Chapter 1
1. Type 2 diabetes mellitus and Hyperlipidemia

1.1 Definition

Diabetes mellitus is a group of related syndrome associated with hyperglycemia and glycosuria. The disorder results from an insulin deficiency state engendered by either decreased insulin production or by a diminished effect of insulin at the cellular level (RECDCDM, 1997).

1.1.1 Distribution and prevalence

Over 120 million people all over the world suffer from diabetes mellitus (Robert and Jose., 1999). The American Diabetes Association has estimated that one person in fourteen either has or will develop one of the many forms of diabetes in his or her lifetime. Of this population, about 90% will develop this disorder in mid to late life (Laine and Caro, 1996).

1.1.2 Types of diabetes mellitus

There are two main types of diabetes

Type 1 or insulin dependent diabetes: About ten percent people with diabetes have type 1 diabetes. Type 1 diabetes is characterized by beta cell destruction, usually leading to absolute insulin deficiency. Its etiology is either immune mediated or related to physical destruction of the pancreas (as in pancreatitis or pancreatic cancer) or idiopathic.

Type 2 or non-insulin dependent diabetes: Type 2 diabetes presents a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency. About 90 percent have type 2 diabetes. Their bodies produce some insulin but it is either inadequate or defective.
1.1.3 Malnutrition related diabetes:

Diabetes among young people with severe malnutrition and starvation is called malnutrition-related diabetes. Although this condition leads to high blood glucose, some of the complications associated with other types of diabetes are absent. Insulin is necessary to control this condition.

1.1.4 Gestational diabetes:

When a woman develops diabetes during pregnancy or if it is first recognized during pregnancy, she is said to have gestational diabetes. Gestational diabetes most often appears during this period of maximum insulin resistance, and ketoacidosis may be seen particularly in patients with type 2 diabetes mellitus (Gabbe, 1985). The incidence of major congenital malformations is increased approximately fourfold among infants of women with gestational diabetes (Fuhrmann et al., 1983).

1.1.5 Nuclear and mitochondrial mutation associated diabetes

Type 2 diabetes mellitus can arise from any one of a number of mutations, either in the nuclear or mitochondrial genomes. Mitochondrial genomic diabetes mellitus is transmitted as a maternal trait because mitochondrial (mt) DNA is of maternal origin. To date 42 different mitochondrial DNA mutations (point mutation, deletion and duplication) have been found to be associated with the type 2 diabetes phenotype (Rotig et al., 1996).

Patients with type 2 diabetes associated mt DNA mutation (Isshiki, 1997), exhibit hyperglycemia that is due to a significantly reduced insulin secretory capacity that progresses with age (Alcolda and Alcolda, 1994). This hyperglycemia is not due to decreased insulin sensitivity (Walker et al., 1995), aberrant insulin receptor or aberrant mobile glucose transporters (Piccolo et al., 1989). These patients develop a fatty liver, which is not a common feature of diabetes mellitus (Hinokio et al., 1995). Mutation, although numerous, account for less than 5 percent of individuals with type 2 diabetes. More than 44 different mutations in maturity onset diabetes of the young (MODY)
Fig. 1 Proposed scheme where by a small error in form of mitochondrial mutation can ultimately have large consequences over a period of time.
have been reported. Mutations in the genes for insulin receptor (Dreyer et al., 1986),
the genes for insulin (Awata et al., 1997) and the genes for the insulin processing
enzymes (Kahn and Halban, 1997) have also been detected and associated with the
early onset forms of the diseases. Fig 1 gives an over view of the complications, which
will arise due to mitochondrial mutation in type 2 diabetes.

1.1.6 Viruses induced diabetes

In human beings, there may be 2 possible roles for viruses in the pathogenesis
of insulin dependent diabetes mellitus. One is acute cytolytic infection of beta cells
(Coxsackis B viruses), which may sometimes induce diabetes in genetically preposed
individuals, and the other one is slow and persistent infection (e.g., congenital
cytomegalovirus and Rubella) which may induce auto-immunity, leading to type 1
diabetes (Yoon, 1990).

1.1.7 Environmental toxins

Environmental genotoxins are known to be potential risk factors for some
form of diabetes mellitus and neurodegenerative diseases. Genotoxins are known to act
as a slow toxin triggering a cascade of cellular events, which culminates in progressive
cell dysfunction and loss of beta cells and neurons. Cycasin, a toxin obtained from the
cycad plant is of special interest since this agent may be implicated in diabetes
mellitus in the Western Pacific Area (Eizirik et al., 1996).

Other diabetic states also may occur that have etiologies differing from those
of type 1 and type 2 diabetes.

- Diseases of the exocrine pancreas (e.g., pancreatitis); trauma; cystic fibrosis;
  endocrinopathies (e.g., acromegaly, Cushing's syndrome, hyperthyroidism,
  pheochromocytoma).

- Drug induced diabetes - Drugs that commonly cause diabetes during
  therapeutic use are the anti-hypersensitive drugs such as vasodilators
diazoxide and corticosteroids. Body builders who take enormous doses of anabolic androgens can develop impaired glucose tolerance. Several drugs including theophylline, aspirin, isoniazid and nalidixic acid can cause transient hyperglycemia in overdoses (Ferner, 1992).

1.1.8 Complications in type 2 diabetes

The complications of diabetes are heterogeneous group of clinical disorders, which can affect the vascular system, kidney, eye, nervous system and other tissues.

1.1.8.1 Macrovascular disease

Diabetes is an important significant risk factor for coronary heart disease, independent of other variables. Coronary heart disease, stroke and peripheral vascular disease occur with a higher frequency in diabetics due to altered lipid profile (Rabago-Velaso et al., 2000). Cardiovascular disease is the primary cause of early mortality in patients with type 2 diabetes. Large-vessel atherosclerosis involving the cerebral, coronary and peripheral vessels of the extremities leads to increased morbidity and mortality (Schaffer, 1991).

1.1.8.2 Microvascular diseases

Microvascular abnormalities and dysfunction are a systemic disease in diabetes. Clinically microangiopathy leads to retinopathy, nephropathy and possibly contributes to neuropathy.

1.1.8.3 Retinopathy

Retinopathy is reported in both patients with type 1 or type 2 diabetes. However, 20 years after the initial diagnosis of diabetes, the risk of proliferative retinopathy is 20 percent in type 2 diabetic patients. The early and most common form, non-proliferative retinopathy, is a result of small micro-neuroysms within the retinal vessels. Hemorrhage and leakage result in the formation of hard exudates and retinal edema. This may be associated with the loss of central vision. Following this initial
stage, occlusion of vessels can lead to ischemia and infraction, resulting in 'cotton-wool' exudates. With the formation of new blood vessels, bleeding and fibrous changes result in retinal retraction and detachment which can lead to loss of vision (Ceramic et al., 1979). Diabetes leads to accelerated death in situ of both retinal pericytes and endothelial cells, the event specific for vascular cells (Mizutani et al., 1996).

The accumulation of sorbitol due to hyperglycemia in the eyes also increases the risk of development of cataracts to one-and-a-half times that of non-diabetics; an increase in the incidence of glaucoma is also reported among diabetics (SRTRG, 1998). Risk factors for the development of diabetic retinopathy include poor glycemic control and increased duration of diabetes.

1.1.8.4 Nephropathy

Nephropathy has an incidence of 4 to 20 percent in those with type 2 diabetes. Albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with type 2 diabetes (Muller, 1996). Glomerular lesion characterized by proteinuria, decreasing glomerular filtration rate and thickening of basement membrane of the renal tubules leads to chronic renal failure (Osterby, 1993). After several years, microalbuminuria may proceed to albuminuria; at this stage renal function starts to decrease. When this is followed by overt proteinuria, progression to end-stage renal disease is irreversible and patients develop nephrotic syndrome, retinopathy, hypertension, hyperlipidemia and atherosclerosis (Donnelly, 1996).

1.1.8.5 Neuropathy

Neuropathy is the most common complication of diabetes. It is diagnosed based on signs and symptoms, and a neurologic assessment. Neuropathic symptoms usually start as a tingling or burning sensation and a loss of vibratory sense. These are noticed in the calves, ankles and feet. Diabetes changes the nerve electrophysiology and shrinkage of axons and schwann cells and causes reduction in myoinositol content (Grenne et al., 1975) of the diabetic nerve and peripheral nerves showing segmental
demyelination (Clements, 1979). As neuropathy progresses, the patient may lose all sensation in these areas. Atherosclerosis of the blood vessels in these areas may further complicate neuropathy by impairing circulation and predisposing the patient to infection and gangrene. Five major hypotheses have been proposed to explain the pathogenesis of diabetic neuropathy (Thurston et al., 1995).

1. Hypoxia /ischemia.
2. Hyperglycemic pseudohypoxia.
4. Fructose and polyol accumulation and osmotic disequilibrium.
5. Non-enzymatic glycation of macromolecules by fructose and glucose.

1.1.8.6 Connective and joint diseases

Limited joint mobility and stiff-hand syndrome occur exclusively in older patients with type 2 diabetes. In all of these conditions, advanced glycation end products as a result of non-enzymatic reaction of glucose with proteins causes stiffening (Rosenbloom and Silverstein, 1996).

1.1.8.7 Central nervous system

Type 2 diabetes is associated with changes in both the barrier and transport function of the cerebral microvessels. Structural changes in cerebral microvessels may account for alterations in hemodynamic variables such as arteriovenous shunting, changes in biophysical properties and biochemical compositions of the endothelial changes in lipid fluidity and composition and alterations of neurotransmitters activity in the cerebral microvessels, notably altered beta adrenergic neurotransmission (Mooradian, 1997).
1.1.8.8 Urinary Tract infection

The urinary tract is the principle site of infection in diabetics. Changes in the bacterial adhesion to the uroepithelium, partly as a result of a changed and lowered Tamm Horsfall protein and granulocyte dysfunction, possibly as a result of an abnormal intracellular calcium metabolism, are involved in the pathogenesis of urinary tract infection in type 2 diabetics (Geerlings et al., 1997).

1.1.8.9 Pulmonary complications

Non-enzymatic glycosylation of the connective tissues in type 2 diabetes results in thickened alveolar epithelial and pulmonary capillary basal lamina, reduced lung volumes, reduced pulmonary diffusion capacity and elastic recoil (Marvisi et al., 1997).

1.1.9 Blood glucose lowering drugs

Oral medications for the treatment of type 2 diabetes mellitus have been in use since the 1950s. Recently, there has been a marked increase in the number and kinds of drugs available. Sulphonylureas, biguanides, alpha glucosidase inhibitors and thiazolidinediones are the four main types of oral medications prescribed for the treatment of type 2 diabetes.

1.1.9.1 Sulfonylureas

These medications, known as oral hypoglycemic agents, have been in use for decades. Sulfonylureas produce hypoglycemia by blocking ATP-sensitive K+ ATP channels in pancreatic cells (Smoak, 1995). Sulfonylureas may cause blood sugar to go too low (hypoglycemia) and prolonged use tends to diminish their effectiveness. Sulfonylureas may play a role in some types of heart problems. The use of sulfonylureas also is often associated with modest weight gain. Newer sulfonylureas include glimepiride and repaglinide.
Glimepiride is similar to other sulfonylureas, but may have fewer side effects. Most importantly, it may be effective over a longer period of time and be safe for people with impaired kidney function.

Repaglinide (chemically not a sulfonylurea but with similar action) is used for treating type 2 diabetic patients. Repaglinide causes a rapid but short-lived release of insulin by the body, so it acts quickly and is useful when taken shortly before meals. Its effects also diminish quickly (REDCMD, 1997).

1.1.9.2 Biguanides

These medications enhance the ability of tissues to take up glucose and reduce the amount of glucose released by the liver. An older biguanide, phenformin, was taken off the market in the 1970s because it caused lactic acidosis, a serious condition in which sugar is incompletely metabolized.

Metformin an oral biguanide, ameliorates hyperglycemia by improving peripheral sensitivity to insulin, reducing gastrointestinal glucose absorption and hepatic glucose production (Guthrie, 1997) It has beneficial effects on serum lipid profiles. Metformin monotherapy significantly improves glycemic control. Gastrointestinal side effects are common, but usually tolerated. Lactic acidosis is minimal provided that contraindication, particularly renal impairment and prescribing guidelines should be followed (Davidson and Peters, 1997).

1.1.9.3 Alphaglucosidase inhibitors

This type of medication reversibly inhibits alpha-glucosidase enzyme in the small intestine which delays cleavage of oligo and disaccharides to monosaccharides (Wolffenbuttel and Graal, 1996).

Acarbose inhibits alpha-glucosidases in the small intestine, an action that delays the digestion and absorption of complex carbohydrates and it is a very safe and
an effective medication, but many people can't tolerate it because of gastrointestinal side effects that include bloating, gas, cramping and diarrhea (Yee and Fong, 1996).

Miglitol acts in a similar fashion but prolonged use results in gastrointestinal side effects. The glucose lowering effects of both of these agents are modest (Wolffenbuttel and Graal, 1996).

1.1.9.4 Thiazolidinediones

These drugs reduce resistance to insulin and they seem to beneficially influence serum cholesterol and triglyceride levels. The first agent of this class to be released was troglitazone. Troglitazone enhances insulin sensitivity and it is shown to have scavenging effect on reactive oxygen (Inoue et al., 1997) Shortly after its release it was associated with liver test abnormalities leading to several fatalities. It was recently removed from the market.

1.1.10 Type 2 diabetes in animal models and avian

In today's world, the investigation of the pathophysiology of many diseases afflicting mankind is added by the use of animals that replicate one or more features of a single disease. Especially valuable are small rodents that have short life span and reproductive cycles compared to man. This is especially important to the study of genetic diseases, which do not occur until midlife or late adulthood. Diseases that are degenerative in nature or that take several decades to become clinically observable are extremely difficult to understand. Needed are observations of sub-cell and cellular changes that precede tissue and organ changes that in turn develop into a clinical condition of note. In complications such as diabetes mellitus, cardiovascular and renal diseases diagnosis is possible only after clinical symptoms appear. Scientists seeking to understand how the diseases develop and the sequence of biological changes that lead to the clinical state must use animal models, whose disease time frame is considerably shorter than that of the humans. The advantages of animal models are of course encountered with limitations. No animal models of diabetes correspond perfectly to
human diseases. The pathophysiology of most animal diabetic syndrome is poorly understood and difficult to relate to human data. The clinical course of animal with this syndrome may be variable and unpredictable. The vascular sequences of animal diabetes syndrome have also proved to be difficult to study. Nonetheless much of our hope for future progress in diabetes research rests in animals.

Stress, infections, mutations and toxins are reported to provoke diabetes both in human and in animals. Certain other manipulation, including pancreatectomy (Von Mehring and Minkowsky, 1890) and lesioning of central nervous system can also produce diabetes. In the majority of cases the agents used to produce experimental diabetes in animals are not thought to play a major role in human pathophysiology. Nonetheless the ensuing animals syndrome mimic the human diseases and thus merit close attention.

In order to assess the hypoglycemic and other anti-diabetic properties of naturally occurring and synthetic drugs, various workers have used animal models. Apart from using normal animals and genetically modified animals, experimentally induced diabetes is also necessary so that the metabolic derangement's in these animals may mimic as to what happens in human diabetes mellitus and subject them both to the effects of the drug in question.

1.1.10.1 Contra–Insulin Hormone

Epinephrine, glucagon, glucocorticoids and growth hormones all have an effect antagonistic to insulin, and when these hormones are produced in excess (due to stress and tumour) glucose tolerance is reduced resulting in hyperglycemia. Studies have reported the induction of hyperglycemia and beta cell hyperplasia in mice, rabbits, rats, following exposure to hydrocortisone or ACTH (Hausberger and Ramsay, 1959; Cavellerio and Mosca, 1953).
1.1.10.2 Hypothalamic diabetes

Hypothalamic lesions can cause obesity both in animals and humans (Hetherington and Ranson, 1940; Bray and Gallagher, 1975). Some humans with hypothalamic obesity have both insulin resistance and type 2 diabetes mellitus. The best-studied animal model for this syndrome is the rats or mouse with lesions of the ventromedial nuclei of the hypothalamus.

1.1.10.3 Virus induced diabetes

A number of different viruses including encephalomyocarditis virus, mengovirus, Coxsackis B and retroviruses can infect and destroy pancreatic beta cells mainly in rodents (Portwood and Taylor, 1990). In the murine models, the development of encephalomyocarditis and Coxsackie B virus induced diabetes is dependent on the genetic background of the host and the genetic makeup of the virus. Mengo-2T virus has caused diabetes in strains of mice resistant to encephalomyocarditis virus-induced diabetes. In contrast to encephalomyocarditis mengovirus, Coxsackis B and retroviruses seem to be somewhat associated with an auto-immune response in the induction of diabetes. Viral particles have been described in several spontaneously diabetic animals, most notably in db db mice (Like and Chicky, 1970), an animal model proved to be useful in studying the viral etiology with reference to diabetes. Most documented viral diabetic syndrome in animals are associated with generalized illness including rubella, encephalitis, myocarditis and food and mouth diseases (Platt, 1959). Majority of them are RNA viruses and all are highly species specific.

1.1.10.4 Genetically modified animal models

1.1.10.4.1 Rats

1.1.10.4.1.1 Goto-Kakizaki (GK)

The Goto-Kakizaki (GK) rats have defects in glucose stimulated insulin secretion. This model of non-obese type 2 diabetes has been shown to have defects in
vitamin D, OXPHOS (Ishimura et al., 1995, Serrada et al., 1995) and normal bioenergetics (Ferreira et al., 1999).

1.1.10.4.1.2 BHE, BHE/cdb

The BHE/cdb rat, a substrain of the heterogeneous BHE rat strain was developed specifically for the study of type 2 diabetes in the absence of obesity (Berdanier, 1994). Prior to the development of glucose intolerance, various hepatic abnormalities in metabolic control have been observed. Among them are 200% increase in de novo fatty acid and cholesterol (Lakshmanan et al., 1977), 40% increase in gluconeogenesis (Berdanier, 1982) and a 20% reduction in the ATP synthesis efficiency (Berdanier and Thomson, 1986).

1.1.10.4.1.3 Zucker, Zucker diabetic fatty

Zucker rats are obese, one colony display glucose intolerance, glycemic, lipemic, fatty liver, hyperphagic, insulin resistant and renal diseases (Carolyn D Berdanier, 1993).

1.1.10.4.1.4 OLETF (Otsuka Long–Evans Tokushima Fatty) rats

An obese model of type 2 diabetes and genetic studies in these animals have identified one diabetogenic, ODB-1 on the X chromosome. Its gene product is unknown. Cholecystokinin (CCK): A receptor gene expression is absent in the pancreas and the hypothalamus in OLETF rats. In the hypothalamus, this could lead to increased obesity and insulin resistance. Insulin resistance appears to be a primary event since it precedes pancreatic $\beta$ cells function (Ishida et al., 1995).

1.1.10.4.1.5 SHR/N–cp

SHR/N–cp mice are obese; males are mildly hypertensive, hyperinsulinemic, hyperlipidemic (Carolyn D Berdanier, 1993).
1.1.10.4.1.6 Long-Evans-Tokushima-Otsuka (LETO)

Male rats could be made obese by either feeding a high-energy cafeteria diet or giving ventromedial hypothalamus lesions. These rats do not develop type 2 diabetes. Whereas female OLETF rats possessing only one copy of the (diabetogenic) ODB-1 gene developed type 2 diabetes when made obese. Thus, this X gene ODB-1 plays a significant role in the development of obesity responsive diabetes in these animals (Carolyn D Berdanier, 1993).

1.1.10.4.2 Mice

1.1.10.4.2.1 KK, yellow KK Toronto K

KK mice are polygenic with large body size, obese, mildly hyperglycemic, hyperinsulinemic and express vascular complication in one colony (Carolyn D Berdanier, 1993)

1.1.10.4.2.2 A", A' yellow

A", A' yellow mice are obese and they carry a dominant trait on chromosome 2, and they are hyperglycemic and hyperinsulinemic (Sleiker et al., 1992).

1.1.10.4.2.3 P1 PB 13/Ld

P1 PB 13/Ld mice are obese, hyperinsulinemic, lipemic and hyperglycemic (Carolyn D Berdanier, 1993).

1.1.10.4.2.4 db^{+/-}

An obese model for type diabetes with a recessive trait on chromosome 4 and the male population are hyperphagic, hypercholesterolemic and hyperinsulinemic (Carolyn D Berdanier, 1993).
1.1.10.4.2.5 C57 BL/6J (ob/ob)

This model carries an autosomal recessive trait on chromosome 6 and they exhibit transient glucose intolerance, hyperinsulinemic and peripheral insulin resistance, due to down-regulation of receptors and increased glucocorticoids (Worley et al., 1994).

1.1.10.4.2.6 New Zealand obese mouse (NZO)

Fructose 1,6 diphosphatase regulation is defective in the NZO mouse. The NZO mouse is a mildly obese animal model of type 2 diabetes with increased gluconeogenesis and hyperglycemia (Andrikopoulous et al., 1993).

1.1.10.4.3 Hamsters

The diabetic Chinese hamster (CHAD) strain is non-obese and is characterized by altered glucose stimulated insulin secretion (Nakajima et al., 1994). It has been recently shown that these alterations in insulin secretion may be due to decreased GLUT 2, which preceded B cell deterioration (Jorns et al., 1996). Transgenic mice expressing a GLUT 2 anti-sense RNA also showed decreased B cell GLUT 2, impaired glucose stimulated insulin secretion, hyperglycemic and impaired glucose tolerance (Valera et al., 1994)

1.1.10.5 Chemical agents capable of inducing diabetes

The use of chemical agents to produce diabetes permits detailed study of the biochemical, hormonal and morphologic events that occur during and after the induction of a diabetic state. Two agents that have been most extensively studied and have yielded the vast majority of information pertinent to human diabetes are alloxan and streptozotocin, both are B -cytotoxins which in diabetogenic doses are relatively free of non specific toxic effects. In contrast to most other chemical agents capable of inducing diabetes, there is a wide range of safety with these compounds. The effective diabetogenic dose (ED50) is 4 to 5 times lower than the lethal dose (LD50).
1.10.5.1 Alloxan

Alloxan is a pyrimidine with structural similarity to uric acid and glucose. The B cell toxicity of alloxan was discovered serendipitously while testing the hepatotoxicity of uric acid derivatives in rats as well as in rabbits. Alloxan has a complex electronic structure and it exists in several tautomeric forms; it is highly unstable in water at neutral pH and reasonably stable in pH less than 3.

Jacobs, (1937) first reported the diabetogenic effect of alloxan. Later Dunn et al., (1943) studied the histological changes in pancreas of rabbits after alloxan injection. Bailey and Bailey, (1943) reported permanent diabetes with alloxan in rabbits. It has been suggested that alloxan itself is not cytogenic but its metabolites may be responsible for the cytotoxic action. Alloxan is known to chelate intracellular zinc, strecher reaction (alpha aminoacid deamination and decarboxylation), deletion of sulphhydril in the β cell (Rerup, 1970). However action of alloxan appears to be mediated by producing damage to the β cell membrane, as suggested by permeability studies (Watkin et al., 1973) rather than through a primary intracellular action.

Mechanism of action

1. Alloxan is rapidly taken up by the β cells and has a direct effect on islet membrane permeability. Morphologic abnormalities have been described, suggesting the disruption of the β cell membrane (Abdel-Rahman et al., 1992).

2. Alloxan derived oxygen radicals disturb intracellular Ca2+ homeostasis by increasing Ca2+ influx, which results in secondary reactions leading to DNA strand breaks and cytotoxicity of β cells (Kim et al., 1994).

3. Iron released from ferritin may be involved in the diabetic action of alloxan (Sakurai and Ogiso, 1995).

4. Alloxan initiates a complete inhibition of electrical activity and hyperpolarization and a decrease in input resistance indicating increased K(+)
conductance. The delayed inhibition results in the accumulation of alloxan or its metabolites within the cell leading to further complications (Carrol et al., 1994).

5. All deleterious effects of alloxan on permeability, transport, intracellular energy generating pathways and insulin secretion are most probably due to free radical formation (Kawada, 1992).

6. Cytotoxic action of alloxan are thought to be caused by the hydroxyl radical (OH+) generated in a cyclic reaction involving alloxan and its reduction products, alloxan radical (HA+) (Sakurai and Ogiso, 1994).

1.1.10.5.2 Alloxan induced complications in animal models

- Endothelial alterations consistent with injury including adhesion of white blood cells, platelet and fibrin like material to the endothelial surface were reported in the aorta of alloxan diabetic rabbits (Hadcock et al., 1991).

- Hepatic regeneration is lower in alloxan induced diabetic rats and the hepatic micro-circulation undergoes changes and evolve at different rate in the central and peripheral zones of the liver (Aznar Aznar et al., 1991).

- Alloxan induced diabetes in rabbits is shown to accelerate the formation of the intercellular matrix of glomerular loops in proliferative glomerulitis resulting in accelerated glomerulosclerosis (Wannibuchi et al., 1991).

- Diabetic hearts demonstrated significant decrease in the rate of contraction (+dP/dt) and relaxation (-dP/dt), heart rates, cardiac work, low mitochondrial respiration rates in saponin skinned fibres (Dzhavadov et al., 1992).

- Morphological changes in islets in alloxan diabetic rats such as multiple necrosis, marked degranulation and extensive vesiculation of the endoplasmic reticulum, golgi complex, mitochondrial enlargement disrupted cristae and mitochondrial ruptures were prominent (Abdel-Rahman et al., 1992).
Alloxan diabetes in dogs appear to have diminished arachidonic acid metabolism and uptake independent of adrenoreceptors and to induce an imbalance between vasoconstrictor and vasodilator cyclooxygenase products, resulting in elevated TXA2 release controlled by adrenergic mechanism which may contribute to an impairment in myocardial micro-circulation (Koltai et al., 1992).

Alloxan treated rats show reduction in body and bone mass, with greater impact on soft tissues. The following adverse effects are reported (Locatto et al., 1993)

1. A decreased intrabecular bone volume.
2. Increased bone collagen glycosylates.
3. Increased resistance of bone collagen to collagenase hydrolysis.
4. Decreased rate of bone resorption.

3H-Cholecalciferol absorption in alloxan diabetic rat intestine was inhibited and there is a reduction in the absorption by the liver (Apukhovskaia et al., 1993).

Protein glycosylation, leading to formation of covalently modified advanced glycosylated products and protein adducts, leads to various complications as observed in alloxan induced diabetes in Sprague-Dawley rats. Protein glycosylation was reported to increase linearly as a function of period of hyperglycemic in all the tissues, except brain. (Kumari and Sahib, 1993)

A significant increase was reported in the NADH-oxidase activity in brain and kidney microsomes of alloxan diabetic rats (Askar and Baquer, 1994).

Alloxan possessed stimulative and toxic dual effects on the electrical activity of mouse pancreatic β cells (Shen et al., 1994).
Alloxan-induced diabetes in male CD-1 mice is preceded by an increase in pancreatic lipid peroxidation leading to permanent damage to pancreatic tissue (Soto et al., 1994).

Muller cells in the retina of the alloxan diabetic rats presented dispersion of nuclear chromatin and electrondense of nuclear granulation, with the presence of increased glycogen, dense bodies and lysosomes in the cytoplasm (Schellini et al., 1995).

Decreased blood microcirculation in the optic head was reported in alloxan diabetic rabbits (Takahashi, 1995).

In alloxan diabetic hamsters, microvascular permeability and an increase in the number of leucocytes sticking to the venucular endothelium were reported to damage endothelial cells (Bertuglia et al., 1995).

Decreased calcium sensitivity in alloxan diabetic rats was reported in various smooth muscles including gastrointestinal tract and this seems to be closely related to calmodulin levels and this may lead to gastrointestinal complications (Ozturk et al., 1996).

Brush border fucose content and membrane hexosamine levels were reported to be significantly reduced in alloxan diabetic rats (Mittal et al., 1996).

Alloxan reduced whole pancreatic blood flow by 50 percent in Sprague-Dawley rats (Jansson, 1996).

In diabetic rabbits the density of nerve plexus becomes markedly sparse and ultra-structural changes showed swelling of axon, irregular distribution of fibrils and degeneration of mitochondria leading to reduction in the corneal sensitivity in diabetes (Li et al., 1997).

Decrease in the catabolic rates for prothrombin and antithrombin was reported in alloxan diabetic rabbits reflecting the decreased rate of plasma protein production by the liver (Hatton et al., 1997).
Addition of alloxan to cultured rat cells increased the rate of oxidative base damage and the lesion frequency of mt DNA was several fold (Drigger et al., 1997).

Alloxan injection in mice promotes early biochemical changes in nervous tissues e.g. decrease in Na⁺,K⁺-ATPase activity and glutathione peroxidase activity (Martinez-Blasco et al., 1998).

Alloxan induced diabetes in rats showed elevated levels of lipid peroxidation in the blood hemolysate and decreased the activities of antioxidant enzymes and reduced glutathione in liver, heart, kidney and pancreas (Matkovics et al., 1998).

Alloxan treatment increased intestinal adenosine deaminase levels significantly in various tissues leading to immune and other metabolic dysfunction in diabetes condition (Singh and Sharma, 1998).

Antioxidant enzymes activities of rat liver and fore brain are reported to be suppressed in alloxan induced diabetes. Suppression of the antioxidant enzymes is predicted to be major factor for diabetes progression (Kosenko et al., 1999).

Superoxide dismutase and glutathione peroxidase were reported to be significantly decreased, while catalase and xanthine oxidase activities were increased in alloxan induced diabetic rat lens, suggesting increased oxidative stress as the important contributory factor in the pathogenesis of diabetic cataract (Cekic et al., 1999).

Increased activity of plasma alpha-amylase, an indicator of pancreatic exocrine toxicity and degranulation of zymogen and duct like structures of exocrine cells, atrophy and disappearance of islet cells were reported in alloxan diabetic mice (Minami et al., 1999).

Hyperglycaemia is reported to impair endothelium-dependent dilation of coronary arteries. Diminished relaxation of diabetic coronaries is worsened by
the inhibition of the synthesis of vasodilator cyclooxygenase products (Kocsis et al., 2000).

Short-term exposure to alloxan leads to a temporarily elevated insulin release from isolated pancreatic islets and this effect is caused by beta-cell necrosis and believed that hydroxyl radicals are the toxic principle (Ebelt et al., 2000).

Alloxan treatment resulted in beta-cell damages with DNA fragmentation, inhibition of glucose-stimulated insulin release, and decrease of cellular ATP level (Rho et al., 2000).

1.1.10.5.3 Streptozotocin

Streptozotocin, an N-niyo derivative of D-glucosamine was isolated from cultures of Streptomyces achromogenes, but subsequently it has been synthesized in the laboratory. Like alloxan, streptozotocin is relatively selective β cytotoxic in certain animal species. Streptozotocin is transported into β cells through the glucose transporters in the cell membrane and attacks mitochondria. Mitochondrial ATP generation is inhibited and the resulting high concentration of intracellular ADP helps its degradation providing hypoxanthine, a substrate of xanthine oxidase, whose activity is high in β cells. Xanthine oxidase catalizing reaction is followed by increased formation of uric acid and O2- radicals, which damage β cells (Kawada, 1992). Within the β cells streptozotocin is believed to reduce the levels of nicotine adenine dinucleotide (NAD) by decreasing its synthesis and increasing its breakdown. A recent report suggests that both alloxan and streptozotocin may act through a common pathway that is dependent on the formation of single stranded breaks in the β cell DNA (Peschke et al., 2000).

1.1.10.6 Avians

The advent of comparative approach to endocrine problems has sharpened the focus of many investigators on the usefulness of projecting the birds in forefront of diabetes mellitus studies. It is well known that injection of alloxan, an mesoxalic acid
derivative produces diabetes by selectively destroying the β cells. In the case of pancreatectomies in birds, alloxan did not produce diabetes in owls, pigeons, chickens (Scott et al., 1945) and ducks (Mirsky, 1945). In some species only temporary elevation of blood glucose was observed but the β cells remained unaltered (Goldner and Gomori, 1945). Guha, (1976) failed to obtain evidence of diabetes after alloxan administration in four species of Indian birds, viz. Balck munia, Crow, King fisher and Parakeets. Alloxan even at higher concentration did not alter the basal or glucose stimulated insulin levels in chicken; neither was the glucose tolerance impaired. No true diabetic syndrome could be observed by both acute and repeated treatment of streptozotocin, ascorbic and dehydroascorbic in birds. The inability of alloxan to disturb carbohydrates in birds may be due to normal elevated blood glucose levels. Normal blood levels in adult chicken’s range from 200 to 250 mg/dl, compared to 80-110 mg/dl in most mammals (Watkins and Cooperstein, 1973). Therefore in birds unlike mammals and other vertebrates, true insulin deficiency syndrome is not observed and they are not vulnerable to the action of the cytotoxic agents.

1.1.11 Current treatment regime for type 2 diabetes

Type 2 diabetes is a common and undiagnosed condition that poses treatment challenges. The introduction of new oral agents within the past three years has expanded the range of possible combination regimens available for treating type 2 diabetes.

Combinations of different oral agents are used for controlling hyperglycemia before insulin therapy becomes necessary (Campbell, 1996). A stepped-care approach to drug therapy may provide the most rational, cost-efficient approach to management of this disease. Ninety percent of patients with diabetes have type 2 diabetes and often require oral agents or insulin for glucose control (Mooradian, 1996).

Alpha-glucosidase inhibitors, such as acarbose and miglitol, are indicated as monotherapy or in combination with sulfonylureas for management of type 2 diabetes (Campbell, 1997).
Troglitazone is prescribed for patients requiring large daily amounts of insulin (more than 30 units per day) whose diabetes is still uncontrolled. A reduction of up to 50 percent in total daily insulin dosage is possible with drug titration (Inzucchi et al., 1998). Troglitazone is also effective when used in combination with other oral agents, thereby potentially delaying the need to start insulin therapy.

Repaglinide is a suitable option for patients with severe sulfa allergy who are not candidates for sulfonylurea therapy (Sparano and Seaton, 1998). The drug is used as monotherapy or in combination with metformin and it is cautiously in elderly patients and in those with renal or hepatic dysfunction (REDCMD., 1997).

1.1.12 Indigenous treatment for type 2 diabetes

Diabetes mellitus is a metabolic disorder as old as mankind and its incidence is considered to be high (4-5%). Since time immemorial, patients with type 2 diabetes have been treated orally with a variety of plant extracts. Bever, (1980) has reported a list of medicinal plants used traditionally in West Africa for treating type 2 diabetes. A review on antidiabetic drugs was published in 1982 (Wishner, 1982). Nagarajan et al., (1982) has reviewed the work done on 75 Indian plants for their anti-diabetic activity. A recent survey of the literature have shown that most of the work on hypoglycemic property of medicinal plants are carried out with crude extracts (Table 1); therefore there is an urgent need for further studies to identify the active natural principle which is responsible for the hypoglycemic action. This is one logical way of searching for new drugs to treat this disorder.
Table 1  
Lists of plants traditionally used for the treatment of type 2 diabetes and experimentally proved to possess hypoglycemic property.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Botanical name</th>
<th>Animal Models</th>
<th>Extracts/ Compounds</th>
<th>Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Capparidaceae</em></td>
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<td></td>
<td><em>Polygonaceae</em></td>
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<td></td>
<td><em>Pandanaceae</em></td>
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<td></td>
<td><em>Lythraceae</em></td>
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<td></td>
<td><em>Euphorbiaceae</em></td>
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<td></td>
<td><em>Asclepidaceae</em></td>
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<tr>
<td>No.</td>
<td>Plant Species</td>
<td>Condition of Animals</td>
<td>Treatment</td>
<td>Reference</td>
<td></td>
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<tr>
<td>10.</td>
<td><em>Parmentiera edulis</em> L.</td>
<td>Alloxan diabetic rats</td>
<td>Hexane, methanol and chloroform whole plant extracts</td>
<td>Perez-Gutierrez et al., 1998</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Treatment/Condition</td>
<td>Compound/Effect</td>
<td>Ref.</td>
<td></td>
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<tr>
<td>16</td>
<td><em>Bauhinias candicans</em> Benth.</td>
<td>Alloxan and streptozotocin diabetic rats</td>
<td>20% aqueous leaf infusion</td>
<td>+ Lemus et al., 1999</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Leguminaceae</em></td>
<td></td>
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<tr>
<td></td>
<td><em>Galega officinalis</em> L.</td>
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<td></td>
<td><em>Papilionaceae</em></td>
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<td></td>
<td><em>Morus alba</em> L.</td>
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<td></td>
<td><em>Urticaceae</em></td>
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<tr>
<td></td>
<td><em>Rubus ulmifolius</em> Schott.</td>
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<tr>
<td></td>
<td><em>Rosaceae</em></td>
<td></td>
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<tr>
<td>17</td>
<td><em>Otholobium pubescens</em> J.W. Grimes</td>
<td>db/db diabetic mouse</td>
<td>Bakuchiol (1)</td>
<td>+ Krenisky et al., 1999</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fabaceae</em></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>18</td>
<td><em>Xanthium strumarium</em> L.</td>
<td>Streptozotocin diabetic rats</td>
<td>Fruits- Caffeic Acid</td>
<td>+ Hsu et al., 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Asteraceae</em></td>
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<td></td>
<td><em>Asteraceae</em></td>
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</tr>
<tr>
<td>20</td>
<td><em>Psoralium decompositum</em> H.Rob Et Brettel</td>
<td>Alloxan diabetic mice</td>
<td>Root water decoction-Sesquiterpenoids.</td>
<td>+ Alarcon-Aguilar et al., 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Asteraceae</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>21</td>
<td><em>Bidens pilosa</em> L.</td>
<td>C57 BL/Ks-db/db diabetic mice</td>
<td>Aqueous alcohol extract of aerial parts (Acetylenic glucosides)</td>
<td>+ Ubillas et al., 2000</td>
<td></td>
</tr>
</tbody>
</table>
1.2 Hyperlipidemia

1.2.1 Definition

Hyperlipidemia is a group of disorders characterized by an excess of fatty substances, such as cholesterol, triglycerides and lipoproteins in the blood (Tai et al., 1999).

1.2.2 Distribution and Prevalence

The incidence of heart disease is 1 out of 100 people. One in ten American women between in the age 45 to 64 years have some form of heart disease, and this increases to one in four women over 65. Heart disease is the main cause of death in Western countries (Massaro et al., 1999). Two million women have had a stroke, and 93,000 women die of stroke each year.

1.2.3 Types of hyperlipidemia

Hyperlipidemia may be caused by genetic factors, as in certain familial diseases, or by secondary factors in acquired hyperlipidemia (Kostner, 1999). There are 6 types of hyperlipidemia, which are differentiated by the type(s) of lipids, and lipoproteins that are elevated in the blood. Some of the types may be due to a primary disorder such as a familial hyperlipidemia, and some are due to secondary causes, which are related to diseases associated with hyperlipidemia, dietary risk factors, and drugs associated with hyperlipidemia.

1.2.4 Complications

1.2.4.1 Atherosclerosis

Atherosclerosis is caused when fatty substances build up inside the artery walls over time (Goldstein and Brown, 1975) and create an occlusion, which restricts proper blood flow (Assmann et al., 1996). Blood platelets and debris also accumulate at
the plaque deposits further narrowing the circulation (Laakso et al., 1993). This causes restricted oxygen supply to peripheral organs like the brain, backpressure on the major arteries that can lead to high blood pressure and congestive heart failure.

1.2.5 Lipids and lipoproteins—in hyperlipidemia

1.2.5.1 Cholesterol

The risk of coronary heart diseases in persons younger than 50 years is strikingly related to the serum total cholesterol levels (NCEP, 1993). Within so-called normal limits, risk has been found to mount over a five-fold range. The contribution of the total serum cholesterol to risk has also been found to be determined by its partition in the various lipoprotein fractions. A relatively large amount of cholesterol in the LDL fraction is atherogenic, where as cholesterol in HDL fraction appears to be protective (Kannel et al., 1979). Evidence linking elevated blood cholesterol levels to atherosclerosis is overwhelming and is associated with coronary artery diseases in continuous, graded and independent fashion (NCEP, 1993).

1.2.5.2 Phospholipids

The increase in the total phospholipids and changes in their fatty acid composition of the aorta with the progression of atherosclerosis is well documented (Hara and Taketomi, 1990). The plasma of hyperlipidemic patients showed the largest increase in the phosphatidylcholine and a decrease in the sphingomyelin. The fatty acid composition of sphingomyelin was characteristic of higher content of unsaturated fatty acids (Engelmann et al., 1992). The shift in the lipid profile indicates severe pathologic state, which can be directly correlated to atherosclerotic lesion in hyperlipidemia.

1.2.5.3 Triglycerides

Evidence now suggests that a relationship exists between elevated blood triglyceride (TRL) levels and the incidence of coronary artery diseases (CAD) (Reed et al., 1986). Patients with moderately increased levels of triglycerides are often at the risk of
CAD, whereas patients with very high levels of plasma triglycerides are usually not afflicted by coronary diseases (Chait, and Brunzell, 1991). Hyperlipidemia is characterized by an overproduction of VLDL and an increased number of LDL particles, which are small and dense and can be readily oxidized. Hyperglyceridemia have different atherogenic potentials (Chait et al., 1993).

a. A direct action of TRL on lipid accumulation by cells of the artery wall.

b. An indirect effect of TRL on the concentration of other lipoproteins, which are pro or antiatherogenic.

c. An effect of TRL on the coagulability of blood.

1.2.5.4 Low Density Lipoproteins (LDL) and Very Low Density Lipoproteins (VLDL)

Low density lipoprotein (LDL) which transports about 75% of the blood's cholesterol to the body's cells, is normally harmless. The normal exogenous and endogenous pathways of LDL metabolism are shown in the Fig. 2.

Key participants in the atherosclerosis are the accumulation of LDL and focal intimal influx and a preferential recruitment of blood monocytes. Both are further enhanced in the presence of hyperlipidemia. When the quantity of intimal LDL and the oxidative potential of the intima exceed the capacity of macrophages to remove, via the non down regulating scavenger receptor, the excess of Ox-LDL particles injure and kill cells including the foam cells, with the formation of the necrotic extracellular lipid core, a key transitional step in lesion progression (Schwartz et al., 1991). This process is the major contributor to the development of coronary heart disease. In addition, the body forms calcium to wall off the inflamed area in the artery. This brittle, calcified area can be sheared off as blood flows through the artery, resulting in injury and the formation of a blood clot. If blockage occurs caused by either the gradual build-up of plaque or by the much more rapid formation of a blood clot will result in heart attack. In addition to that, endothelial cells derived relaxing factor (EDRF) mediated relaxation
of blood vessels is impaired in vessels exposed to lipoprotein in vitro and in arteries (Tirziu et al., 1995).

1.2.5.5 High Density Lipoproteins (HDL)

HDL particles are found in plasma and lymphs. HDL particles are predominantly involved in reverse cholesterol transport and they mediate the removal of cellular cholesterol by two mechanisms (Eisenberg, 1984).

- The passive description of membrane cholesterol to nascent HDL particles.
- Through the interaction of apo Al with cellular cholesterol to the plasma membrane.

In addition to reverse cholesterol transport, HDL acts as a peripheral oxidative substrate over LDL particles and might protect LDL particles from oxidation (Gordon et al., 1977) High levels of high density lipoprotein (HDL) are as important for health as low LDL levels. High HDL levels appear to protect arteries from dangerous narrowing and so help prevent heart attacks.

1.2.5.6 Other Lipoproteins

Lipoprotein(a) - The molecules have a structure similar to LDL and carry a protein that may deter the body's ability to dissolve blood clots and so may contribute to heart attacks. On the other hand, high levels of Lp(a) may merely be by-products of long-term injury to the arteries that serve only as markers of late-stage atherosclerosis (Netea et al., 1999).

Apolipoprotein A-1 - Apolipoprotein A-1 has been associated with healthy hearts and may be partially responsible for the lower risk for heart disease associated with high levels of HDL (Dammerman and Breslow, 1995).
Apolipoprotein B - Apolipoprotein B (apo B) is associated with high levels of LDL, and one study reported that it may be more effective than other lipids for predicting heart disease in women (Arden et al., 1999).

1.2.6 Heart disease risk factors

Risk factors are habits or traits that make a person more likely to develop a disease. These include:

1.2.6.1 High Blood Cholesterol

High blood cholesterol is another very important risk factor for coronary heart disease. Higher the blood cholesterol levels, higher the heart disease risk (Reed et al., 1986).

1.2.6.2 High Blood Pressure

High blood pressure also known as hypertension is another major risk factor for coronary heart disease and the most important risk factor for stroke and heart failure (Papadakis et al., 1999).

1.2.6.3 Diabetes

Diabetes, or high blood sugar, is a serious disorder that raises the risk of coronary heart disease. Diabetics are more apt to have high blood pressure and high blood cholesterol (Maeda et al., 1993).

1.2.6.4 Obesity and Overweight

Recent studies have shown the predictive power of abdominal distribution of adipose tissue for the development of cardiovascular diseases (Bjorntorp, 1970).
1.2.6.5 Smoking

Cigarette smoking is one of the major risk factors for cardiovascular diseases. Smoking also boosts the risk of stroke. Smoking seems to affect HDL-C and HDL-ester cholesterol levels and lowers the LCAT activity in smokers compared to non-smokers (Dirican et al., 1999).

1.2.6.6 Physical Inactivity

Physical inactivity increases the risk of heart disease. It contributes directly to heart-related problems and increases the chances of developing other risk factors, such as high blood pressure and diabetes (Grundy, 1999).

1.2.6.7 Stress

Commonly reported incident preceding a heart attack is an emotionally upsetting event, particularly one that involves anger (Rashid et al., 2000).

1.2.7 Cholesterol lowering drugs

1.2.7.1 Bile-Acid Binding Resins

These drugs work by binding bile, a substance made by the liver using cholesterol as one of the primary manufacturing components. Because the drugs bind with bile acids in the digestive tract, they are then excreted with the faeces rather than being absorbed into the body. The liver as a result, must take more cholesterol from the circulation to continue constructing bile acids, resulting in decreased LDL levels (Garg and Grundy, 1994). Bile-acid binding resins include cholestyramine and colestipol. Although these drugs pose no major risks, they do have side effects, including constipation, heartburn and other gastrointestinal problems. They also interfere with medications used to treat hypoglycemia (Bandisode and Boshell, 1974).
1.2.7.1.1 Probucol

Probucol lowers LDL-cholesterol levels by 10% to 15%. Unfortunately, it also lowers HDL levels by 20% to 30% (Bagdade et al., 1995). It is generally used for certain genetic disorders that cause high cholesterol levels or when other cholesterol-lowering drugs are ineffective or cannot be used. Common side effects include gastrointestinal discomforts such as diarrhea, bloating, nausea, and dizziness (Lau et al., 2000).

1.2.7.1.2 Nicotinic Acid (Niacin)

Nicotinic acid or niacin is vitamin B3. When used in high doses, it is extremely effective in reducing triglyceride levels and it raises HDL levels higher than any other anti-cholesterol drug (Garg and Grundy, 1990) and it also lowers LDL-cholesterol and lipoprotein(a) (Nakaya et al., 1999).

1.2.7.2 HMG CoA reductase inhibitors

1.2.7.2.1 Statins

Statins inhibit the liver enzyme HMG CoA reductase (Sasaki et al., 1999). They have proven to be the most effective drugs for the treatment of high cholesterol (Alfon et al., 1999). Statins are particularly effective for lowering LDL levels, they also raise HDL levels, but to a lesser extent than other anti-cholesterol drugs (Bagdade, 1990). They are reported to increase nitric oxide, a substance important for flexibility and tone in blood vessel walls, thereby allowing blood to flow more freely. They may even have some preventive effect on blood clotting (Alfon et al., 1999). Statins include lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin and cerivastatin. All are effective and safe.

1.2.7.2.2 Fibrin Acid Derivatives

Fibrin acid derivatives or fibrates are prescribed to lower triglyceride levels and increase HDL and they are also reported to produce modest reductions in LDL.
levels and decreases VLDL synthesis (Kinoshita, 1999). Gemfibrozil is the standard
fibrate; a newer member of this group is fenofibrate. They are reported to interact with
a number of drugs used for diabetes, certain antibiotics and grapefruit juice.

1.2.8 Hyperlipidemia in animal models

1.2.8.1 Rico rats

The genetically hypercholesterolemic RICO rat shows a different lipoprotein
spectrum than man after the addition of cholesterol to its food, because approximately
70% of the plasma cholesterol is carried by HDL (Lutton., 1999). It is good model for
testing a food substance or a drug specific for a key enzyme involved in cholesterol
metabolism.

1.2.8.2 JCR : LA-corpulent rat

The JCR:LA-corpulent rat is one of the strains incorporating the corpulent (cp)
gene. Animals homozygous for the cp gene are obese, insulin-resistant and
hyperlipidemic (decreased unesterified cholesterol and hypertriglyceride) (Dolphin et al.,
1990).

1.2.8.3 E*3-Leiden transgenic mice

Hyperlipidemic apolipoprotein (APo) E*3 Leiden mice have impaired
chylomicron and VLDL remnants metabolism. Cholesterol fed E*3 Leiden mice showed
increased levels of cholesterol and triglycerides (Van Vlijmen et al., 1999)

1.2.8.4 New Zealand White (NZW) Rabbits

New Zealand White (NZW) rabbits have low plasma total cholesterol
concentrations, high cholesterol ester transfer protein, low hepatic lipase activity, and
lack an analogue of human apoprotein A-II providing a unique system in which to
assess the effects of human transgenes on plasma lipoprotein and atherosclerosis
susceptibility (Brousseau and Hieg, 1999).
1.2.8.5 Watanabe Heritable Hyperlipidemic (WHHL) Rabbits

An animal model of familial hypercholesterolemia- HDL deficiency, atherosclerotic lesion in the coronary arteries (Matsuo et al., 1995), accumulation of subendothelial macrophage-derived foam cells, few functional LDL receptors (Shimano et al., 1990; Rosenfield et al., 1992) and show five fold increase in the neutral glycosphingolipids in the aorta (Hara and Taketomi., 1991).

1.2.8.6 St. Thoma's Mixed Hyperlipidemic (STMH) Rabbits

St. Thoma's Mixed Hyperlipidemic (STMH) rabbits are a putative model for familial combined hyperlipidemia. It exhibits elevation in cholesterol and triglycerides levels (Arden et al., 1999).

1.2.8.7 Atherosclerosis prone Japanese quail

Cholesterol fed HAP quail showed HDL-predominant pattern and a marked increase in the cholesterol ester levels. (Nagata et al., 1997).

1.2.8.8 Hamster

Hyperlipidemic diet fed hamsters showed characteristic proliferation of the subendothelial matrix and the appearance of liposome like structure in the intima. Like in human atherosclerotic plaque there was a progressive accumulation of extracellular unesterified cholesterol, calcium deposits and necrosis. Hamsters appear to be a suitable model for studying the molecular cellular events leading to obstructive coronary atherosclerosis (Sima et al., 1990).

1.2.8.9 Artificial-cholesterol induced hyperlipidemia

Quantitative and qualitative alteration in the steroid composition in the bile and feces were accompanied by changes in the intestinal morphology (Sable et al., 1990).
Proliferation of the subendothelial matrix and the appearance of liposome like structures in the intima and appearance of smooth muscle cells in the intima and adherence and penetration of the monocytes through the endothelium are reported in artificial cholesterol fed hamsters (Sima et al., 1990).

Modest numbers of blood monocytes are reported to be attached temporarily to the endothelium of large arteries in high cholesterol fed swine (Kim et al., 1990).

Continuous feeding hypercholesterolaemic inducing diet increased the bile flow, biliary secretion of bile acids and inorganic electrolytes in the Wistar rats (Monte and Jimenez, 1993).

High cholesterol is reported to enhance the content of diene conjugate, the activity of phospholipase A2 in rat liver and decrease in the hepatic cholesterol esterases (Chirkin et al., 1994).

Atherogenic diet fed rats showed increased cholesterol content in the aorta and in the liver thereby increasing the atherosclerotic lesion in the coronary arteries (Matsuo et al., 1995).

Increases in the frequency of endothelial cell turnover and endothelial permeability to large molecules in the aorta are reported in the cholesterol fed rats (Lin and Ding, 1996).

High cholesterol diet decreases the activity of glutathione peroxidase with increased formation of peroxide in the colon (Tseng et al., 1996).

Cholesterol feeding in rats increased the residence time of LDL in the lesioned aortic arch (Tozer and Carew, 1997).

Cholesterol feeding doubled the bile acid pool sizes with increased cholic acid synthesis and decreased the activity of cholesterol 7 alpha-hydroxylase (Xu et al., 1998).

Cholesterol enriched diet caused a significant increase in the total LDL, HDL-cholesterol, plasma MDA and post heparin total and hepatic lipase activities
with marked alteration in the aortic wall with the appearance of large multiple atheromatous (Ismail et al., 1999).

Fecal bile acid output that reflected bile acid synthesis increased 2 to 2.4 times while the classic bile acid synthesis was inhibited in the cholesterol fed rabbits (Xu et al., 2000).

1.2.9 **Current treatment regime for hyperlipidemia**

1.2.9.1 **Pharmacologic Therapy**

Niacin is a first choice when drugs are required because of its low cost and its efficacy in altering multiple lipid fractions. The combination of diet, bile acid sequestrants and niacin reduced progression of atherosclerosis and appearance of new lesions in patients with and without coronary bypass grafts. Nicotinic acid and fibric acid derivatives, which are known to decrease triglycerides and increase HDL-C, is prescribed for CHD patients with mixed hyperlipidemia and low HDL levels. All medications have side effects and potential risks must be balanced with potential for benefit before their use can be justified. Lifestyle factors that significantly aggravate hypertriglyceridemia and low HDL-C levels are obesity, smoking and sedentary lifestyle. Treatment should be individualized and targeted to the causative factor(s).

1.2.9.2 **Obesity/Overweight and Excess Calories**

Frequently, weight loss alone can significantly decrease plasma triglycerides and combined with a program of regular exercise, HDL-C levels may increase 10 to 20 percent.

1.2.9.3 **Carbohydrate**

A high carbohydrate diet has been shown to increase plasma triglycerides and decrease HDL-C levels. These diets lead to the production of large buoyant VLDL particles, which are thought to be less atherogenic compared to dense VLDL particles.
The step-one and step-two diets should emphasize complex carbohydrates and fiber for the treatment of elevated triglycerides.

1.2.9.4 Fish Oil

Diets high in fish are recommended because they are associated with reduced CHD risk. Fish oils and omega-3 fatty acids result in decreased triglycerides and may increase LDL-C and/or apolipoprotein B level(s) (Kim et al., 1990).

1.2.9.5 Exercise

Exercise increases HDL-C and decreases plasma triglycerides and the risk of CHD. In general, intervention studies report a 10 to 20 percent increase in HDL-C in response to an exercise program. A program of regular exercise is important in achieving and maintaining a healthy weight (Wynadham, 1979).

1.2.9.6 Cigarette Smoking

A recent study suggests that passive smoking also decreases HDL-C. Smoking cessation increases HDL-C and reduces CHD risk (Dirican et al., 1999).

1.2.10 Indigenous treatment for hyperlipidemia

Recently, the industrial world has seen increase in problems due to hyperlipidemia and nearly 85 percent mortality in type 2 diabetic patients is due to dyslipidemia (Somogyi, 1993). There is, consequently, an increasing demand for a medical treatment, with a low frequency of side effects for this problem. The currently available hypolipidemic agents lack the desired properties of an ideal drug and often result in patient’s non-compliance. Traditionally medicinal plants are used to treat hyperlipidemia (Birgitte et al., 1995; Ritu Mathur et al., 1996; Ram et al., 1997; Sharma et al., 1997) and they are reported to be effective. Table 2 lists few plants, which are reported to be used to treat hyperlipidemia.
Table 2  
Lists of plants traditionally used and experimentally proved to possess hypolipidemic property.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Botanical name</th>
<th>Animal Models</th>
<th>Extracts/ Compounds</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Terminalia arjuna</em> Wet A. Combretaceae</td>
<td>Diet-induced hyperlipidaemic rabbits</td>
<td>50% ethanolic bark extract</td>
<td>+</td>
<td>Ram <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>2.</td>
<td><em>Coffea arabica</em> L. Rubiaceae</td>
<td>Hypercholesterolemic rats</td>
<td>Diet containing beans</td>
<td>+</td>
<td>al Kanhal, 1997</td>
</tr>
</tbody>
</table>
| 8. | **Olea europaea L.**
|    | **Oleaceae** | Hypercholesterolemic
|    |              | insulin-resistant sand
|    |              | rats.  
|    |              | 10 per cent leaf
decoction  
|    |              | +  
|    |              | **Bennani et al., 1999**  
| 9. | **Cicer arietinum L.**
|    | **Fabaceae** | Hypercholesterolemic
|    |              | rats fed a diet
diet containing heated
|    |              | chickpea  
|    |              | +  
|    |              | **Zulet et al., 1999**  
| 10. | **Eugenia uniflora L.**
|     | **Myrtaceae** | Hyperglycemic and
|     |              | hypertriglyceridemic
|     |              | mice.  
|     |              | EtOH (70%) leaf extract  
|     |              | +  
|     |              | **Arai et al., 1999**  
| 11. | **Salvadora persica L.**
|     | **Salvadoraceae** | Hypercholesterolemia
|     |              | rat.  
|     |              | Aqueous stem extract  
|     |              | +  
|     |              | **Galati et al., 1999**  
| 12. | **Suaeda fruticosa**
|     | **Forsk. Chenopodiaceae** | Hypercholesterolaeemic
|     |              | and insulin-resistant
|     |              | sand rat  
|     |              | Aqueous leaf extract  
|     |              | +  
|     |              | **Bennani et al., 1999**  
| 12. | **Curcuma comosa**
|     | **Roxb Zingerberaceae** | Hypercholesterolemic
|     |              | hamster  
|     |              | Ethyl acetate rhizome
|     |              | extract  
|     |              | +  
|     |              | **Piyachaturawat et al., 1999**  
| 13. | **Cucuma longa L.**
|     | **Scitaminaceae** | Cholesterol fed rabbits
|     |              | Ethanol aqueous
|     |              | rhizome extract  
|     |              | +  
|     |              | **Ramirez et al., 1999**  
| 14. | **Moringa oleifera**
|     | **Lam. Moringaceae** | High-fat diet fed Wistar
|     |              | rats  
|     |              | Crude leaf extract  
|     |              | +  
|     |              | **Ghazi et al., 2000**  

Scope of the present investigation

Our ancestors were solely dependent on plants to fulfill their dietary requirements for curing various ailments and their diet was exceedingly rich in phytochemicals as a result they had low risk of any chronic diseases, from cancer to type 2 diabetes. Recently there has been an explosion of research concerning the health benefits of phytochemical in food and medicinal plants.

Over 343 plant extracts and some proteins, flavanoids, steroids, terpenoids are known to be used for the treatment of type 2 diabetes (Atta-Ur-Rahman and Khurshid Zaman, 1989). Plant drugs are frequently considered to be less toxic and free from side effects. Long term use of oral synthetic hypoglycemic agents are reported to increase serum lipids (Shipp et al., 1964) and produce serious side effects including hematological, cutaneous, gastro-intestinal reactions, hypoglycemic coma and disturbances of liver and kidney (Katin and Schecter, 1991). Although insulin has become one of the most important therapeutic agents known in the medicine, efforts continue to find insulin substitute from plant source for the treatment of type 2 diabetes. But still information on insulin substitute from plant source is sparse. Therefore, search for more effective and safer hypoglycemic agents from plants, which also possess hypolipidemic property, has continued to be an area of active research.

In this context Ziziphus mauritiana Lam. (Rhamnaceae), a plant known for its sour edible fruits, its leaves are reported to possess hypoglycemic property (Mohapatra et al., 1976) and traditionally used to treat diabetic patients. The leaves are used for the treatment when they turn into mild yellow colour (Aydin et al., 1995) and the alkaloid extracts were proved to be responsible for the hypoglycemic property of leaves of Z. mauritiana (Mohapatra et al., 1976).

A detailed study in-terms of identifying the alkaloid from the leaves of Z. mauritiana and studying its hypoglycemic and hypolipidemic property, is one step further for a search of a drug which is more effective and safer and that can be used to
to treat hyperglycemic and hyperlipidemic conditions which are manifested in type 2 diabetes.

**Objectives of the study**

- To identify, isolate and characterize the alkaloid from the leaves of *Ziziphus mauritiana* Lam. responsible for the hypoglycemic property using Electronic spectrum, Infrared spectrum, GC-Mass spectrum, ¹H NMR and ¹³C NMR.

- To investigate its hypoglycemic property in non-diabetic and alloxan diabetic rats with respect to dose dependent effect, serum insulin levels and serum glucose tolerance tests.

- To evaluate the long-term effect of the alkaloid on alloxan induced diabetic rats. Various biochemical profiles such as serum glucose, serum and tissue proteins, serum urea, liver glycogen, serum and tissue lipids, enzyme markers, antioxidant enzymes, lipid peroxides and enzymes related to carbohydrate and lipid metabolism will be assayed. All the effects shall be compared with that of a standard hypoglycemic drug, glibenclamide.

- To investigate the hypolipidemic effect of the alkaloid on cholesterol fed rats. Various biochemical profile such as serum and tissue lipids, lipid peroxides, enzyme markers, antioxidant enzyme, faecal sterols and bile acid excretion and enzymes related to lipid metabolism will be assayed and the effects shall be compared with that of a standard hypolipidemic drug, gemfibrozil.

- To evaluate the effect of the alkaloid in acute and chronic study in normal rat models by monitoring various biochemical, hematological and histopathological changes.