey et al, 1990). The presence of higher glucose or glycated protein concentration enhances lipid peroxidation (Kawamura et al, 1994) and reversely, lipid peroxides may increase the extent of advanced glycation end-products (Hicks et al, 1989). Oxidative stress in DM was thought to be a result of free radicals generated during autoxidation of glucose (Miyata et al, 1999). Increased levels of ROS in type-II DM was implicated to contribute a hypercoagulable state (Collier et al, 1992), and most recently, evidence was provided for the accumulation of oxidation products prior to the development of diabetes (Matteucci and Giampietro, 2000). The causes of enhanced free radical production are hyperglycemia (Hammes et al, 1997) and hyperinsulinemia (Paolisso and Giugliano, 1996).

1.1.8.1. Mechanisms of oxygen free radicals’ production by hyperglycemia

Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations (Hammes et al, 1997). Free radical production caused by hyperglycemia may occur via at least four different routes: i) increased glycolysis (Vaag et al, 1992); ii) intercellular activation of sorbitol (polyol) pathway (Williamson et al, 1993); iii) auto-oxidation of glucose (Wolff et al, 1991) and iv) non-enzymatic protein glycation (Ceriello et al, 1992).

1.1.8.1.1. Increased Glycolysis

Hyperglycemia seems to enhance non-oxidative metabolism (glucose conversion to lactate) by increasing glucose-6-phosphate (G6P) (Vaag et al, 1992). Increased glucose metabolism to lactate is associated with an increase in NADH / NAD+ ratio (Williamson et al, 1993). Under this condition of markedly accelerated glycolysis, oxidation of glyceraldehydes 3-phosphate (GAP) to 1,3-diphosphoglycerate (1,3-DPG) by glyceraldehyde 3-phosphate dehydrogenase appears to become the rate limiting step in glycolysis (Kobayashi and Neely, 1979) and this reaction is coupled with reduction of NAD+ to NADH. In the cytosol, NADH is oxidized to NAD+ by lactate dehydrogenase (LDH), coupled to reduction of pyruvate to lactate. Thus, the increase in the ratio of NADH / NAD+ will reflect increased
lactate / pyruvate ratio (Williamson et al, 1993). The mechanism by which an increased rate of glycolysis increases free cytosolic NADH / NAD+ ratio (redox imbalance) appears to result from a disequilibrium between the rate of oxidation of GAP to 1,3-DPG and the rate of reduction of pyruvate (Kobayashi and Neely, 1979). This result indicates that, the increased glycolysis as a consequence of hyperglycemia is closely related to an increase in NADH / NAD+ ratio due to impaired oxidation of NADH to NAD+.

1.1.8.1.2. Increased activity of sorbitol pathway

The increased glucose flux via sorbitol pathway (a pathway of a minor significant under normal glycemic condition) which leads to the accumulation of both sorbitol and fructose is thought to be one of the main metabolic disturbances related to diabetic hyperglycemia (Ciuchi et al, 1996).

In this pathway, glucose is reduced to sorbitol by aldose reductase (AR), coupled with oxidation of NADH / NAD+. Sorbitol is then oxidized to fructose coupled with reduction of NAD+ to NADH by sorbitol dehydrogenase (SDH) (Cameron et al, 1997). Previous studies suggested several hypotheses for tissue injury caused by increased sorbitol pathway activity: 1) the decreased availability of NADPH (required for maintenance of reduced glutathione) which is oxidized to NADP+ through reduction of glucose to sorbitol by aldose reductase (Tilton et al, 1995). Furthermore, the competition between aldose reductase and glutathione reductase for NADPH cofactor depletes reduced glutathione (Ciuchi et al, 1996).

Attention has been focused on this GSH depletion, because it can play a role in increased oxygen free radicals production, which is thought to lead to oxidative tissue damage in diabetes (Brownlee, 1994) and increased NADH / NAD+ ratio, which is related to accelerated oxidation of sorbitol to fructose by NAD+-dependent sorbitol dehydrogenase (Tesfamariam and Cohen, 1992). It is reported that NADH produced in the cytosol by oxidation of sorbitol to fructose can remain there temporarily but for a long run it has to be transported into the mitochondria to be oxidized by respiratory chain causing generation of superoxide radical and other oxygen reactive species derived from it (Williamson et al. 1993; Ceriello et al, 1996). Thus, an increase in the cytosolic NADH may be accompanied by increased load of mitochondrial NADH, which in turn leads to increased oxygen radicals generation.

1.1.8.1.3. Glucose auto-oxidation

Glucose can be auto-oxidized in a cell-free system under physiological conditions via enediol tautomer formation which generates hydrogen peroxide; reactive intermediate such as hydroxyl and superoxide radicals, and ketoaldehydes (Brownlee, 1994; Packer, 1993).
Transition metals such as iron are believed to be of crucial importance in the cascade of these reactions, as they catalyze auto-oxidation of glucose (Packer, 1993). In this review, several studies have reported that glucose auto-oxidation can actually occur and could be responsible for increased oxygen radicals in diabetes (Monnier, 1990; Baynes, 1991; Santini et al, 1997).

1.1.8.1.4. Non-enzymatic protein glycation (glycosylation)

Non-enzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Schiff bases and more stable Amadori products are formed (Vlassara et al, 1994). Advanced glycation end products (AGEs) are then produced by auto-oxidation of Amadori product (The Diabetes Control and Complication Trials Research Group, 1993). AGEs elicit their cellular effects by binding to specific cellular receptors (Esposito et al, 1989; Yang et al, 1991; Schmidt et al, 1994; Vlassara et al, 1996), one of which, RAGEs (receptor for AGEs), has been identified on endothelial cells (Neeper et al, 1992; Abel et al, 1995; Ritthaler et al, 1995), monocytes / macrophages, mesangial cells, neurons and smooth muscle cells. Interaction of AGEs with endothelial surface RAGEs generates intracellular oxidative stress and therapy modulating cellular functions, even in the presence of intact antioxidant mechanisms (Wautier et al, 1994; Yan et al, 1994; Schmidt et al, 1995; Wautier et al, 1996). This process is probably enhanced and amplified when antioxidant defense mechanisms are reduced (Bierhaus et al, 1997).

Mechanisms of oxygen free radicals production by hyper-insulinemia

Decline in physical fitness, increase in body fatness and upper body fat distribution are frequently associated with hyperinsulinemia and insulin resistance (De-Fronzo et al, 1991).

Several lines of evidence seem to indicate the relationship between hyperinsulinemia and free radical production. Krieger-Brauer and Kather reported that in intact human fat cells, exposure to insulin leads to a time-and dose-dependent accumulation of hydrogen peroxidase in the suspension medium (Krieger-Brauer and Kather, 1992). This effect, which has been related to the presence of a membrane-bound NADPH oxidase was found to persist after cell disruption and not to require ATP indicating that the receptor kinase step was bypassed. In addition, increased insulin concentration in rats following intraperitoneal injection of dextrose has been found to be associated with increased free radical production (Habib et al, 1994). Since fasting hyperinsulinemia is considered to be a hallmark of insulin resistance (De-Fronzo et al, 1991), a relationship between insulin resistance and plasma free radical concentration can not be excluded (Ceriello and Pirisi, 1995; Ceriello, 2000). The genesis of free radical concentration in insulin resistant conditions (Paolisso and Giugliano, 1996) might be due to an insulin-mediated overdrive of the sympathetic nervous system activities.
1.1.8.1.5. An insulin-mediated overdrive of the sympathetic nervous system activities

Hyperinsulinemia is responsible for the overdrive of the sympathetic nervous system (Rowe et al, 1981). In diabetic animals, catecholamines may increase free radical production through induction of the metabolic rate and auto-oxidation (Singal et al, 1983).

1.1.8.1.6. Elevation in plasma non-esterified fatty acid concentration

Insulin resistance has been shown to be associated with elevated fasting plasma non-esterified fatty acid (NEFA) concentration (De-Fronzo et al, 1991; Randle et al, 1994). Recently, Toborek and Henning showed that fatty acids cause an increase in oxidative stress in cultured endothelial cells and an initial decrease in reduced glutathione concentrations after 6h of exposure to the incubation medium (Toborek and Henning, 1994). Traverse et al, 1998 and Betteridge, 2000 reported that the imbalance of generation and scavenging of free radicals play an important role in determining tissue damage associated with diabetes. Lipid peroxidation is the primary cellular damage resulting from free radical reactions. Also, significant changes in cellular lipid structures are generally occurring in diabetic states (Armstrong D and Al-Awadi, 1991; Toborek et al, 1992). In these states, the structure changes are oxidative in nature due to peroxidation of the lipids, which defined as peroxidative deterioration of unsaturated fatty acids of cellular membrane phospholipids, via intermediate radical reactions (Rungby et al, 1992; Cameron et al, 1994), with a result of producing hydroperoxides. The net effect of these combined reactions is the generation of highly toxic peroxyl radicals (ROO•) which generate new lipid hydroperoxides because of their close proximity in biomembranes to other lipids (Betteridge, 2000; Sakamoto, 1985; Kajanachumpol et al, 1997).

1.1.8.2. Mechanisms of oxygen free radical production by hypo-insulinemia

Hypoinsulinemia increases the activity of fatty acyl-CoA oxidase that indicates 3-oxidation of fatty acids resulting in increased production of H$_2$O$_2$ (Horie et al, 1981). Kakkar et al, 1995 and Tatsuki et al, 1997 recorded significant increase of erythrocytic and pancreatic catalase (CAT) activity in streptozotocin diabetic rats and ascribed this increase to the accentuated oxidative stress in diabetes. However, Matkovics et al, 1998 demonstrated a significant decrease of CAT activity in erythrocyte hemolysates of streptozotocin diabetic rats.

1.1.8.3. Changes in antioxidant enzyme activities due to diabetes

Several studies examined the tissue levels of the enzymatic antioxidant defenses in diabetes with varying results. Piper et al, 1995 demonstrated that, in experimental diabetes the activity of catalase was increased in vascular tissues with absence of any significant
changes in the activity of the other major antioxidant enzymes (superoxide dismutase and glutathione peroxidase). In addition, Wohaieb and Godin, 1987 showed increased activities of catalase and superoxide dismutase (SOD) in the pancreas of diabetic rats, while the liver showed a generalized decrease in the activities of catalase, SOD and glutathione peroxidase (GSH-Px). In the past study, the increase in the activities of both CAT and SOD occurred in the tissue with the lowest antioxidant enzymatic activities (pancreas) before onset of diabetes, suggesting a compensatory response to an increase in endogenous oxidant radicals in pancreas by diabetes. A decrease in the concentration of reduced glutathione (GSH) has been observed in erythrocytes from diabetic subjects, as a result of decreases in activities of the enzymes involved in GSH synthesis (such as γ-glutamycystein synthetase) or in the transport rate of oxidized glutathione (GSSG) from erythrocytes (Murakami et al, 1989) and enhanced sorbitol pathway (Ciuchi et al, 1996). In addition, a decrease in the activity of glutathione reductase (GSSG-R) which acts to reduce GSSG to GSH, has also been reported (Tagami et al, 1992). Kazuhiro et al, 1989 and Matkovics et al, 1998 elucidated that GSSG-R activity decreases in erythrocyte hemolysates of streptozotocin diabetic rats and attributed this decrease to the enzyme glycation by the uncontrolled hyperglycemia (Jain and McVie, 1994). Also, Jos et al, 1990 and Dominguez et al, 1998 reported a significant decrease of erythrocyte GSH-Px activity in diabetic children and adolescents compared to control subjects. They attributed this decrease to a decline in blood glutathione content in those diabetics, since GSH is a substrate and cofactor for this enzyme. Therefore, low GSH content indicates low GSH-Px activity, which may produce increased oxidative stress propensity. Moreover, enzyme inactivation either through glycation process (Arai et al, 1987) or under conditions of increased oxidative stress might also contribute to low GSH-Px activity (Lyons, 1991).

1.1.8.4. Oxidative stress in diabetes

Both radical and non-radical oxidants can induce lipid peroxidation particularly of those lipoproteins that contain unsaturated fatty acids. A product of the reaction between a superoxide anion and nitric oxide, known as peroxynitrite, is a particularly powerful oxidant of low-density lipoproteins (LDLs) (Violi et al, 1999). The evidence for oxidative damage in diabetes has been reported by Sato et al, 1979. The authors reported that the average level of lipid peroxides in plasma is higher in diabetic patients than in normal people and the diabetic patients with angiopathy had higher lipid peroxide levels than other diabetic patients. Further suggestion is that the high levels of lipid peroxide in plasma may cause an increase in lipid peroxide levels in the intima of the blood vessel, which may then initiate atherosclerosis.
Recent studies have found increased and similar in vitro oxidizability of LDL fractions of plasma from diabetic patients and identified autoantibodies against oxidatively modified LDL in type I diabetic patients again suggesting that LDL oxidation occurs in-vivo in diabetes (Jain et al, 1998). Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders (Armstrong et al, 1982) including naturally occurring (Nishigaki et al, 1981) and chemically induced diabetes mellitus (Rerup, 1970; Higuchi, 1982).

Consequently, mechanisms in the formation of lipid hydroperoxides and biologically active metabolites, together with their effect on cellular structure and function are becoming of increasing importance to the study of diabetogenesis (Crabbe, 1987). Lipid hydroperoxides (LHP) produced from a variety of long-chain polyunsaturated fatty acid precursors via intermediate radical reactions, involve oxygen and metal cations (iron and copper). The net result of these combined reactions is the generation of highly reactive and cytotoxic lipid radicals, which generate new LHP because of their close proximity in biomembranes to other lipids. Extracellularly, lipid hydroperoxides are transported in the systemic circulation by low- and high-density lipoproteins (Nishigaki et al, 1981). When released locally, LHP produce structural damage (Berdanier, 1988) Peroxidative regulation occurs through intervention by lipid and water-soluble antioxidants, as well as by specific antioxidant enzymes, i.e., dioxide (1-) dismutase, peroxidase and catalase. The formation of LHP and their metabolites are important in clinical medicine because they alter membrane structure and function, especially in the retinal portion of eye which is very sensitive to oxidative stress. For example, a steady decline is observed in the electroretinogram not only in the streptozotocin (STZ) model (Pautler and Ennis, 1980) but also when synthetic LHP is injected into the vitreous of experimental animals (Armstrong et al, 1982). These changes are irreversible.

Moreover, support for the concept of increased oxidative stress (increased generation of free radicals) in diabetes is derived principally from in vitro experiments (Giugliano and Cariello, 1996; Wolff, 1993). The primary causal factor is hyperglycaemia and this operates via several mechanisms, although the individual contribution of each mechanism to hyperoxidative stress remains undefined, as does also the dose response relationship between hyperglycaemia and overall oxidative stress in diabetes. Glycooxidation of glucose generates reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radical (Giugliano and Cariello, 1996). These accelerate the formation of advanced glycosylation end-products (AGEs) which in turn generate more free radicals (Giugliano and Cariello, 1996; Vlassara et al, 1994). Increased cellular uptake of glucose stimulates protein kinase C
activity (Lee et al, 1989) which, amongst other effects, activates peroxidase enzymes and the cyclo- oxygenase (COX) pathway (Lee et al, 1989; Feener and King, 1997), with resultant over-production of oxidative molecules. By elevating endothelial cell calcium, hyperglycaemia also stimulates the synthesis of NO (Cohen, 1993; Poston and Taylor, 1995), but in the presence of superoxide, nitric oxide (NO) is converted into the highly potent oxidant molecule peroxynitrite (ONOO-) (Beckman et al, 1990).

Antioxidant defenses may also be impaired in diabetes, thereby contributing to net oxidative stress. Decreased tissue concentrations of antioxidants, such as vitamin E, SOD and CAT, have also been demonstrated in vitro (Wohaieb and Godin, 1987). Although there are extreme difficulties in measuring free radicals in vivo, some support for the notion of increased oxidative stress in diabetes and its association with poor metabolic control and coronary heart disease has been derived from observations in patients with diabetes mellitus (Nourooz-Zadeh et al, 1997; Griffin et al, 1997). Increased oxidative stress may provide a plausible pathobiological basis for the direct association between hyperglycaemia and increased cardiovascular persuasive risk in diabetes mellitus (Barrett-Connor, 1997; Lehto et al, 1997).

In spite of recent persuasive evidence (Griendling and Alexander, 1997), definitive clinical proof for the role of oxidative stress in the pathogenesis of atherosclerosis in both diabetic and non diabetic subjects remains outstanding. In addition, there was an inverse relationship between insulin action and oxidative stress or hypofibrinolysis. Insulin resistance and increased oxidative stress have been observed in obese Type II diabetic patients (Skrha et al, 1996). The relationship between insulin action and oxidative stress was therefore suggested. This finding of an inverse relationship between plasma malondialdehyde concentration and glucose disposal rate during hyperinsulinemic clamp is in agreement with this suggestion. A decrease of oxidative stress could therefore improve insulin action in subjects with insulin resistance. Drugs acting like scavengers of oxygen radicals are promising tool in the treatment of patients with increased oxidative stress.

On the other hand, lipid peroxide levels in plasma of diabetic patients have been found to be significantly higher than in healthy individuals (Kaji et al, 1985). Furthermore, Sato et al, 1979 reported an increase in thiobarbituric acid reaction in these patients especially in poorly controlled diabetic and diabetica with angiopathy. This elevation has been considered as a cause of organ or tissue degeneration. Significantly higher values of thiobarbituric acid reactive substances (TBARS), which provide an indirect measurement of lipid peroxidation and decreased erythrocyte antioxidant enzyme activities, have been
observed in serum of adult diabetic patients (Arai et al, 1987; Sharma et al, 2000), heart, pancreas and blood of Streptozotocin diabetic rats (Kakkar et al, 1995).

On other instance, TBARS is considered as an indicator of free radical production. An increase in TBARS level in liver may therefore be due to increased oxidative stress that might promote DNA and protein alterations (Wolff et al, 1991) including changes in the enzyme activities implicated in lipid metabolism and free radicals scavenging process (Douillet et al, 1998). Also, increased oxidative stress in diabetes mellitus may be a reason for such decrease in erythrocytes count. Hyperglycemia can burden the cells with extra free radicals (Fujiwara et al, 1989). This, coupled with reduced GSH content secondary to its increased utilization in diabetic erythrocytes (Jain et al, 1994) can cause peroxidative breakdown of phospholipids fatty acids in the erythrocytes membrane. This is supported by the fact that erythrocytes of diabetic patients are more susceptible to lipid peroxidation when treated with hydrogen peroxide in vitro (Matkovics et al, 1982; Uzel et al, 1987). In addition, the decrease in hematocrit percentage may be attributed to the reduction in the total red blood cell count and the failure in blood osmoregulation and plasma osmolarity (Holt et al, 1982; Wong et al, 1983).

1.1.8.5. Oxidative modification of macromolecules (Proteins and lipoproteins)

Oxidative damage to biologically important macromolecules may occur by means of non-radical oxidants such as hydrogen peroxide, hypochlorous acid or singlet oxygen, and by radical oxygen species like superoxide anion and hydroxyl radicals. These oxidants attack double bonds in unsaturated fatty acids resulting in the formation of lipid peroxides. Study of lipid peroxidation is, however, hampered by instability of the peroxidation products and the complexity of assays (Gutteridge and Halliwell, 1990). Oxygen radicals, particularly the very aggressive hydroxyl radical, can also oxidize apolipoproteins and other plasma proteins, the products of which are much more stable than lipid peroxides. The decrease in the total protein concentration in serum of diabetic rats may be ascribed to: i) decreased amino acids uptake (Garber, 1980), ii) greatly decreased concentration of variety of essential amino acids (Brosnan et al, 1984), iii) increased conversion rate of glycogenic amino acids to CO₂ and H₂O (Mortimore and Mandon, 1970) and iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA (Peavy et al, 1985). Furthermore, Wanke and Wong, 1991 attributed the decrease of albumin concentration in experimental diabetes to the presence of inhibitor(s) of albumin promoter activity in the liver.
1.1.9. The mechanism of Alloxan action

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) was first described by Brugnatelli in 1818. Wöhler and Liebig used the name “alloxan” and described its synthesis by uric acid oxidation (Lenzen and Panten 1988). The diabetogenic properties of this drug were reported many years later by Dunn, Sheehan and McLethie, 1943, who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic Islets. Since then, alloxan diabetes has been commonly utilized as an animal model of Insulin Dependent Diabetes Mellitus (IDDM).

Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human Islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik et al. 1994). The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg b.w. (Gruppuso et al. 1990, Boylan et al. 1992). When alloxan is given intraperitoneally or subcutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg b.w. may be insufficient for inducing diabetes in the rat (Katsumata et al. 1992, 1993). However, fasted animals are more susceptible to alloxan (Katsumata et al. 1992, Szkudelski et al. 1998), whereas increased blood glucose provides partial protection (Bansal et al. 1980, Szkudelski et al. 1998).

The mechanism of alloxan action has been intensively studied, predominantly in vitro, and is now characterized quite well. Using isolated Islets (Weaver et al. 1978b) and perfused rat pancreas (Kliber et al. 1996) it was demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose. This phenomenon appeared just after alloxan treatment and was not observed after repetitive exposure of Islets to this diabetogenic agent (Weaver et al. 1978b). The sudden rise in blood insulin concentration was also observed in vivo just after alloxan injection to rats (Szkudelski et al. 1998). Alloxan-induced insulin release is, however, of short duration and is followed by complete suppression of the Islet response to glucose, even when high concentrations (16.6 mM) of this sugar were used (Kliber et al. 1996). Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 °C is about 1.5 min and is longer at lower temperatures (Lenzen and Munday 1991). On the other hand, when a diabetogenic dose is used, the time of alloxan decomposition is sufficient to allow it to reach the pancreas in deleterious amounts.
The action of alloxan in the pancreas is preceded by its rapid uptake by the B cells (Weaver et al. 1978a, Boquist et al. 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Another aspect concerns the formation of reactive oxygen species (Heikkila et al. 1976). A similar uptake of alloxan also takes place in the liver. However, the liver and other tissues are more resistant to reactive oxygen species in comparison to pancreatic Beta cells and this resistance protects them against alloxan toxicity (Malaisse et al. 1982, Tiedge et al. 1997). The formation of reactive oxygen species is preceded by alloxan reduction. In Beta cells of the pancreas its reduction occurs in the presence of different reducing agents. Since alloxan exhibits a high affinity to the SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein-bound sulfahydryl groups (including SH containing enzymes) are very susceptible to its action (Lenzen and Munday 1991). However, other reducing agents such as ascorbate may also participate in this reduction (Zhang et al. 1992). Lenzen et al. (1987) proposed that one of the SH-containing compounds essential for proper glucose-induced insulin secretion is glucokinase (EC 2.7.1.2), being very vulnerable to alloxan. Alloxan reacts with two -SH groups in the sugarbinding side of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. Glucose can protect glucokinase against the inactivation hindering the access of alloxan to the -SH groups of the enzyme (Lenzen et al. 1987, 1988, Lenzen and Mirzaie-Petri 1991). Dialuric acid is formed as a result of alloxan reduction. It is then re-oxidized back to alloxan establishing a redox cycle for the generation of superoxide radicals (Munday 1988). The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals (HA•) and an unidentified "compound 305" (maximum absorption at 305 nm) is formed. The latter appears when alloxan is reduced by GSH (Sakurai and Ogiso 1991). Superoxide radicals are able to liberate ferric ions from ferritin and reduce them to ferrous ions. Fe3+ can also be reduced by alloxan radicals (Sakurai and Ogiso 1995). Moreover, superoxide radicals undergo dismutation to hydrogen peroxide:

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

This reaction may occur spontaneously or may be catalyzed by superoxide dismutase (EC 1.15.1.1) (Malaisse 1982). In the presence of Fe2+ and hydrogen peroxide, highly reactive hydroxyl radicals are then formed according to the Fenton reaction:

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \]

The action of hydroxyl radicals following alloxan treatment was demonstrated in both in vitro (Grankvist 1981, Munday 1988) and in vivo (Kurahashi et al. 1993).
One of the targets of the reactive oxygen species is DNA of pancreatic Islets. Its fragmentation takes place in beta cells exposed to alloxan (Takasu et al. 1991a, Sakurai and Ogiso 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADP ribosylation initiated by alloxan (Sandler and Swenne 1983, LeDoux et al. 1988). Superoxide dismutase, catalase (EC 1.11.1.6) (Grankvist et al. 1979, Grankvist 1981, Jörns et al. 1999) and non-enzymatic scavengers of hydroxyl radicals (Ebelt et al. 2000) were also found to protect against alloxan toxicity. Therefore, chemicals rendering anti-oxidative properties and inhibiting poly ADP-ribosylation can attenuate alloxan toxicity. It has been argued that glucose counteracts alloxan cytotoxicity in vitro and in vivo. This ability, however, is not only the result of the protection of glucokinase. The protective effect of glucose against necrotic death of beta cells may be due to interaction of the sugar with the glucose transporter GLUT2 resulting in limited alloxan uptake (Jörns et al. 1997). It has been previously proposed that the action of glucose is also related to its metabolism and to the increased generation of reducing equivalents (NADH and NADPH) accelerating the recirculation of glutathione. GSH is known to provide protection against free radicals (Donnini et al. 1996). It may thus divert hydrogen peroxide from the pathway leading to the formation of hydroxyl radicals (Malaisse 1982, Malaisse-Lagae et al. 1983, Pipeleers and van de Winkel 1986):

\[
\text{GSSG} + 2 \text{NADPH} \rightarrow 2 \text{GSH} + 2 \text{NADP} + \text{H}_2\text{O}_2 + 2 \text{GSH} \rightarrow \text{GSSG} + 2 \text{H}_2\text{O}
\]

Moreover, Sakurai and Ogiso (1991) observed that the in vitro generation of hydroxyl radicals in the presence of alloxan strongly depends on GSH concentration. GSH in low concentrations potentiated the formation of these radicals, whereas the oxygen consumption, autoxidation of dialuric acid and formation of hydroxyl radicals were significantly inhibited in higher concentrations. GSH at high concentrations can also inhibit HA• generation and directly neutralize hydroxyl radicals. Thiyl radicals (GS•) formed in this reaction are then converted to GSSG:

\[
\text{GSH} + \text{OH}• \rightarrow \text{GS}• + \text{H}_2\text{O}
\]
\[
\text{GS}• + \text{GS}• \rightarrow \text{GSSG}
\]

Indeed, in rat Islets incubated with alloxan the GSH content and GSH/GSSG ratio were decreased (Malaisse et al. 1982), whereas glucose evoked the opposite effect. In the in vivo experiment, glucose given to rats 20 min prior to alloxan partially restricted alloxan induced increase in the activity of glutathione peroxidase (EC 1.11.1.9) and mitigated the
drop of liver nonprotein -SH groups (especially reduced glutathione) (Szkudelski et al. 1998). The protective action of this sugar is, however, strongly glucose and alloxan dose-dependent (Harman and Fischer 1982, Gorray et al. 1983).

It has been proposed that disturbances in intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan. This concept was confirmed by in vitro and in vivo experiments demonstrating that alloxan elevates cytosolic free Ca$^{2+}$ concentration in pancreatic Beta cells (Kim et al. 1994, Park et al. 1995). This effect arises from several events: alloxan-induced calcium influx from extracellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx may result from the ability of alloxan to depolarize pancreatic Beta cells (Dean and Matthews 1972). Depolarization of the cell membrane opens voltage dependent calcium channels and enhances calcium entry into cells. Alloxan was also found to exert a stimulatory effect on mitochondrial Ca$^{2+}$ efflux with simultaneous inhibitory action on Ca$^{2+}$ uptake by mitochondria (Nelson and Boquist 1982, Lenzen et al. 1992). The restriction of calcium removal from the cells due to alloxan-induced inhibition of liver plasma membrane Ca$^{2+}$-ATPase was also reported (Seckin et al. 1993). The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by H$_2$O$_2$ since hydrogen peroxide itself exerts a similar effect on calcium concentration in Beta cells (Park et al. 1995). Thus, the previously mentioned sudden rise in insulin release from Beta cells treated with alloxan (Weaver et al. 1978b, Kliber et al. 1996) may be one of the effects of alloxan induced augmentation in cytosolic Ca$^{2+}$ concentration (Weaver et al. 1978b, Kim et al. 1994). The exaggerated concentration of this ion contributes to supra-physiological insulin release and together with reactive oxygen species, causes damage of pancreatic Beta cells. The results of experiments with calcium channel antagonists have confirmed the important role of cytosolic calcium in the cytotoxic action of alloxan. Pretreatment of rats with verapamil prevented the alloxan-induced increase in Beta cell Ca$^{2+}$ concentration and abolished the stimulatory effect of alloxan on insulin release (Kim et al. 1994). The calcium channel antagonists (verapamil and diltiazem) also suppressed hyperglycemia and the onset of alloxan induced diabetes in rats (Katsumata et al. 1992, Kim et al. 1994).

Summing up, the toxic action of alloxan on pancreatic Beta cells, described many years ago by Dunn et al. (1943), are the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis.
Many investigators suggested that the selectivity of alloxan action is not quite satisfactory. Recent experiments confirmed this objection. The diabetogenic dose of alloxan was found to decrease -SH groups accompanied by a simultaneous rise in glutathione peroxidase activity in the rat liver two minutes after its administration (Szkudelski et al. 1998). At the same time, the blood insulin concentration increased dramatically. This exaggerated insulinemia did not evoke, however, any significant reduction of blood glucose suggesting impaired peripheral insulin sensitivity in the short time after alloxan treatment (Szkudelski et al. 1998) was not found. It was also observed that alloxan intensified basal and epinephrine-induced lipolysis in isolated rat adipocytes and insulin failed to restrict this effect (Kandulska et al. 1999).

Thus, using alloxan to evoke diabetes, animals should be examined after proper period of time to minimize side effects of alloxan. It should also be emphasized that the range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic causing the loss of many animals. This loss is most likely due to kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered (Lenzen et al. 1996).

1.1.1. Classification of Oral Hypoglycemic Agents

1. Sulfonamide Group
   (a) 1st Generation: e.g. Tolbutamide, Acetohexamide, Tolazamide, Chloropropamide
   (b) 2nd Generation: e.g. Glyburide (Glibenclamide), Glipizide, Gliclazide, Glimepiride

2. Benzoic Acid Derivatives: e.g. Repaglinide, Nateglinide.

3. Biguanide Group: e.g. Phenformin, Metformin.

4. A- Glucosidase Inhibitors: e.g. Acarbose (oligosaccharide of microbial origin), Miglitol (Dextroxy njirimycin Derivative).

5. Miscellaneous
   (a) Thiazolidinedione derivatives: e.g. Rosiglitazone, Pioglitazone
   (b) Linogliride fumarate (Pyrolidine Nucleus)

1.1.10. Sulfonamide Group: Sulfonyl Ureas cause hypoglycemia by stimulating insulin release from pancreatic β-cells. The acute administration of sulfonyl ureas to Type-II DM patients increases insulin release from the pancreas. Sulfonyl ureas also may further increase insulin levels by reducing hepatic clearance of the hormone. Sulfonyl ureas also stimulate release of somatostatin and they may supress the secretion of Glucagon slightly as glucagon is now known to have a significant physiological role in the regulation of glucose and ketone body metabolism, but it is only of minor therapeutic interest for the short term management.
of Hypoglycemia (Krall, 1985). The concentration of insulin receptors increases in the monocytes adipocytes and erythrocytes of type-II DM patients who receive oral hypoglycemic agents (Olefsky and Reaven, 1976).

Sulfonyl ureas enhance 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. Attempts to describe the long-term blood glucose lowering effect of Sulfonyl ureas to specific changes in insulin action on target tissues are confounded by the effects of a lowered prevailing blood glucose level.

**a) Glibenclamide:** This 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. Moreover, it is claimed that the incidence of secondary failure to Glibenclamide is remarkably low. A minimal therapeutic plasma level is sufficient for its action. Glibenclamide is also believed to result in the release of duodenal insulin releasing agent (DDIRA—a gut factor) which is probably responsible for the sustained effect of drug.

Studies have clearly delineated the different methods of action of sulphonylureas during acute and chronic phases of therapy. The kinetics of insulin secretion involves four stages: 1. Synthesis, 2. Transport, 3. Storage and 4. Release. Short-term administration of sulphonylureas results in enhanced insulin release while paradoxically insulin biosynthesis is inhibited. The sulphonylureas during short-term treatment prime the beta cells and make them responsive to glucose stimulus (beta cytotoxic effect). On the other hand, chronic administration of sulphonylureas does not result in enhanced insulin release. But the extra-pancreatic effects and increased insulin sensitivity manifests. This beneficial effect on the receptors also occurs during the short-term sulphonylureas therapy.

The augmented insulin response (during acute phases of sulphonylureas therapy) corrected the hyperglycemia and sets the serum glucose level within the physiological range so that subsequent maintenance of euglycemia may be achieved solely through the extra-pancreatic effect of the drug (chronic phase of sulphonylureas therapy), without the necessity of further enhancement of insulin release. It is probable that the drug preferentially reserves its insulin releasing effect only in the setting of profound hyperglycemia. Since the insulin levels during chronic sulphonylureas therapy are just comparable to the pretreatment levels and yet glycemic control achieved, a phenomenon not encountered at the pretreatment stage.
of the disease with the same amount of circulating insulin, it might be inferred that the defect of significance in NIDDM is one at the “receptor” and the “post receptor” level in addition to the quantity of circulating insulin.

1.1.10.2. Benzoic Acid Derivatives: Repaglinide / Nateglinide stimulates insulin release by closing ATP dependent potassium channels in pancreatic beta cells. Drugs thus resemble physiological secretagogues which also lower the conductance of the K⁺ channels. Reduced K⁺ conductance cause membrane depolarisation and influx of Ca²⁺ through voltage sensitive Ca²⁺ channels (Aguilar-Bryan et al, 1995; Horton et al, 2000).

1.1.10.3. Biguanide Group: They have been used in NIDDM patients as adjuncts in insulin therapy and they are active only in patients with some endogenous insulin secretion. The Biguanides normalise blood sugars by several proposed mechanism. These including reduction in gastro intestinal absorption of glucose, stimulation of anaerobic glycolysis, inhibition of Gluconeogenesis, stimulaton of tissue glucose uptake and increasing insulin receptor binding. Also its ability to reduce hepatic glucose production is thought to be most important (Stumroll et al, 1995). Phenformin exerts its action on both carbohydrate and lipid metabolism. Phenformin by-passes the pancreas with direct antihyperglycemic action. It is highly effective first line drug therapy particularly in obese NIDDM and is also synergistic in combination.

1.1.10.4. α-Glucosidase Inhibitors: They reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of intestinal brush border alpha-Glucosidase. Inhibition of this enzyme slows the absorption of carbohydrates, the postprandial rise in plasms glucose is blunted in both normal and diabetic studies. Acarbose and Miglitol also competitively inhibit glucoamylase and sucrose, but have weak effects on pancreatic alpha-amylase. They reduce postprandial plasma glucose levels in both Type-I and II DM. α-Glucosidase Inhibitors have profound effects on hemoglobin levels in severe hyperglycemic type-II DM (Bressler and Johnson, 1992).

1.1.10.5. Thiazolidinedione Derivatives: They contain a thiazole ring which has the antidiabetic effect. Thiazolidinedione Secnidazole is the selective agonists for nuclear peroxisome proliferator-activated receptor Gamma (PPARγ). These drugs bind to PPARγ which in turn activates insulin responsive genes that regulate carbohydrate and lipid metabolism. They exert their principal effects by lowering the insulin resistance in peripheral tissue and effect to lower glucose production by the liver. Thiazolidinedione increase glucose transportation into muscle and adipose tissue by enhancing the synthesis and translocation of
specific forms of the glucose transporter proteins. They also can activate genes that regulate free fatty acid metabolism in peripheral tissue.

1.1.11. Limitations of currently available antidiabetic therapy

- Sulfonyl ureas may cause hypoglycemic reactions, including coma. Glyburide (glibenclamide) has been reported to result in hypoglycemia in up to 20% to 30% of users, whereas another long-acting sulfonylurea, glimepiride, results in hypoglycemia in only 2% to 4% of users.
- Sulfonyl ureas are effective only if β-cells are functional; Severe hypoglycemia in the elderly can present as an acute neurological emergency that may mimic a cerebrovascular accident.
- Other side effects of sulfonylureas include nausea and vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, and dermatological reactions.
- Patients with renal impairment should not receive Biguanides. Other contraindications include hepatic disease, a past history of lactic acidosis (of any cause), cardiac failure requiring pharmacological therapy, or chronic hypoxic lung disease.
- Acute side effects of Biguanides include diarrhoea, abdominal discomfort, nausea, metallic taste, and anorexia.
- Thiazolidinediones are very prone to cause hepatotoxicity and can cause weight gain and oedema.
- α-Glucosidase inhibitor causes flatulence and diarrhoea.

1.1.12. Importance of Herbal Products in Diabetes mellitus

In modern medicine no satisfactory effective therapy is still available to cure DM. Although insulin therapy, oral hypoglycemic agents, restricted diet, exercises either singly or in combination constitute a major regimen of therapy available for the present day diabetic patients. In a large no. of cases, treatment with traditional medicine in the form of plant extracts has been reported to give remarkable good results (Sumana and Suryawanshi, 2001). The presence of hypoglycemic property in leaves and flowers of Vinca Rosea has been reported (Chopra et al, 1956). Shroti et al (1963) reported a mild hypoglycemic effect of crude aqueous extract of Vinca Rosea whole plant except roots on alloxan induced diabetic rabbits. A large number of plants were screened in India and elsewhere for their hypoglycemic activity; Mulberry is one of such plants having tremendous therapeutic applications. Extracts of various parts of the plant viz. methanolic extract of root bark (Hikino et al, 1985), ethanol insoluble extract of the leaves (Chen et al, 1995), aqueous extract of the
leaves of Shoot cultures (Kelkar et al, 1996) and the leaves (Kim et al, 1999) were reported to possess antidiabetic effect.

Many herbal products, described for the care of DM in ancient literature of Ayurveda in India, their hypoglycemic action on animals and humans have also been reviewed (Gupta, 1994; Shukla et al, 2000). Artemisia herba alba is reported to have hypoglycemic effect (Twaij and Al-Badr, 1988). Extracts of ripe leaves, tender leaves, fruits and flowers of Azadiracta indica have been reported to possess antidiabetic and antiviral activity (Rao et al, 1969; Bhattacharji et al, 1953). Plant products investigated for antidiabetic effect have been exhaustively reviewed (Ivorra et al, 1989) such as Phyllanthous amarus (Srividya and Periwal, 1995), Pterocarpus marsupium (Manickam et al, 1997), Salacia oblonga (Augusti et al, 1995) etc. Leaves of Coccinia indica (Basu, 1992), Catharanthus roseus (Rastogi Ram and Mehrotra, 1980-84), Momordica charantia (Day et al, 1990), Gymnema sylvestre (Rastogi Ram and Mehrotra, 1990-94) and also the various plant extracts of Aegle marmelose (Sumana and Suryawanshi, 2001), Eugenia jambolana (Upadhyay et al, 1996), Cassia auriculata (Pari et al, 2001), Withania somnifera (Andallu et al, 2000) and Curcuma longa (Nischino et al, 2000), Alium cepa, Alium sativum, Ficus bengalensis and Trignella foenum graecum (Twaij and Al-Badr, 1988) have been reported to have potent hypoglycemic and antidiabetic activity.

In the last few decades, there has been an exponential growth in the field of herbal medicine or phytotherapy (Current Science, 2001). It is getting popularized in developing and developed countries owing to its capacity for treatment of various ailments chiefly because of prohibitive cost of allopathic drugs, unavailability in remote areas and also popular belief that herbal remedies are without any adverse side effects, therefore preferable. Moreover, as is well known, the herbal medicine system has originated as a result of continuous trial and use of various plant parts by the people and the information so generated has been passed from one generation to another. This resurgence and popularity of herbal products led to quantum jump in the number of herbal products swarming the market without any regard for their quality, efficacy and often-new combination of plants are released in the market as a proclaimed remedy for a particular disease. Unfortunately, no accurate information regarding the rational use of drug is available (Dikshit et al, 1990). So, there is a great challenge of conducting clinical research in herbal drugs, bioassays for biological standards, and pharmacological, toxicological evaluation of various animal models for toxicity safety evaluation (Haley et al, 1987).
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.
1.2. PLANTS’ PROFILE

1.2.1. SOLANUM NIGRUM LINN.

1.2.1.1. Introduction

*S. nigrum* Linn. (European Black Nightshade or locally just "black nightshade", Duscle, Garden Nightshade, Hound's Berry, Petty Morel, Wonder Berry, Small-fruited black nightshade or popolo) is a species in the *Solanum* genus, native to Eurasia and introduced in the America, Australasia and South Africa. It is distributed throughout India, Ceylon – All temperate and tropical regions of the world. In India, the plant is found in dry situation as a weed on roadsides and waste lands particularly in Deccan, Malabar, Odisha and Punjab (http://en.wikipedia.org/wiki/Solanum_nigrum).

Habitat according to altitude: Low altitude, interior valleys
Watering conditions: Somewhat dry areas where the drought may last 3 - 5 months. Precipitations of 400 - 800 mm. are concentrated in winter.
Light conditions: Some shadow, some protection against direct sunlight, some shadow from vegetation, filtering about 20 - 40 % of light.

1.2.1.2. Classification

*S. nigrum* is classified under the family Solanaceae and order Solanales. *S. nigrum* was known by botanical synonym *S. rubrum* Mill.

Kingdom : *Plantae*
Sub Kingdom : *Tracheobionta* – Vascular plants
Superdivision : *Spermatophyta* – Seed plants
Division : *Magnoliophyta* – Flowering plants
Class : *Magnoliopsida* – Dicotyledons
Subclass : *Asteridae*
(unranked) : *Angiosperms*
(unranked) : *Eudicots*
(unranked) : *Asterids*
Order : *Solanales*
Family : *Solanaceae*
Genus : *Solanum*
Species : *S. nigrum*
1.2.1.3. **Botanical description:** (Kirtikar and Basu, 1999) and (S. nigrum plant profile, New South Wales Flora Online)

Black nightshade is a fairly common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats. It has a height of 30–120 cm (12-48”), leaves 4-7.5 cm (1 1/2-3”) long and 2–5 cm wide (1-2 1/2”); ovate to heart-shaped, with wavy or large-toothed edges; both surfaces hairy or hairless; petiole 1–3 cm (1/2-1”) long with a winged upper portion. Stem erect, glabrous or more or less pubscent, much divaricately branched. Leaves numerous, ovate-lanceolate, subacute or acuminate, glabrous, thin, entire, sinuate toothed, tapering into the petiole; petioles 2 cm, long. The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers. Flowers small, in extra-axillary subumbellate 3-8-flowered cymes; peduncles 6-20mm long, slender; pedicles 6-10mm long, very slender. Calyx 3mm long, glabrous or nearly so; lobes 5, oblong, obtuse, 1.25mm long, not enlarged in fruit. Corolla 4-8mm long, divided more than 1/2 way down into 5 oblong subacute lobes. Filaments short, flattened, hairy at the base; anthers 2.5mm long, yellow, oblong, obtuse, notched at the apex. Ovary globose, glabrous; style cylindric, hairy. Berry 6mm diameter, globose, usually purplish black, but sometimes red or yellow, smooth, shining. Seeds discoid 1.5 mm diameter, minutely pitted, yellow. In India, another strain is found with berries that turn red when ripe. Sometimes S. nigrum is confused for deadly nightshade, a different Solanaceae species altogether.
1.2.2. MOLLUGO PENTAPHYLLA LINN.

1.2.2.1. Introduction

*M. pentaphylla* Linn. commonly known as carpet weed (English), Pitta saga (Odia) is a perennial herb found throughout India, Ceylon, Malacca, China, Japan, Fiji and also cultivated in some part of Odisha, India. (Kirtikar and Basu, 1999)

1.2.2.2. Distributional range

*M. pentaphylla* is distributed throughout the tropics and subtropics of the Old World, from India to New Caledonia and Micronesia, S.E.Asia, China, Japan, India, Vietnam, Indonesia, Malaysia, Philippines but is rare in Australia. (Kirtikar and Basu, 1999; CSIR, 1948-1976; Hnatiuk, 1990; Keay and Hepper, 1953–1972)

1.2.2.3. Classification (CSIR, 1948-1976; Merrill, 1922–1926; Steenis, 1948; Verma and Mudgal et al, 1993)

*M. pentaphylla* is classified under the family *Molluginaceae* and order *Caryophyllales*. The family molluginaceae contains 16 genera and about 100 species. The family molluginaceae was often included within the family Aizoaceae, but now it is generally accepted that the 16 genera of molluginaceae are not related to the other genera of Aizoaceae (Edwards et al., 2000).

*M. pentaphylla* was known by different botanical synonyms such as *Mollugo stricta* L., *Mollugo sumatrana* Gand.

Kingdom : *Plantae* – Plants
Subkingdom : *Tracheobionta* – Vascular plants
Superdivision : *Spermatophyta* – Seed plants
Division : *Magnoliophyta* – Flowering plants
Class : *Magnoliopsida* – Dicotyledons
Subclass : *Caryophyllidae*
Order : *Caryophyllales*
Family : *Molluginaceae* – Carpet-weed family
Genus : *Mollugo* L. – carpetweed
Species : *M. pentaphylla* L. – *Mollugo*

1.2.2.4. Botanical description

Annual, diffuse, glabrous, 15-30cm high; Stems numerous, with many more or less quadrangular leafy dichotomously arranged branches. Leaves 1.3 – 3.8 cm by 3 – 6mm, in whorls of 2-9, linear - lanceolate to obovate, obtuse or acute, sometimes apiculate, much narrowed at the base; petioles obscure. Flowers white, numerous, in lax corymbose terminal
cymes; peduncles and pedicels filiform; bracts lanceolate, scarious. Calyx glabrous; sepals 1.5-2.5 mm long, broadly elliptic-oblong, obtuse, parallel-nerved. Stamens usually 3. Styles 3, short, linear. Capsules subglobose, as long as or slightly longer than the sepals, with thin walls. Seeds numerous, roundish reniform, compressed, covered with raised tubercular points, dark brown. (Kirtikar and Basu, 1999)

1.2.2.5. Other description (Lavit Kham, 2004; Soerjani et al, 1987; Tavatchai and Maxwell, 1994)

**Biology:** *M. pentaphylla* is propagated by seeds, which are dispersed by water. It is widespread in anthropogenic habitats, and is therefore probably not at risk of genetic erosion.

**Phenology:** Flowering May to November: fruiting June to December.

**Ecology:** In cultivated areas, in regions with or without a pronounced dry season, in sunny or somewhat shaded, often sandy or stony sites; in fields; gardens; premises; railroad bands, teak forests; except lowland irrigated and rice fields.

**Agricultural importance:** A weed of minor importance.

**Cultural control:** It may be difficult to control by tillage because of ready from cut stems.

**Biological control:** *Gibbago Trianthema*, the causal agent of leaf spot on *M. pentaphylla*, was isolated from diseased plants collected in Texas, USA. Plants sprayed with conidia were killed within 9 days. In host-range studies, the fungus was pathogenic only to *M. pentaphylla*. This fungus may be a useful agent for the biological control of this species.

**Chemical control:** Propanil at 2 kg/ha applied two weeds after rice emergence or application 2-4-D at 500 g/ha or Almix at 4g/ha.

**Ecology / Cultivation:** *M. pentaphylla* occurs in semi-arid to humid regions, mostly locally abundant as a minor weed in cultivated areas, including rice fields.

**Remarks:** *M. pentaphylla* is closely related to *Glinus*, and they are differentiated by the presence of a fili-form appendage and conspicuous of *Glinus*. 
Fig. 1.07 The Plant, Aerial parts and Leaf of *M. pentaphylla*