CHAPTER II

Literature review

2.1. Etiological classification of diabetes mellitus (currently recommended by WHO and ADA) (Peter H, et al., 2005).

(i) Type 1 diabetes (previously called as insulin-Dependent Diabetes Mellitus) involves absolute insulin deficiency due to an autoimmune destruction of the insulin producing β-cells in the islets of Langerhans; (ii) Type 2 diabetes (previously called as Non-Insulin-Dependent Diabetes Mellitus) is characterized by relative insulin deficiency due to decreased effect of insulin in the target tissues e.g. muscles and adipose tissue (insulin resistance) or due to secretory defect of insulin with or without insulin resistance; (iii) Gestational Diabetes (GDM) is characterized by carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy, and (iv) Other specific types of diabetes. In these forms of diabetes, the underlying defect or disease process can be identified in a relatively specific way or those that have other distinctive, distinguishing features, associated with particular diseases or syndromes with a distinct etiology (Table 2.1).

Impaired glucose tolerance (IGT): It is a stage of impaired glucose regulation that is present in individuals whose glucose tolerance is above the conventional normal range but lower than the level considered diagnostic of diabetes (Gavin JR III, et al., 1997; WHO, 1999). IGT cannot be defined on the basis of fasting glucose concentrations; an OGTT is needed to categorize such individuals. Persons with IGT do have a high risk of developing diabetes, although it is not so in all individuals (Edelstein SL, et al., 1997).

Impaired Fasting Glucose (IFG): It is also a stage of impaired glucose homeostasis. This category was introduced in the 1997 ADA and 1999 WHO classification to include individuals whose fasting glucose levels were above normal but below those diagnostic for diabetes (Gavin JR III, 1997; WHO 1999).
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HNF4α, hepatic nuclear factor 4α; MODY, maturity onset diabetes of the young; HNF1α, hepatic nuclear factor 1α; IPF1, insulin promoting factor 1; HNF3β, hepatic nuclear factor 3β.
2.1.1. **Etiology and clinical presentation of Type 1, Type 2 and few specific types of diabetes mellitus.**

(i) **Type 1 Diabetes Mellitus.** The etiology of Type 1 diabetes is multifactorial. The extremely wide geographic and racial variability in both the incidence and prevalence of Type 1 diabetes suggests that genetics play a significant role in the etiology of the disease. The single most important genetic determinant is the histocompatibility locus (HLA) on chromosome 6, in particular HLA-DR (DR3 and DR4) and HLA-DQ (DQ B1 * 0302 and DQ B1 * 0201) alleles (Eisenbarth GS, et al, 1994). However, Type 1 diabetes is a polygenic disorder with at least 15 different genes contributing in varying degrees. Further, the discordance in development of Type 1 diabetes between identical twins (30%) indicates that the etiology is only partly genetic. Epidemiologic studies demonstrating seasonality for the incidence of Type 1 diabetes, as well as outbreaks and secular trends, provide further evidence of nongenetic environmental involvement in the pathogenesis of Type 1 diabetes (Hagglof B, et al, 1990; Bodansky HJ, et al, 1992; Elliott RB, 1992; Kaprio J, et al, 1992; Karvonen, et al, 1993). Risk factors for which evidence exists dietary triggers (Cow’s milk, caffeine and nitrates), enterovirus infections during pregnancy, and possibly psycho-social events in early childhood (Wilander E, et al, 1975; Scott FW, et al, 1988; Tuomilehto J, et al, 1990; Dahlquist GG, et al, 1990; Martin JM, et al, 1991; Gerstein HC, 1994; Virtanen SM, et al, 1994; Akerblom HK, et al, 1999). There is no evidence that immunization have a causative or protective role in the development of Type 1 diabetes (Harada M, et al, 1990; Bloom L, et al, 1991; Hummel M, et al, 1996; Heijbel H, et al, 1997).

Type 1 diabetes is a chronic disease characterized by hyperglycaemia due to absolute deficiency of insulin secretion. The clinical representation ranges from mild nonspecific symptoms or no symptoms to coma. Although Type 1 diabetes usually develops before 30 years of age, it can occur at any age. At presentation, most patients are thin and have experienced weight loss, polyuria (including enuresis in children), polydipsia, and fatigue. Approximately 25% of individuals with Type 1 diabetes present with diabetes ketoacidosis (Pinkey JH, et al, 1994).

Most individuals with Type 1 diabetes have immune-mediated disease. This form of diabetes is caused by cellular-mediated autoimmune destruction of the insulin-
producing β-cells of the pancreas. An environmental insult, such as a virus, exposure to an allergen, or both, is believed to initiate the process in genetically susceptible individuals. This external influence precipitates an inflammatory response in the pancreas known as insulitis (Foster DW, 1988). Activated T-lymphocytes infiltrate the islet cells in the pancreas. Macrophages and T-cells appear to be implicated in β-cell destruction via localized release of cytokines (Gale EA, 1996). Cytotoxic amounts of nitric oxide and reactive oxygen intermediates are also released, contributing to free radical damage to the β-cells. The initial steps in free-radical induced islet cell death involve breaks in DNA strands and the activation of the enzyme poly (ADP-ribose) polymerase (PARP). PARP is involved in DNA repair and consumes large amounts of NAD+ in the process. The depletion of intracellular NAD+ pools leads to islet cell death (Heller B, et al., 1997). The inflammatory response is autoimmune mediated and takes place on the surface of the insulin-producing β-cells such that these cells are no longer recognized by the immune system. Antibodies against the β-cells are produced, resulting in their destruction and the clinical appearance of diabetes. This destruction is thought to occur slowly, over the course of several years in many cases (Foster DW, 1988). Some viruses seem to attack and destroy the β-cells directly, rather than initiating an autoimmune reaction (Mijac V, et al., 1995). A Venezuelan study conducted by Mijac, et al. reported a mumps infection prior to the onset of diabetes in 42.5% of subjects with IDDM vs. a 12.5% incidence in control subjects (Mijac V, 1995). Elevated levels of Coxsackie virus IgM antibodies have been reported in patients with newly diagnosed type 1 diabetes (Schmerthaner G, 1995). Large prospective studies have also found that exposure to enterovirus infections either in utero or during childhood may initiate β-cell damage and subsequent type 1 diabetes (Schmerthaner G, 1995). Other viral infections, including rubella and chicken pox, had no statistically significant correlation (Mijac V, et al., 1995). There is a smaller group of people with Type 1 diabetes who have no evidence of autoimmunity or other known etiology, a condition termed as “idiopathic Type 1 diabetes.” These patients often have severe but varying levels of insulin deficiency and are prone to diabetic ketoacidosis. (Vardi P, et al., 1991; Verge CF, et al., 1996; Kulmala P, et al., 1998).

Molecular studies have revealed that (i) the classical Caucasian autoimmunity favouring AH 8.1 (HLA-A1 B and DR3) is rare in Indian population and has been
replaced by a variant AH 8.1v that differs from the Caucasian AH 8.1 at several gene loci, (ii) AH 8.2 (HLA-26 B8 DR3) is the most common DR3 positive haplotype in this population and resembles the Indian AH 8.1v rather than Caucasian AH 8.1, and (iii) there are additional HLA-DR3 haplotypes HLA-A24 B8 DR3 (AH 8.3), A3 B8 DR3 (AH 8.4) and A31 B8 DR3 (AH 8.5) that occur in the Indian population (Mehra Nk, et al., 2002; Witt CS, et al., 2002 and Kanga U, et al., 2004).

(ii) Type 2 Diabetes Mellitus. Type 2 diabetes is a complex heterogenous disorder with both genetic and environmental determinants and characterized by elevated levels of plasma glucose, caused by impairment in both insulin secretion and action. Twin studies, which show that concordance for Type 2 diabetes is twice as high in monozygotic twins as in dizygotic twins (34-80% vs 16-40%) (Anand S, Yusuf S, 1998), provide strong evidence for heritability. This is further supported by the finding that Type 2 diabetes is more common in certain ethnic groups and in offsprings of parents with diabetes (Rewers M, Hamman RF, 1995). Familial aggregation of the disease is another source of evidence for a genetic contribution to the disease although; families also share common environmental traits. The odd ratio for offspring of a single affected parent is 2.5 compared to those with no parental diabetes history and this increases to 6.62 if both parents are affected (Mohan V, et al., 2003).

With the exception of rare forms of Type 2 diabetes [such as Maturity-onset diabetes of the young (MODY) and diabetes deafness syndrome], which account for <1% of all cases of diabetes, the specific genetic defects implicated in most cases of diabetes are unknown (Kahn CR, et al., 1996). Nevertheless, studies in nondiabetic relatives of individual with Type 2 diabetes suggest that insulin resistance is a primary inherited defect that occurs early in the course of Type 2 diabetes (Eriksson J, et al., 1989). Insulin secretion by the pancreatic β-cell is initially sufficient to compensate for insulin resistance, thereby maintaining normal blood glucose levels. However, in patients destined to develop Type 2 diabetes, insulin secretion eventually fails, leading to hyperglycaemia and clinical diabetes (Warram JH, et al., 1990). Obesity contributes to the development of diabetes by further increasing insulin resistance; the effect may be particularly pronounced in people with family history of diabetes. Other environmental

As Asian Indians have an increased susceptibility to diabetes and have increased insulin resistance, they are a unique population for carrying out genetic studies. There appears to be certain genes which predispose Indians to diabetes while other genes (eg. Pro 12 Ala polymorphism of PPAR gamma gene) which afford protection against diabetes and insulin resistance to Caucasians, do not appear to protect Indians. Further studies are needed to unravel the genetics of diabetes in South Asians (Mohan V, et al., 1985, Mohan V, et al., 1986, Yajnik CS, et al., 2002; Joshi R, 2003).

(iii) Gestational Diabetes Mellitus. Pregnancy is associated with profound hormonal changes that have a direct effect on carbohydrate tolerance. In early pregnancy, progesterone and estrogen rise but counterbalance each other in terms of insulin action, in that progesterone causes insulin resistance and estrogen is protective (Ryan EA, et al., 1988). Once the second trimester is entered, human placental lactogen (hPL), cortisol, and prolactin (but particularly hPL) all rise, causing decreased phosphorylation of insulin receptor substrate-1 (Friedman JE, et al., 1999) and profound insulin resistance. (Ryan EA, et al., 1988; Kuhl C, 1998). In most subjects, pancreatic insulin secretion rises to match this need, (Agardh CD, et al., 1996) but in those with underlying β-cell defects,
hyperglycaemia ensues (Catalano PM, et al., 1991; Catalano PM, et al., 1993; Nicholls JSD, et al., 1995; Bowes SB, et al., 1996; Kautzky-Willer A, et al., 1997). The insulin resistance of pregnancy is exaggerated in those with gestational diabetes, especially if fasting hyperglycaemia is present, (Ryan EA, et al., 1985; Catalano PM, et al., 1999) and is related to additional defective tyrosine phosphorylation of the insulin receptor β-subunit (Friedman JE, et al., 1999). It is the defect in insulin secretion that is likely the most critical in determining carbohydrate tolerance. Postpartum women with a history of gestational diabetes typically return to euglycaemia, but defects in insulin secretion and action are still evident (Ward WK, et al., 1985, Ryan EA, et al., 1995). Thus, underlying defects in both insulin secretion and action are likely present before pregnancy; if faced with the stress of insulin resistance induced by hormonal changes during gestation, GDM results.

(iv) Other Specific Types of Diabetes Mellitus. Maturity-onset diabetes of the young (MODY) is a relatively rare monogenic disorder characterized by non-insulin-dependent diabetes with autosomal dominant inheritance and an age at onset of 25 years or younger. Patients are non-obese and their hyperglycaemia is due to impaired glucose-induced secretion of insulin. Five types of MODY have been described, with single gene defects localized to chromosomes 20, 7, 12, 13, and 17. Except for MODY 2, all types involve mutations of a nuclear transcription factor that regulates islet gene expression (Mohan V, et al., 1986; Froguel P, et al. 1993; Yamagata K, et al., 1996; Stoffers DA, et al., 1997, Horikawa Y, et al., 1997; Malecki M, et al., 1999).

a. MODY 1 includes 74 members of a pedigree known as the R-W family, who are descendants of a German couple who immigrated to Michigan in 1861. Their extremely rare genetic defect was shown to be a nonsense mutation of a nuclear transcription factor found in liver as well as in pancreatic β-cells. This gene has been termed hepatocyte nuclear factor 4 alpha (HNF-4α) and is found on chromosome 20. How it reduces glucose-induced insulin secretion has not yet been clarified. Six families with mutations of this gene have been reported.

b. MODY 2 have been described in all parts of the world, and at least 26 different mutations of the glucokinase gene on chromosome 7 have been identified. Reduced
sensitivity of pancreatic β-cell glucokinase to plasma glucose causes impaired insulin
secretion, resulting in fasting hyperglycaemia and mild diabetes. Most of these patients
have a benign course without long-term complications and respond well to diet or oral
agents.

c. MODY 3 are caused by mutations of the hepatocyte nuclear factor-1 alpha, whose
gene is located on chromosome 12. Approximately two-thirds of all known cases of
MODY are due to MODY 3, with 41 different mutations reported in 61 families. This
transcription factor is expressed in pancreatic β-cells as well as in liver and is a weak
transactivator of the insulin gene. This may explain how mutations of hepatocyte nuclear
factor-1 alpha (HNF-1α) impair glucose-induced insulin secretion. Unlike most type 2
diabetes, there is no associated insulin resistance, but the clinical course of these two
disorders is otherwise similar regarding prevalence of micro-angiopathy and failure to
continue to respond to oral agents with time.

d. MODY 4 results from mutation of a pancreatic nuclear transcription factor known as
insulin promoter factor-1 (IPF-1), whose gene is on chromosome 13. It mediates insulin
gene transcription as well as regulates expression of other β-cell-specific genes such as
glucokinase and the glucose transporter-2. When both alleles of this gene are
nonfunctioning, a genesis of the entire pancreas results; but in the presence of a
heterozygous mutation of IPF-1, a mild form of MODY has been described in a family in
whom affected individuals developed diabetes at a later age (mean onset at 35 years) than
occurs with the other forms of MODY in which onset generally occurs before the age of
25 years.

e. MODY 5 have recently been reported in a Japanese family with a mutation of hepatic
nuclear transcription factor-1 Beta (HNF-1β), which acts with the 1 alpha factor to
regulate gene expression in pancreatic islets. This mutation caused a moderately severe
form of MODY with progression to insulin treatment and severe diabetic complications
in those affected. In addition, a nephropathy was seen in affected individuals prior to the
onset of diabetes, suggesting that decreased levels of this transcription factor in the
kidney, where it is also normally expressed in high levels, may contribute to renal
dysfunction.
In Asian Indians, type 2 diabetes occurs earlier and overlap with MODY, but the genetics of the latter is unknown. Anuradha S, et al., (2005) performed a study to estimate the prevalence of a common polymorphism of the HNF1-α gene, the Ala 98 Val, in five different types of diabetes in Asian Indians including MODY and a control group of glucose tolerant subjects to evaluate its role in conferring risk of diabetes in Asian Indians. When the age of onset for the disease with the three associated genotypes were compared, it was found that the mean age of onset was earliest in the homozygote Val/Val genotype (mean age of 24.8 yr) compared to Ala/Val (mean age of 29.9 yr), and Ala/Ala (mean age of 35.7 yr). The Val/Val genotype thus appears to trigger the condition almost 11 year earlier than those with the Ala/Ala genotype. This shows that in Asian Indians, the Ala98Val polymorphism of HNF-α gene is associated with MODY and with earlier age at onset of type 2 diabetes (Anuradha S, et al., 2005).

(v) Diabetes due to mutant insulins. This is a very rare subtype of non-obese type 2 diabetes, with no more than ten families having been described. Since affected individuals were heterozygous and possessed one normal insulin gene, diabetes was mild, did not appear until middle age, and showed autosomal dominant genetic transmission. There is generally no evidence of clinical insulin resistance and these patients respond well to standard therapy.

(vi) Diabetes due to mutant insulin receptors. Defects in the insulin receptor gene have been found in more than 40 people with diabetes, but most have extreme insulin resistance associated with acanthosis nigricans.

(vii) Diabetes mellitus associated with a mutation of mitochondrial DNA. Diabetes due to a mutation of mitochondrial DNA that impairs the transfer of leucine into mitochondrial proteins has been described in Japanese families as well as in isolated case reports in Caucasians. Most patients have a mild form of diabetes that responds to oral hypoglycaemic agents, some a nonimmune form of type 1 diabetes. Two-thirds of patients with this subtype of diabetes have a hearing loss, and a smaller proportion had a syndrome of myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). A leucine transfer defect has also recently been found in a family with maternally transmitted diabetes.
Obese type 2 patients. This most common form of diabetes is secondary to extrapancreatic factors that produce insensitivity to endogenous insulin. When an associated defect of insulin production prevents adequate compensation for this insulin resistance, nonketotic mild diabetes occurs. The primary problem is a "target organ" disorder resulting in ineffective insulin action that can secondarily influence pancreatic β-cell function. Hyperplasia of pancreatic β-cells is often present and probably accounts for the fasting hyperinsulinism and exaggerated insulin and proinsulin responses to glucose and other stimuli seen in the milder forms of this disorder. In more severe cases, especially after several years' duration of diabetes, failure of β-cell secretion may result. Chronic deposition of amyloid in the islets may combine with inherited genetic defects to progressively impair β-cell function. Obesity is generally associated with abdominal distribution of fat, producing an abnormally high waist-to-hip ratio. This "visceral" obesity, due to accumulation of fat in the omental and mesenteric regions, correlates with insulin resistance; subcutaneous abdominal fat has little if any association with insulin insensitivity. Visceral metabolites released into the portal circulation alter liver metabolism and increase hepatic glucose output more than peripheral fat mobilization into systemic veins. Exercise may affect the deposition of visceral fat as suggested by CT scans of Japanese wrestlers, whose daily vigorous exercise program prevents accumulation of visceral fat, and they have normal serum lipids and euglycaemia despite daily intakes of 5000-7000 kcal and development of massive subcutaneous obesity. A major cause of the observed resistance to insulin in target tissues of obese patients is believed to be a postreceptor defect in insulin action. This is associated with overdistended visceral fat deposits, and there is a reduced ability to clear nutrients from the circulation after meals. The resulting hyperinsulinism can further enhance insulin resistance by down-regulation of insulin receptors. Moreover, when hyperglycaemia develops, hexosamines accumulate in the muscle and fat tissue to further inhibit glucose transport. This contributes to further defects in postreceptor insulin action, thereby aggravating hyperglycaemia. When exercise increases blood flow to muscle as well as increasing muscle mass, and when overfeeding is corrected so that storage depots become less saturated, the cycle is interrupted. There is improvement in insulin sensitivity, which
is further restored toward normal by a reduction of both the hyperinsulinism and the hyperglycaemia.

Recently, a new factor termed "resistin" has been identified in animal models of obesity; it is a peptide specifically expressed in and secreted by fat cells. Its serum levels are increased markedly in both genetic and diet-induced obesity. Neutralization of this factor with antibodies improves insulin action in obese animals, and injection of resistin into normal mice impairs insulin action. This novel peptide may have the key to better understanding of the relationship of insulin resistance to obesity (Mudaliar, 2007).

(ix) Malnutrition-Mediated Diabetes Mellitus. This form of diabetes includes two forms: fibrocalculous pancreatic diabetes and protein-deficient diabetes and are well described in developing, tropical countries (Mohan V, et al., 1991).

Fibrocalculous pancreatic diabetes is characterized by the presence of pancreatic calculi, a history of recurrent abdominal pain, and concomitant evidence of pancreatic exocrine dysfunction. Although the hyperglycaemia is usually quite severe, ketosis does not develop, but about 80% of those affected require insulin to control hyperglycaemia.

Protein-deficient pancreatic diabetes has no pathognomonic features. It occurs in malnourished young adults, who are said to require large doses of insulin to control hyperglycaemia. The implication of this requirement is that this condition is associated with marked insulin resistance. The pathogenetic mechanism and its relationship to protein-calorie malnutrition are unknown.

2.2. Epidemiology of diabetes in India

Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million had diabetes in the year 2000 (Wild S, et al., 2004). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree R, et al., 2006).

Although the definitive figures are not available, the incidence of Type 1 diabetes in Southern region of India has been reported to be 10.5 cases/ 100,000 per year (Ramachandran A, et al., 1996).
Studies show a rising trend in the prevalence of diabetes across different parts of India (Ramachandran A, et al., 1988; Rao PV, et al., 1989; Ramachandran A, et al., 1997). The National Urban Diabetes Survey (NUDS) revealed that the prevalence in the southern part of India is higher—13.5% in Chennai, 12.4%, in Bangalore, and 16.6% in Hyderabad; compared to eastern India (Kolkata), 11.7%; northern India (New Delhi), 11.6%; western India (Mumbai) 9.3%, and Guwahati-8.2% (Mohan V, et al., 2007). The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14% with a high risk of conversion to diabetes (Alberti KG, et al., 1998; Ramachandran A, et al., 2001). A study done in western India showed age standardized prevalence of 8.6% in urban population (Gupta A, et al., 2003). A more recent study reported a high prevalence (9.3%) in rural Maharashtra (Deo SS, et al., 2006). The Amrita Diabetes and Endocrine Population Survey (ADEPS) (Menon VU, et al., 2006), a community based cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5%. Further support for the rising prevalence of diabetes comes from the results obtained from the Chennai Urban Rural Epidemiology Study (CURES) (Deepa M, et al., 2003). This study revealed that within a span of 14 years (1989 to 2004), the prevalence of diabetes increased significantly by 72.3% in Chennai. The CURES (Mohan V, et al., 2006) also reported a temporal shift in the age at diagnosis to a younger group when compared to the NUDS study published just five years earlier (Ramachandran A, et al., 2001). A study from Delhi also reports a high prevalence of insulin resistance in post pubertal children which was associated with excess body fat and abdominal adiposity (Misra A, et al., 2004). Studies on prevalence have also revealed a very high prevalence of undiagnosed diabetes in the community. While in CURES, the prevalence of known diabetes was 6.1 per cent that of undiagnosed diabetes was 9.1 per cent respectively (Menon V, et al., 2006). The prevalence of diabetes in India Study (PODIS) (Sadikot SM, et al., 2004) revealed that the prevalence in the urban areas is much higher than the rural areas. Several studies have shown that pre-diabetic states [Impaired glucose tolerance (IGT) and impaired fasting glucose (IMG)] are high risk stages for cardiovascular disease (Novoa FJ, et al., 2005; Peterson JL, et al., 2005). The PODIS also reported that the prevalence of IGT was significantly high in both rural and urban populations (Sadikot SM, et al., 2004). A recent study has
reported a decreased prevalence of IGT in an urban population compared to earlier studies done in the same city (Mohan V, et al., 2006) (16.8% in 2000 to 10.2% in 2004). This could suggest that the diabetes epidemic in urban India may be slowing down or it may also suggest that there could be a rapid progression from the normal state through IGT to diabetes, which could imply a rapid increase in the diabetes epidemic or a worsening diabetogenic environment.

Both macrovascular and microvascular complications cause significant morbidity and mortality among diabetic subjects (Zargar AH, et al., 1999). Asian Indians appear to have a greater predilection for cardiovascular complications whereas the prevalence of microvascular complications appears to be lower than in Europeans (ICMR, WHO, 2006).

**Evolution of diabetes epidemic in India**

The first national study on the prevalence of Type 2 diabetes in India was done between 1972 and 1975 by the Indian Council of Medical Research (ICMR, New Delhi). Screening was done in about 35,000 individuals above 14 year of age, using 50g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1 % in urban population and 1.5 % in the rural population. In those above 40 years of age, the prevalence was 5 % in urban and 2.8% in rural areas (Ahuja MS, 1979).

Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in south India reported a prevalence of 5% (Ramachandran A et al., 1988). The prevalence of impaired glucose tolerance in the same study was 2%. A national rural diabetes survey was done between 1985 and 1991 in different parts of the country in selected rural populations (Sridhar GR, 2002). This study which used the 1985 WHO criteria to diagnose diabetes, reported a crude prevalence of 2.8 per cent (Sridhar GR, 2002). The Eluru survey which looked at the prevalence of known diabetes in four villages in Andhra Pradesh showed a prevalence of 1.5%. The prevalence of known diabetes was 6.1% in individuals aged above 40 yr which was unexpectedly high at that time for a rural area with low socio-economic status and decreased health awareness (Rao PV, et al., 1989). A study done in 1988 in Chennai reported a prevalence of 8.2% in the urban and 2.4% in the rural areas (Ramachandran A,
et al., 1992). A subsequent study in the same urban area done after years showed an age standardized prevalence of 11.6% indicating a rising trend in prevalence of diabetes (Ramachandran A, et al., 1997). A very high prevalence of 16.3% was reported in Thiruvanathapuram in Kerala State in the year 1999. In the same year, a prevalence of 8.2% was reported from Guwahati (Shah SK, et al., 1999). A cross-sectional population survey was done in the Kashmir valley in 2000 and the prevalence of 'known diabetes' among adults aged >40 yr was found to be 1.9 per cent (Zargar AH, et al., 2000).

The National Urban Diabetes Survey (NUDS), a population based study was conducted in six metropolitan cities across India and recruited 11,216 subjects aged 20 yr and above representative of all socio-economic strata (Ramachandran A, et al., 2001). An oral glucose tolerance test was done using capillary glucose and diabetes was defined using the WHO criteria (Alberti KG, et al., 1998). The study reported that the age standardized prevalence of type 2 diabetes was 12.1%. This study also revealed that the prevalence in the southern part of India to be higher-13.5% in Chennai, 12.4%, in Bangalore, and 16.6% in Hyderabad; compared to eastern India (Kolkata), 11.7%; northern India (New Delhi), 11.6%; and western India (Mumbai), 9.3%. The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14% with a high risk of conversion to diabetes.

A study done in western India showed age standardized prevalence of 8.6% in urban populationd (Gupta A, et al., 2003). A more recent study reported a high prevalence (9.3%) in rural Maharashtra (Deo SS, et al., 2006). The Amrita Diabetes and Endocrine Population Survey (ADEPS) (Menon VU, et al., 2006), a community based cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5%.

Further support for the rising prevalence of diabetes comes from the results obtained from the CURES (Deepa M, et al., 2003). This study was conducted on a representative population of Chennai which was based on the model of systematic random sampling, wherein of the 155 wards of the corporation of Chennai, 46 were selected to represent all the 10 zones. A total of 26,001 individuals were selected from these 46 wards for the phase 1 of CURES and a fasting capillary glucose measurement was obtained in all. Phase 2 focused on the study of complications of diabetes in the self-
reported diabetic subjects identified in Phase 1, while Phase 3 recruited every tenth subject (n=2600) screened in phase 1 for an oral glucose tolerance test. Phase 3 had a response rate of 90.4 % (ie. 2350/2600 subjects participated).

This study gave a unique opportunity to compare prevalence rates of diabetes in Chennai city which is the only region in India that has had repeated well-conducted epidemiology studies on prevalence of diabetes over the past two decades. Thus, datas obtained from CURES were compared with three earlier epidemiological studies (Ramachandran A, et al., 1992, 1997 and 2001) carried out in the same city using similar methods.

The overall crude prevalence of diabetes using WHO criteria (Alberti KG and Zimmet PZ, 1998) in CURES was 15.5 % (age-standardized:14.3%), while that of IGT was 10.6% (age-standardized:10.2%). From 1989-1995, the prevalence of diabetes in Chennai increased by 39.8% (8.3 to 11.6%); from 1995-2000 by 16.3% (11.6 to 13.5%) and from 2000-2004, by 6.0% (13.5 to 14.3%). Thus within a span of 14 years, the prevalence of diabetes increased significantly by 72.3% (P<0.001).

Shift in age of onset of diabetes
The CURES (Mohan V, et al., 2006) also reported a temporal shift in the age at diagnosis to a younger group when compared to the NUDS study published just five years earlier (Ramachandran A, et al., 2001). This is a disturbing finding as the earlier age of onset combined with increasing prevalence of diabetes could have adverse effects on nation’s health and economy. A study from Delhi also reports a high prevalence of insulin resistance in post pubertal children which was associated with excess body fat and abdominal adiposity (Misra A, et al., 2004).

Undiagnosed diabetes- the hidden danger
Studies on prevalence have also revealed a very high prevalence of undiagnosed diabetes in the community. While in CURES, the prevalence of known diabetes was 6.1 per cent and that of undiagnosed diabetes was 9.1 per cent respectively (Menon VU, et al., 2006). The Kashmir valley study showed that the prevalence of undiagnosed diabetes was 4.25 per cent, which was more than double to that of the known diabetes (1.9%) (Zargar AH,
The individuals who are unaware of their disease status are left untreated and are thus more prone to microvascular as well as macrovascular complications. Hence, it is necessary to detect the large pool of undiagnosed diabetic subjects in India and offer early therapy to these individuals.

Urban-rural differences in diabetes prevalence
The ICMR study reported that the prevalence was 2.1 per cent in urban and 1.5 per cent in rural areas (Ahuja MMS, 1979), a later study showed that the prevalence was three times higher among the urban (8.2%) compared to the rural population (2.4%) (Ramachandran A, et al., 1992). A study done in southern Kerala looked at the variations in the prevalence of type 2 diabetes among different geographic divisions within a region (Kutty VR, et al., 2000). The prevalence of diabetes was the highest in the urban (12.4%) areas, followed by the midland (8.1%), highland (5.8%) and coastal division (2.5%).

The prevalence of diabetes in India Study (PODIS) was carried out in 108 centres (49 urban and 59 rural) to look at the urban-rural differences in the prevalence of type 2 diabetes and glucose intolerance (Sadikot SM, et al., 2004). Capillary blood was used to estimate glucose levels and glucose intolerance was defined according to the WHO 1999 as well as the American Diabetes Association (ADA) 1997 criteria (Sadikot SM, et al., 2004). According to the ADA criteria, the prevalence of diabetes was 4.7% in the urban compared to the 2.0% in the rural population while the prevalence of diabetes according to the WHO criteria was 5.6 and 2.7% among urban rural areas respectively.

The WHO-ICMR National NCD risk factors surveillance
In order to obtain continuous surveillance of NCD risk factors in India, the WHO and the ICMR took up NCD Risk Factor Surveillance in five States of India, representing different geographical locations (north, south, east and west/central India) (ICMR,WHO, 2006). About 40,000 individuals aged 15 to 64 yr with equal representation from urban, peri-urban (slum) and rural areas were recruited for the study (ICMR, WHO, 2006). The overall frequency of self reported diabetes study was 4.5%. Urban area had the highest prevalence (7.3%), followed by peri-urban/slum (3.2%) and rural areas (3.1%).
**Prediabetes-the harbinger of future diabetes**

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) collectively called as pre-diabetic states, have a high risk of conversion to diabetes. Several studies have shown that these pre-diabetic states are also high risk stages for cardiovascular disease (Novoa FJ, et al., 2005; Peterson JL, et al., 2005). Hence data on IGT and IFG are also urgently needed as they are indicators of future diabetes prevalence and burden on the nation. The NUDS results indicate that the prevalence of OGT was higher than that of type 2 diabetes in four out of six cities studied (Ramachandran A, et al., 2001). The prevalence of IGT was 16.8% in Chennai, 14.9% in Bengaluru (formerly Bangalore), 29.8% in Hyderabad, 10% in Kolkata, 10.8% in Mumbai and 8.6% in New Delhi. The ADEPS done in Kerala showed that 11.2% of the subjects had either IFG or IGT (Menon VU, et al., 2006). The PODIS reported that the prevalence of IGT was significantly high in both rural and urban populations (Sadikot SM, et al., 2004). A recent study has reported a decreased prevalence of IGT in an urban population compared to earlier studies done in the same city (Mohan V, et al., 2006) (16.8% in 2000 to 10.2% in 2004). This could suggest that the diabetes epidemic in urban India may be slowing down or it may also suggest that there could be a rapid progression from the normal state through IGT to diabetes, which could imply a rapid increase in the diabetes epidemic or a worsening diabetogenic environment.

**Burden of diabetes related complications in India**

Both macrovascular and microvascular complications cause significant morbidity and mortality among diabetic subjects (Zargar AH, et al., 1999). The Chennai Urban Population Study (CUPS) and CURES provided valuable data from India on the complications related to diabetes. The prevalence of coronary artery disease was 21.4% among diabetic subjects compared to 9.1% in subjects with normal glucose tolerance (Mohan V, et al., 2001). The prevalence of CVD in IGT subjects were 14.9% in the same study. It was also seen that the diabetic subjects had increased subclinical atherosclerosis as measured by intimal medial thickness (IMT) at every age point compared to subjects with normal glucose tolerance (Mohan V, et al., 2000). A recent study showed that carotid intimal medial thickness increased with worsening grades of glucose tolerance as
well as with increase in the number of components of metabolic syndrome (Mohan V, et al., 2006). The prevalence of peripheral vascular disease (PVD) was 6.3% among diabetic subjects compared to 2.7% in non-diabetic subjects (Premalata G, et al., 2000), and these figures are lower than the prevalence reported in western populations (Melton LJ, et al., 1980). This is probably due to lower age at onset for diagnosis of type 2 diabetes in India. It is well known that PVD is more common in older individuals. The CURES Eye study is the largest population based data on the prevalence of diabetic retinopathy done in India. This study showed that the overall prevalence was 17.6%, which was lower when compared to the reports from the west (ICMR, WHO, 2006). A recent population based study reported that the prevalence of overt nephropathy was 2.2% in Indians while microalbuminuria was present in 26.9%. Glycated haemoglobin, duration of diabetes and systolic blood pressure were independently associated with diabetic nephropathy (Ranjit UI, et al., CURES I press). Overall, Asian Indians appear to have a greater predilection for cardiovascular complications whereas the prevalence of microvascular complications appears to be lower than in Europeans.

A recent follow up of the original CUPS cohort showed that the overall mortality rates were nearly three-fold higher (18.9 per 1000 person/year) in people with diabetes compared to non diabetic subjects (5.3 per 1000 person/year, P=0.004) (Mohan V, et al., 2006). The hazard ratio (HR) for all cause mortality for diabetes was found to be 3.6 compared to non diabetic subjects. The study also showed that mortality due to cardiovascular (diabetic subjects: 52.9% vs. non diabetic subjects 24.2%, P=0.042) and renal (diabetic subjects 23.5% vs. non diabetic subjects 6.1%, P=0.072) causes was higher among diabetic subjects.

**Causes of the rise in prevalence of diabetes**

(i) Genetic predisposition. Several studies on migrant Indians across the globe have shown that Asian Indians have an increased risk for developing type 2 diabetes and related metabolic abnormalities compared to other ethnic groups (Mohan V, et al., 1986; McKeigue PM et al., 1991; Abet N et al., 2001). Although the exact reasons are still not clear, certain unique clinical and biochemical characteristics of this ethnic group collectively called as the “Asian Indian phenotype” is considered to be one of the major
factors contributing to the increased predilection towards diabetes (Joshi R, 2003; Deepa R, et al., 2006). Despite having lower prevalence of obesity as defined by body mass index (BMI), Asian Indians tend to have greater waist circumference and waist to hip ratios (Ramachandran A, et al., 1997) thus having a greater degree of central obesity. Again, Asian Indians have more total abdominal and visceral fat for any given BMI (Raji A, et al., 2001) and for any given body fat they have increased insulin resistance (Chandalia M, et al., 1999). Moreover, they have lower levels of the protective adipokine adiponectin and have increased levels of adipose tissue metabolites (Abet N, et al., 2004). Studies on neonates suggested that Indian babies are born smaller but relatively fatter compared to Caucasian babies and are referred to as “the thin fat Indian baby” (Yajnik CS, 2002; Yajnik CS, et al., 2003). A recent study confirmed this finding and suggested that the “thin fat phenotype” in neonates persisted in childhood and could be a forerunner of the diabetogenic adult phenotype (Krishnaveni GV, et al., 2005). These findings suggest that Asian Indians are more prone to diabetes and related metabolic abnormalities. Genetic factors that determine body fat distribution and glucose metabolism have to be fully elucidated for the better understanding of the biochemical and molecular mechanisms behind the aetiopathogenesis of diabetes. Studies have shown that while some genes seem to confer increased susceptibility to diabetes in Indians (Abet N, et al., 2005; Vimalaswaran KS, et al., 2005), some protective genes in Europeans do not appear to protect Indians (Radha V, et al., 2006).

(ii) The epidemiological transition. The dramatic rise in the prevalence of type 2 diabetes and related disorders like obesity, hypertension and the metabolic syndrome could be related to the rapid changes in life style that has occurred during the last 50 yr. Although this “epidemiological transition”, which includes improved nutrition, better hygiene, control of many communicable diseases and improved access to quality healthcare have resulted in increased longevity, it has also led to the rapid rise of the new-age diseases like obesity, diabetes and heart disease. The intrusion of western culture into the lives of traditional indigenous communities has also had devastating results in terms of the rise in diabetes and related metabolic disorders. The explosion of type 2 diabetes in Native American and Pacific Island communities are classical examples of
this phenomenon. Another way to explain the diabetes epidemic in these and other ethnic
groups like Africans and Asian Indians is through Neel’s ‘thrifty genotype’ hypothesis
(Neel JV, et al., 1998). This hypothesis proposes that some genes are selected over
previous millennia to allow survival in times of famine by efficiently storing all available
energy during times of fast. However, these very genes lead to obesity and type 2
diabetes when exposed to a constant high energy diet. In virtually all populations, higher
fat diets and decreased physical activity and sedentary occupational habits have
accompanied the process of modernization which has resulted in the doubling of the
prevalence of obesity and type 2 diabetes in less than a generation.

Increase in the prevalence of type 2 diabetes may also result due to migration (a
move from one environment to another, either external or internal), which brings with it
marked social and cultural changes. Misra and colleagues (Misra A, et al., 2001) reported
that migration from rural areas to urban slums in a metropolitan city in India led to
obesity, glucose intolerance, and dyslipidaemia. Many epidemiological studies on
diabetes in migrant populations, mostly in people originating from developing counties,
have reported a higher prevalence of diabetes than the host populations of those countries.

(iii) ‘Fast food culture’ and ‘Sedentarism’ (The main drivers of diabetes epidemic in
India). In order to assess the effect of affluence on the prevalence of diabetes in India, the
Chennai Urban Population Study (CUPS) (Shanti Rani CS, et al., 1999) was undertaken.
The CUPS is a population-based study involving two residential areas representing the
lower and middle income group in Chennai in South India. All individuals aged more
than 20 yr living in these two colonies were requested to participate in the study. Of the
total of 1399 eligible subjects (age>20 yr), 1262 (90.2%) participated in the study. The
study subjects underwent a glucose tolerance test (GTT) and were categorized as having
normal glucose tolerance (NGT), IGT or diabetes.

The overall prevalence of diabetes was 12 per cent in the population above the
age of 20 yr (Mohan V, et al., 2001). The age-standardized prevalence rate of diabetes
was significantly higher in the middle income group compared to the lower income group
(12.4 vs 6.5%, respectively). The prevalence of obesity and other cardiovascular risk
factors were also markedly higher in the middle income group than the low income group.
Moreover, the fast food culture' which has overwhelmed our cities and towns is also a major driver of the diabetes epidemic. The fast-foods’ that are fat and calorie rich are easily available in the numerous food joints. As a majority of the immigrants in Indian cities depend on these unhealthy ‘junk’ foods, this may be a major factor in the rising prevalence of diabetes and cardiovascular diseases in urban slums. One point worth emphasizing is that diabetes can no longer be considered as a disease of the rich. The prevalence of diabetes is now rapidly increasing among the poor in the urban slum dwellers, the middle class and even in the rural areas. This is due to rapid changes in physical activity and dietary habits even among the poorer sections of the society. Unfortunately the poor diabetic subjects delay taking treatment leading to increased risk of complications (Ramachandran A, et al., 2002). Moreover, as the epidemic matures and reaches the next state of transition, the rich and affluent will rapidly change their activity patterns and start making healthier food choices and ultimately the diabetes and heart disease will decrease in this section of the society. This has been demonstrated in the developed world where the prevalence of diabetes and cardiovascular diseases are higher among the lower socio-economic group and in rural areas compared to higher socio-economic group and urban areas (Robbins J, et al., 2001; Rabi DM, et al., 2006; Mainous AG, et al., 2004).

The next factor driving the epidemic is what has been referred to as ‘sedentarism’ or the adoption of sedentary behaviour. Over the past few decades, a huge number of the working population has shifted from manual labor associated with the agriculture sector to physically less demanding office jobs. With the advent of highly addictive computer and video games, sedentarism is now affecting the children and youth as they tend to spend more time in front of television sets or computers than playing outdoors. The evidence for the effects of physical inactivity on the prevalence of diabetes and cardiovascular diseases can be seen in CUPS (Mohan V, et al., 2003; Mohan V, et al., 2005). It was observed that the prevalence of diabetes was almost three times higher in individuals with light physical activity compared to those having heavy physical activity (23.2 vs. 8.1%, P<0.001) (Mohan V, et al., 2003). It was also noted that prevalence of metabolic syndrome and hypertension was also significantly higher among people with light physical activity (Mohan V, et al., 2003). Overall, individuals with
light-grade physical activity had 2.4 times higher chances of developing coronary artery disease compared to heavy grade physical activity group (Mohan V, et al., 2005). Hence early identification of the risk factors associated with diabetes and appropriate interventions aimed at preventing the onset of diabetes and its complications are urgently required.

**Early identification and prevention of at risk individuals.** Several prospective studies have shown that measures of lifestyle modification help in preventing the onset of diabetes (Li G, et al., 2002; Tuomilehto J, et al., 2001; Knowler WC, 2002). The Indian Diabetes Prevention Programme (IDPP), a preventive study done in India based on the Diabetes Prevention Program (DPP) has clearly documented the importance of physical activity in the prevention of diabetes (Ramachandran A, et al., 2006). Early identification of the high risk individuals would help in taking appropriate intervention in the form of dietary changes and increasing physical activity, thus helping to prevent, or at least delay, the onset of diabetes. This means that identification of at risk individuals is extremely important to prevent diabetes in India. Recently, risk scores based on simple anthropometric and demographic variables have been devised to detect high risk individuals (Spijkerman AM, et al., 2004). But it is also evident that a common risk score cannot be applied for all ethnic groups (Glumer C, et al., 2004). Hence ethnic specific risk scores are extremely important in identification of at risk individuals in a particular ethnic group. Recently, the Indian Diabetes Risk Score (IDRS) have been developed using four simple variables namely, age, family history, regular exercise and waist circumference (Mohan V, et al., 2005). The individuals were classified as having high risk (score >60), moderate risk (score 30-50) and low risk (score <30) out of a total score of 100. IDRS has a sensitivity and specificity of over 60% for a cut-off >60 and can be used to do a selective screening for Indian population. A recent study showed that IDRS not only predicted diabetes, but also identified individuals with higher cardiovascular risk i.e., those with metabolic syndrome even at a stage when they have normal glucose tolerance (Mohan V, et al., 2006). This simple and cost effective IDRS could thus serve as a tool for a primary care physician or a health worker to identify at risk individuals for both diabetes and cardiovascular diseases.
Diabetes prevention through community empowerment. The CURES has demonstrated that the awareness about diabetes in urban areas is extremely low (Mohan D, et al., 2005). Nearly 25% of the residents were not even aware of a condition called diabetes. Moreover, even among the diabetic subjects, the knowledge and awareness about complications was poor and less than 50% knew that diabetes is preventable.

A recent study has shown how increasing awareness and empowerment of community can possibly help in the prevention of diabetes and other non communicable disorders (Mohan D, et al., 2005). Mass awareness programmes like public lectures, video clippings and distribution of educational pamphlets were carried out in a residential colony in Chennai for three years continuously. A follow up study was done 7 yr after the baseline study. It was found that there was a 277% increase in the proportion of walkers from baseline to follow up. The proportion of individuals who exercised increased from 14.2 to 58.7 per cent (Mohan V, et al., 2006). The colony residents motivated by the awareness programmes constructed a park with the help of civic authorities which is being now used regularly not only by the residents but also by neighboring colonies.

Prevention Awareness Counseling and Evaluation (PACE) Diabetes programme is a large awareness and prevention programme underway in Chennai (Suresh S, et al., 2005). The aim of this programme which is funded by the Chennai Willington Corporate Foundation, a non government organization (NGO) in Chennai, is to create massive public awareness about diabetes and related disorders reaching out to about a million people and conduct large scale opportunistic screening of at least 100,000 people. Awareness programme are being organized in public places like banks, shopping complexes, cinema halls, places of worship, bus stands, railway stations, schools, colleges, etc. The PACE project is already having a large impact in the form of increased diabetes awareness. Mass awareness programme not only help in the prevention of diabetes, but also help in increasing the awareness about other non communicable diseases.
2.3. New glucose lowering agents.

Current therapies for type 2 diabetes are often associated with inadequate control of postprandial hyperglycaemia (especially with the sulphonylureas, metformin and TZDs), weight gain (sulphonylureas, meglitiniides, TZDs and insulin), and loss of efficacy over time (a problem with all the current oral agents). A better understanding of physiological responses to meals has lead to the development of new agents whose therapeutic action is based on the enhancement of gastrointestinal (GI) hormone action.

Incretins such as glucagons-like peptide-1 (GLP-1) are naturally occurring hormones released from the GI tract in response to the ingestion of food. GLP-1 is released from the L-cells located in the distal ileum and colon, in response to food containing carbohydrates and fats (Riddle MC, Drucker DJ, 2006). The pleiotropic effects of GLP-1 include enhancement of glucose-dependent insulin secretion from the pancreas, suppression of inappropriately elevated glucagons secretion, delaying gastric emptying, reducing appetite, preserving β-cell function, and increasing β-cell mass (in animal models) (Galwitz B, 2005). Importantly, GLP-1 does not suppress normal counter-regulatory increase in glucagons secretion during hypoglycaemia. It is now well known that meal-stimulated circulating levels of GLP-1 are reduced in type 2 diabetes. Thus GLP-1 would seem an appropriate therapeutic agent in patients with type 2 diabetes. However, GLP-1 only has a plasma half life of approximately 2 min before being degraded by dipeptidyl peptidase IV (DPP IV); therefore, its utility as a pharmacologic agent is limited. Exenatide is a synthetic GLP-1 analogue which has a longer half-life because it is not recognized by DPP-IV, thus making it suitable for clinical use (Nauck MA, Meier JJ, 2005).

Exenatide (Byetta®): Exenatide is a 39-amino acid peptide incretin mimetic so named because it mimics the action of GLP-1. It is the synthetic version of exendin-4, an incretin mimetic isolated from the saliva of the Gila monster lizard (Ruggles JA, et al., 2004). Approximately 53% of the 39-amino acid sequence of GLP-1 is similar to exenatide. Exenatide is the first agent in the class known as incretin mimetics that has been approved for use in the USA as adjunctive therapy to improve glycaemic control in patients with type 2 diabetes who have not achieved adequate glycaemic control with either sulphonylurea or metformin monotherapy or with a Sulphonylurea+ metformin
combination therapy (Ruggles, et al., 2004; Defronzo RA et al., 2005; Kendall DM, et al., 2005). Its actions result in a slowing of gastric emptying, stimulation of insulin secretion, inhibition of glucagons secretion, improved control of postprandial hyperglycaemia, and of body weight. In three recent 30 wk double, blind, placebo controlled studies (Buse JB, et al., 2004; Defronzo RA, et al., 2005; Kendall DM, et al., 2005), in over 1400 patients with a mean HbA1c of approximately 8.5% and mean body weight of about 99kg, the addition of exenatide 5μg and 10μg (SQ b.i.d.) (to either sulphonylurea or metformin monotherapy or sulphonylurea + metformin combination therapy) resulted in a reduction of HbA1c by 0.6 and 0.9% and body weight by 3.1 and 4.2 kg respectively as compared to placebo. Long-term extension data in 265 patients reveal that the decrease in HbA1c is maintained at week 82 (-1.1% from baselines) and the weight progressively continues to decrease over time (-4.5 kg from baseline). In addition, there were beneficial effects on the lipid profile with small but significant reductions in LDL and triglycerides and an increase in HDL cholesterol. The most commonly reported side effects with exenatide therapy are GI complaints, most commonly nausea. The incidence of nausea is dose-dependent and was consistently seen among patients in the three clinical trials. Nausea occurred most frequently during weeks 0-8 and was generally mild-moderate in nature. Severe nausea ranged from 2.7-6% and withdrawal from the study due to nausea ranged from 1.8-4%. The combination of metformin and exenatide did not result in an increased incidence of nausea. Of note, the weight loss seen with exenatide treatment was not attributable to nausea. Mild to moderate hypoglycaemia occurred more frequently in the exenatide treatment groups when a sulphonylurea was included in the treatment regimen. However, in the exenatide + metformin study, the incidence was the same as the placebo group. Approximately 45% of the patients receiving exenatide were positive for anti-exenatide antibodies, with the majority of titres being in the low range (<1/125). The presence of these titres did not appear to have a predictive effect on glycaemic response or adverse events. Whether these beneficial effects of exenatide are maintained in the longer term and more importantly, whether it has effects on pancreatic β-cell regeneration in humans remain to be determined.

Other GLP-1 analogues: Liraglutide (NN2211) is an acylated human GLP-1 analogue which binds non-covalently to albumin. Since it exhibits a more prolonged
pharmacokinetic profile relative to native GLP-1 or exenatide, this drug can be administered once daily. Similar to exenatide, nausea is the most common adverse effect associated with liraglutide administered. In a recent 12 wk trials in 193 patients with type 2 diabetes, 0.75 mg liraglutide SQ daily caused equivalent placebo-adjusted reductions of HbA1c compared with glimepiride (0.75 and 0.74%) from mean baseline values of 7.4-7.9%. In addition, liraglutide treatment was associated with a placebo-adjusted weight reduction of 0.39 kg, whereas patients treated with glimepiride experienced a mean weight gain of 0.94 kg (Madsbad S, et al., 2004). In another study, once-daily liraglutide (0.45, 0.6 and 0.75mg SQ) improved glycaemic control and weight, in a comparable degree to metformin (Feinglos MN, et al., 2005).

Other albumin-based GLP-1 agonists under investigation include CJC-1131, a DPP-IV-resistant GLP-1 analogue modified with a reactive chemical link that forms a covalent bond with a single amino acid residue within human serum albumin, and Albugon, a recombinant albumin/GLP-1 hybrid protein (Holst JJ, 2004). The ability to link a GLP-1 peptide domain conferring GLP-1R activation to albumin or other proteins that exhibit a more prolonged circulating half life should enable the development of longer-acting GLP-1R agonists suitable for once daily or even weekly administration (Holst JJ, 2004).

**DPP-IV inhibitors:** Orally administered DPP-IV inhibitors are currently in development as glucose lowering agents. Of these, vildagliptin (LAF 237-Novartis) and sitagliptin (Merck) are in late stages of clinical development.

**Vildagliptin:** In a recent study, vildagliptin at a dosage of 50 mg/day was compared with placebo for 12 wk (with a further 40 wk extension) in 107 patients with type 2 diabetes on metformin therapy (Ahren B, et al., 2004). The placebo-adjusted reduction of HbA1c from the mean baseline values of 7.7% was 0.6%. At 1 yr, compared to the placebo, vildagliptin (50mg) reduced the prandial glucose by 43mg/dl, fasting glucose by 20mg/dl, fasting insulin levels by 40 pmol/l and HbA1c by 1.1%. In this study, in contrast to exenatide, no significant between-treatment differences in change of weight occurred. Mathematical modeling studies have suggested that vildagliptin treatment might improve β-cell function (Ahren B, et al., 2005).
Sitagliptin (MK-0431-Januvia®): Sitagliptin is the other oral DPP-IV inhibitor in late clinical development. In human studies, sitagliptin once a day increases the postprandial rise in active GLP-1 concentrations without causing hypoglycaemia in normoglycaemic healthy male volunteers (Bergman AJ, et al., 2005). Sitagliptin was recently approved for use in the US as monotherapy and in combination with metformin or a TZD.

Pramlintide (Symlin®): The mechanism of action of pramlintide does not involve GLP-1 like exenatide and the DPP-IV inhibitors. Pramlintide is an analogue of another gut hormone—Amylin, which is a 37-amino acid peptide co-secreted along with insulin from pancreatic β-cells (Schmitz O, et al., 2004). In the pancreatic β-cells, amylin is processed by prohormone convertase, the same enzyme that processes insulin, and is packaged with insulin into the same secretory granules. Both β-cell peptides have similar diurnal patterns, with low fasting levels and robust increases in response to meals (Young AA. 1997). However, amylin circulates at lower plasma levels than insulin (molar ratio of ~ 1:20). Amylin is virtually absent in patients with type 1 diabetes and it is insufficient at mealtime in insulin requiring patients with type 2 diabetes. However, native amylin is not a suitable therapeutic agent because of its poor solubility and its tendency to aggregate. Hence, an amylin analogue, pramlintide has been developed as a potential pharmaceutical agent with important gluco-regulatory actions in humans. Pramlintide slows gastric emptying and suppresses glucagons secretion during the prandial/postprandial period in order to slow and reduce the entry of glucose into the circulation (Young AA. 1997). These actions, in conjunction with those of insulin, help reduce fluctuations in circulating glucose levels to a greater degree than is possible with insulin treatment alone. In clinical studies, pramlintide treatment as an adjunct to insulin decreased HbA1c levels between 0.39 to 0.62% and body weight by 0.5 to 1.4 kg (Hollander PA, et al., 2003; Kruger DF, Gloster MA, 2004). The combined improvement of glycaemic and weight control makes pramlintide, as an adjunct to insulin therapy, a potentially useful treatment option in overweight and obese patients with type 2 diabetes.

Pramlintide is currently approved for use in the USA as adjunctive treatment to mealtime insulin therapy in patients with type 1 and type 2 diabetes who have been unable to achieve desired glucose control despite optimal insulin therapy (with or without concurrent sulphonylurea or metformin therapy for type 2 diabetes). Because of the
effects of pramlintide on gastric emptying, the drug is contraindicated in patients with gastroparesis and also it should not be used in patients taking drugs that alter GI motility or who use the α-glucosidase inhibitors (acarbose, miglitol). The most common side effect with pramlintide therapy is mild to moderate nausea which appears to be dose-related and decreases over time.

Pramlintide has been associated with an increased risk of insulin-induced severe hypoglycaemia, particularly in patients with type 1 diabetes within 3 hr following a pramlintide injection. Appropriate patient selection, careful patient instruction, and insulin dose adjustments are critical elements for reducing this risk. The initial dose is 60μg given SQ immediately prior to major meals (>250 kcal or containing >30 g of carbohydrate). The dose of pre-prandial rapid-acting or short-acting insulin (including premixed 70/30 or 75/25 preparations) is reduced by 50% and it is recommended that blood glucose be monitored more frequently. If no clinically significant nausea has occurred for 3-7 days, the dose is increased to 120μg SQ prior to major meals. If the 120μg dose is not tolerated due to nausea, a stable dose of pramlintide has been reached and nausea has subsided, the dose of insulin may be adjusted to optimize glycaemic control.

The use of the above pharmacologic agents which augment the effects of gut hormones is clearly associated with improved glucose control and in the case of exenatide, liraglutide and pramlintide with the added benefit of weight loss. However, at the present time, it is unclear as to what extent the various effects of these agents are mediated through central effects on the brain or peripheral effects in the GI system and also whether these beneficial effects are sustained over the long term (Riddle MC, Drucker DJ, 2006). It is possible that that use of injectable peptides (exenatide, liraglutide and pramlintide) is associated with immunogenicity and the development of neutralizing antibodies that diminish their efficacy over time in some patients. It also remains to be determined whether the use of some of these agents (exenatide, liraglutide and vildagliptin) will protect β-cells and promote their regeneration as seen in animal studies. On the other hand, recent reports of hyperglycaemia and nesidioblastosis associated with increased circulating levels of GLP-1 in some patients after gastric bypass surgery highlight the possible, unwanted long-term consequences of prolonged activation of the
GLP-1 receptor in humans (Riddle MC, Drucker DJ, 2006). Long-term studies are needed to answer the above questions and determine the future role of these agents in the treatment of type 2 diabetes.

2.4. Plants with possible beneficial effects in the treatment of diabetes.
Many plants have been investigated in the last two decades in response to the World Health Organization's 1980 request that researchers re-examine traditional medicines. There are enumerable plants known for their uses in folk medicine to treat symptoms of diabetes. It is beyond the scope of this thesis to mention all those plants with possible beneficial activities in the treatment of diabetes. Humble effort has been done to mention some of them. The following tables (Table 2.2., 2.3., 2.4 and 2.5.) illustrates about various plants known for their anti-diabetic action as reported by some researchers mentioned in the tables. Their contributions in the research of natural therapies for the benefit of grief-stricken diabetic patients are invaluable and praiseworthy.

These literatures show that many of our important pharmacopoeia drugs were known and used long before they were introduced into the conventional medicine and before their actions were investigated on scientific lines. Therefore, efforts have to be initiated for its improvement and development as a branch of medicine. Much more can be done in furthering the cause of indigenous foods in the treatment of diabetes mellitus and other diseases and making it really useful to the common people in India and other developing countries.
Plants known for their anti-diabetic effects.

Table 2.2.

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Common Name</th>
<th>Plant parts used, active principle and mode of action</th>
<th>Research studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Butea monosperma</em> (Papilionaceae)</td>
<td>Palash</td>
<td>Flowers kept in water overnight and water taken every day for 1 to 2 months (?)</td>
<td>Shiva, 1998.</td>
</tr>
<tr>
<td><em>Cassia auriculata</em> (Caesalpiniaceae)</td>
<td>Mature tea tree</td>
<td>Seed powder (Tannins), decoction of flower buds</td>
<td>Shiva, 1998</td>
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<td>Botanical Name</td>
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<td>Plant parts used, active principle and mode of action</td>
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<td><em>Jambolana</em></td>
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<tr>
<td><em>Madhuca indica</em></td>
<td>Honey tree, Mahua of South India</td>
<td>Bark</td>
<td>Shiva, 1998.</td>
</tr>
<tr>
<td>Botanical name</td>
<td>Common Name</td>
<td>Plant parts used, active principle and mode of action</td>
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2.5. Animal models in the study of diabetes

The use of animals rather than humans, in diabetes research has multiple advantages. Study of the multifactorial genetics of diabetes is feasible in animals since inbreeding, maintenance of accurate genealogies, and observation of multiple generations in a short period are possible. Some animals, such as the autosomal recessive db/db mouse, develop different degrees of diabetes when bred on different genetic backgrounds, (Coleman DL, et al., 1967) permitting a better understanding of the role of a single mutation and of polygenic factors in the production of the diabetes syndrome. In addition, careful inbreeding studies of diabetic animals, such as NOD (non-obese diabetic) mouse, have permitted recognition of multiple genes controlling expression of the diabetic syndrome in these animals (Hattori M, et al., 1986). In the exploration of the pathogenesis of diabetes, animal models permit better analysis of the biochemical and anatomical alterations in organs inaccessible in humans.

The etiology of diabetes in many animal models is homogeneous, permitting the isolation of one of the many pathogenetic factors that influence the development of diabetes in humans. Moreover, a selected etiologic factor can be induced and studied in depth by a specified manipulation. Diabetes may be induced by diet/nutrition (Shafrir E, et al., 1999), virus (Craighead JE, et al., 1968, Hayashi K, et al., 1974), chemical agents (Dunn JS, et al., 1943, Rerup CC, et al., 1970) or partial pancreatectomy. (Shafrir E. 1992). Aloxan (AXN) and streptozotocin (STZ) diabetic animals are most widely used for screening the compounds including natural products for their insulinomimetic, insulinotropic and other hypoglycaemic/antihyperglycaemic activities. (Young DA, et al., 1991; Katovitch MJ, et al., 1991; Jones RB, et al., 1997; Shafrir E, et al., 1998; PeleTounian A, et al., 1998; Reed MJ, et al., 1999; Bates SH, et al., 2000; Nuss JM, et al., 2000; Zhang BB, et al., 2000). Use of transgenic mice allows even finer experimental focus and targeting of etiologic factors that induce diabetes (Plum L, et al., 2005). Recently, a new animal model of type 2 diabetes has been produced by combination of STZ and nicotinamide (NAD) (Masiello P, et al., 1998). Some investigators have attempted to replicate the disease process that naturally occurs in human beings from the insulin resistance to type 2 diabetes in outbred animals (Reaven GM, et al., 1991). More recently, a novel type 2 diabetic rat model has been developed by combination of short
term high fat diet feeding followed by low dose STZ treatment (Srinivasan K, et al., 2005). This rat model interestingly exhibits stable long lasting hyperglycaemia and the symptoms of type 2 diabetes like polyuria, polydipsia, polyphagia and diabetic complications such as hypertension.

However, none of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying etiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Careful selection of appropriate animal model, interpretation and extrapolation of the results obtained from these animal models to humans are critical for the investigators using animal models in the study of diabetes mellitus.

**Fructose-induced animal model of diabetes**


**Fructose metabolism.** The hepatic metabolism of fructose has important effects on both glucose and lipid metabolism. Absorbed fructose is delivered to the liver via the portal vein (Mayes PA, 1993). Fructose is phosphorylated in the liver by adenosine triphosphate to form fructose-1-phosthate. The reaction is catalyzed by the enzyme fructokinase. Fructose-1-phosthate is split by aldolase B into glyceraldehyde and dihydroxyacetone phosphate. Both can be converted to glyceraldehydes-3-phosphate. Thus, the fructose molecule is metabolized into 2 triose phosphate that bypass the main rate-controlling step in glycolysis, 6-phosphofructokinase. In contrast, hepatic glucose metabolism is limited by the capacity to store glucose as glycogen and, more importantly, by the inhibition of glycolysis and further glucose uptake resulting from the effects of citrate and ATP to inhibit phosphofructokinase. The products of fructose metabolism in the glycolytic pathway of the liver are glucose, glycogen, lactate, and pyruvate. Because
fructose uptake by the liver is not inhibited at the level of phosphofructokinase, fructose consumption results in larger increases of circulating lactate than does consumption of a comparable amount of glucose.

Infusing small amounts of fructose intraportally in dogs appears to have a catalytic action that increases hepatic glucose uptake (Shiota M, et al., 1998), an effect likely to be mediated by hepatic glucokinase. More recently, a low-dose infusion of fructose has been shown to increase carbon flux through glycogen synthase and thereby stimulate glycogen synthesis in humans (Petersen KF, 2001). Low-dose fructose has also been found to restore the ability of hyperglycaemia to regulate hepatic glucose production (Hawkuns MA, 1999) and the addition of 7.5g fructose to the standard 75g glucose reduced the glycemic response to OGTT in adults with type 2 diabetes (Moore MC, 2000). Thus small (catalytic) amounts of oral fructose may be beneficial in improving glycaemic control in type 2 diabetes. In addition, fructose ingestion results in smaller postprandial glycaemic excursions compared with glucose and glucose-containing carbohydrates (starches) that are rapidly absorbed as glucose (Glinsmann WH, 1993); however, increased blood fructose concentrations could also contribute to glycation and diabetic complications.

In contrast with low doses of fructose, when much larger amounts of fructose are consumed (eg, in sucrose and HFCS-sweetened beverages) fructose continues to enter the glycolytic pathway distal to phosphofructokinase, and triacylglycerol production is facilitated. Fructose can provide carbon atoms for both the glycerol and the acyl portions of acylglycerol molecules (Mayes PA, 1993). Thus, unlike glucose metabolism, in which the uptake of glucose is negatively regulated at the level of phosphofructokinase, high concentrations of fructose, can serve as a relatively unregulated source of acetyl-CoA. Indeed, studies in human subjects have shown that fructose ingestion results in markedly increased rates of de novo lipogenesis (Schwarz JM, 1993; Schwarz JM, 1994), whereas de novo lipogenesis does not increase in response to eucaloric glucose ingestion (Hellerstein MK, et al., 1996). Thus, fructose is more lipogenic than is glucose, an effect that might be exacerbated in subjects with existing hyperlipidaemia (Jeppesen J, et al., 1995) or insulin resistance or type 2 diabetes (Abraha A, et al., 1998). In addition, as discussed below, fructose does not stimulate the production of 2 key hormones, insulin
and leptin, which are involved in the long-term regulation of energy homeostasis. Therefore, the decrease in insulin responses to meals and leptin production associated with chronic consumption of diets high in fructose may have deleterious long-term effects on the regulation of energy intake and body adiposity.

Although energy intake, body weight, and adiposity all increase in animals consuming high-fructose diets (Kanarek B, et al., 1982; Rizkalla SW, et al., 1993; Kasim-Karakas SE, et al., 1996), considerably less information is available about humans. The effects of dietary fructose on weight gain have been reported in 3 studies in human subjects. Drinking 1150g soda sweetened with HFCS for 3 weeks resulted in significant increases in ad libitum energy intake and body weight compared with the same amount of soda aspartame in male and female subjects (Tordoff MG, Alleva AM, 1990). Body weight also increased in a group of 14 middle-aged men, 11 with type 2 diabetes mellitus and 3 with type 1 diabetes mellitus, who incorporated 50-60g fructose/day into their diets for 24 weeks (Anderson JW, et al., 1989). More recently, the effects of consumption of either sucrose, which consists of 50% fructose, or artificial sweetened on ad-libitum food intake and body weight were measured in over weights. Individuals who consumed large amounts of sucrose (28% of energy) showed an increase in energy intake, body weight, fat mass, and blood pressure after the 10-weeks intervention (Astrup A, et al., 2002). Thus, in these limited studies of fructose or sucrose feeding in humans, the subjects did not compensate for energy consumed as fructose by reducing ad libitum energy intake from other sources. Although these studies were not designed to test the effects of fructose on weight gain, the observation of increased body weight associated with fructose ingestion is of interest. One explanation for this observation could be that fructose ingestion did not increase the production of 2 hormones, insulin and leptin that have key roles in the long-term regulation of food intake and energy expenditure.

Fructose, unlike glucose, does not stimulate insulin secretion from pancreatic β-cells (Grant AM, Christie MR, 1980; Curry DL, 1989). The lack of stimulation by fructose is likely due to the low concentrations of the fructose transporter GLUT5 in β-cells (Sato Y, Ito T, Udaka N, et al., 1996). Insulin is involved in the regulation of body adiposity via its actions in the central nervous system (CNS) to inhibit food intake and increase energy expenditure (Woods SC, et al., 1996; Schwartz MW, 2000). Briefly,
insulin receptors are localized in CNS areas involved in the control of food intake and energy homeostasis. Insulin administration into the CNS inhibits food intake in animals, including nonhuman primates. Insulin does not enter the brain, but is transported into the CNS via a saturable receptor-mediated process. Using compartmental modeling, Kaiyala et al., (Kaiyala KJ, 2000) showed that the obesity, induced by a high-fat diet was associated with a 60% reduction of the transport of insulin into the CNS in dogs. This impairment of central insulin transport was inversely related to an increase in body weight in response to high-fat feeding. Knocking out the insulin receptor in neurons resulted in hyperphagia and obesity in mice (Bruning JC, et al., 2000). Thus, reduced insulin delivery into the CNS or disruption of the insulin-signaling pathways in the CNS may result in weight gain and the development of obesity.

There is considerable evidence in support of the hypothesis that insulin signaling in the CNS lowers food intake and that insulin functions as a negative feedback signal of recent energy intake and body adiposity. However, because of the known anabolic effects of insulin to stimulate lipid synthesis and promote fat storage, there is a widespread belief that insulin induces weight gain and obesity. This misconception has led to the promotion of numerous diets suggesting that weight loss can be achieved by avoiding foods that stimulate insulin secretion. However, the proponents of such diets do not distinguish between normal insulin responses to meals in which circulating insulin concentrations increase and quickly return to fasting concentrations and the chronic hyperinsulinaemia secondary to β-cell adaptation to insulin resistance. Reduced glucose-stimulated insulin secretion has been shown to be prognostic of greater future weight gain; therefore, increased insulin secretion in response to meals is unlikely to contribute to weight gain and obesity (Schwartz MW, et al., 1995).

A major breakthrough in obesity research came with the cloning of the defective gene (ob) responsible for hyperphagia and obesity in an obese diabetic mouse strain (Zhang Y, et al., 1994). The gene is expressed in adipose tissue and its protein product, leptin, functions as a circulating signal from body fat store to the CNS, where it acts to limit adiposity by inhibiting food intake and increasing energy expenditure (Caro JF, et al., 1996, Rohner-Jeanrenaud B, 1996). The effects of insulin and leptin on food intake appear to share a common signaling pathway via activation of phosphatidylinositol-3-
kinase (Niswender KD, 2001). The increase in energy expenditure in rodents may be mediated by activation of the sympathetic nervous system (Haynes WG, 1997). Leptin administration decreases food intake and activates the sympathetic nervous system in rhesus monkeys (Havel PJ; 1997 Tang-Christensen M, 1999), indicating that leptin has similar biological effects in primates. In addition, human subjects have been identified with hyperphagia and marked obesity, resulting from a failure to produce leptin (Montague CT, et al., 1997) or from defects in the leptin receptor (Clement K, 1998), and leptin administration decreases the hyperphagia and body adiposity resulting from leptin deficiency (Farooqi IS, et al., 1999). Relative leptin deficiency, associated with heterozygous leptin gene mutations, was also shown recently to have a significant biological effect, resulting in increased body adiposity in humans (Farooqi SI, et al., 2001). Decreases in circulating leptin concentrations correlate with increased sensations of hunger during prolonged energy restriction in women (Keim NL, et al., 1998), and leptin administration can reduce appetite in humans (Westerterp-Plantenga MS, et al., 2001). Together, the available strongly suggests an important role for leptin in the regulation of energy balance in humans (Havel PJ, 1998).

**Fructose consumption and insulin resistance.** Diets high in fructose induce insulin resistance in rodents (Reiser S, Halffrisch J, 1977; Halffrisch J, 1979; Zavaroni I, et al., 1980) and in dogs (Martinez FJ, et al., 1994). For example, Thorburn, et al., (Thrbum AW, et al., 1989) fed rats a diet containing 35% of energy as fructose for 4 weeks and found reduced insulin sensitivity associated with impaired hepatic insulin action and whole-body glucose disposal. Both copper-deficient and copper-replete rats showed adverse changes in glucose metabolism when fed diets containing fructose for 2 weeks, whereas rats fed a diet a comparable amount of starch had no observable effects (Fields M, et al., 1996). In a study in hamsters fed a diet with either a high-fructose or a high-sucrose carbohydrate source for 2 weeks, the rate of glucose disappearance after intravenous glucose administration decreased to a greater degree after fructose consumption than after consumption of the sucrose diet, which supplied only 50% as much fructose (Kasim-Karakas SE, et al., 1996). Although fructose does not stimulate insulin secretion in the short term (Curry DL, 1989), the insulin resistance and obesity
induced by long-term fructose feeding in experimental animals induces compensatory hyperinsulinaemia. Blakely, et al., (Blakely SR, et al., 1981) showed significant increases in fasting serum insulin and fasting serum glucose concentrations in rats that consumed 15% of energy as fructose for 15 months compared with cornstarch-fed rats, even though no differences in body weight or food intake between the 2 groups were observed. The effects of dietary fructose on insulin action in humans are not as well documented. In 1980, Beck-Nielsen, et al., (Beck-Nielsen H, et al., 1980) investigated whether the reduction in insulin sensitivity induced by sucrose consumption is related to the glucose or fructose components of the diet. They found that 7 day of high-glucose feeding induced no significant changes in insulin sensitivity, whereas high-fructose feeding was accompanied by both reductions in insulin binding and insulin sensitivity. Other investigators found that diets containing 15% of energy as fructose produced undesirable changes in glucose metabolism in both normal and hyperinsulinaemic men (Hallfrisch J, et al., 1983).

The classic relation between insulin resistance, increased fasting plasma insulin concentrations and intolerance has been hypothesized to be mediated by changes in ambient nonesterified fatty acid concentrations (McGarry JD, 1994). Elevated nonesterified fatty acid concentrations are one of the metabolic consequences of a chronic positive energy balance and increased body adiposity (Jequir E, Tappy L, 1999). If, fructose consumption leads to increased body weight as a result of decreased insulin secretion and reduced leptin production, an increase in circulating nonesterified fatty acids might follow. The exposure to increased concentrations of nonesterified fatty acids may reduce insulin sensitivity by increasing the intramyocellular lipid content (Virkamaki A, et al., 2001). Increased portal delivery of nonesterified fatty acids, particularly from visceral adipose tissue, could also lead to impaired carbohydrate metabolism, because elevated portal nonesterified fatty acid concentrations increase hepatic glucose production (Rebrin K, et al., 1995; Steil GM, et al., 1998). In addition, over time, increased nonesterified fatty acid concentrations may have a deleterious effect on β-cell function (Bergman RN, Ader M, 2000).

An increased supply of nonesterified fatty acids in the liver also leads to an increase in the production of VLDL triacylglycerol (Arner P, 2001). Fructose
consumption has been shown to induce hypertriacylglycerolaemia. Because insulin resistance and reduced insulin binding have been reported in hypertriacylglycerolaemic persons (Bieger WP, et al., 1984), this may be one mechanism by which fructose diets promote insulin resistance. Administration of benfluorex, a hypolipidaemic agent, reversed the insulin resistance induced by fructose feeding in rats. The improvement was associated with the normalization of triacylglycerol concentrations (Storlien LH, et al., 1993). However, 3 months of gemfibrozil administration to 24 persons with high endogenous triacylglycerol resulted in marked decreases in both plasma triacylglycerol and nonesterified fatty acid concentrations but did not enhance insulin-mediated glucose disposal and did not lower plasma insulin concentrations (Jeng CY, et al., 1996). Therefore, the role of triacylglycerol in the development of insulin resistance remains controversial. On the other hand, postprandial hypertriacylglycerolaemia after fructose ingestion is exacerbated in subjects with higher fasting insulin concentrations (Abraha A, et al., 1998), suggesting an interaction between insulin resistance and the lipogenic effects of fructose.

Another potential mechanism leading to insulin resistance could involve decreased production of the adipocyte protein, adiponectin, because reduced circulating concentrations of these hormones are associated with insulin resistance independently of body adiposity (Weyer C, et al., 2001).

**Fructose consumption and lipids.** There are numerous studies in which dietary fructose has been shown to induce hyperlipidaemia in rodents (Herman RH, et al. 1970; Storlien LH, et al., 1993; Okazaki M, et al., 1994; Inoue I, et al., 1995). Herman et al., reported that rats fed a high-fructose diet had sustained elevations in serum triacylglycerol. Circulating triacylglycerol concentrations rose and remained elevated during the entire time fructose was fed (100d) and fell promptly when a standard chow diet was instituted. The same investigators also concluded that there was a greater capacity of human liver to metabolize fructose to lipid compared with glucose because high-sucrose diets led to elevated serum triacylglycerol concentrations in humans, whereas the same amount of glucose resulted in lowered concentrations of serum triacylglycerol. Fields and Lewis (Fields M, Lewis CG, 1999) fed rats copper-adequate or copper-deficient, high-fat diets
with fructose or starch as the sole carbohydrate source. The combination of the high-fat diet with fructose resulted in increased circulating triacylglycerol, and fructose with copper deficiency resulted in significant increases in blood cholesterol. Hyperlipidaemia did not develop when starch was combined with a high-fat diet (Fields M, Lewis CG, 1999). Glucose and fructose are metabolized differently. Hellerstein (Hellerstein MK, 1996) showed that there is little de novo lipogenesis from glucose under eucaloric conditions in humans. In contrast, Schwarz, et al., (Schwarz JM, et al., 1993, 1994) reported 3- to 15-fold increases in fractional de novo lipogenesis from fructose above fasting concentrations in obese and lean subjects (Schwarz JM, et al., 1993) and nearly 30% of circulating triacylglycerol palmitate after fructose ingestion resulted from de novo lipogenesis derived from fructose.

Fructose is the component of sucrose that is considered to be responsible for some of the adverse effects of this disaccharide on blood triacylglycerol (Reiser S, 1985). After extensive work on the metabolic effects of sucrose at the Beltsville Human Nutrition Research Center, Hallfrisch et al., (Hallfrisch JM, et al., 1983) fed 12 hyperinsulinaemic men and 12 male control subjects' diets containing 0%, 7.5%, and 15% of energy from fructose for 5 weeks each in a crossover study. Total plasma cholesterol and LDL-cholesterol concentrations were higher when the men consumed 7.5% or 15% of energy as fructose than as starch. Plasma triacylglycerol concentration in the hyperinsulinaemic subjects increased as the amount of fructose increased. In 1989 Reiser, et al. (Reiser S, et al., 1989) reported results from another 5-weeks crossover study in which 10 hyperinsulinaemic and 11 nonhyperinsulinaemic men consumed diets containing 20% of energy as fructose or as high-amylose cornstarch. Triacylglycerol and cholesterol concentrations increased in both groups of subjects when they consumed fructose, but not cornstarch. Thus, consumption of fructose compared with the same amount of high-amylose cornstarch, produced undesirable changes in cardiovascular risk factors in both hyperinsulinaemic and nonhyperinsulinaemic men.

Not all studies that have evaluated the effects of fructose have reported increased lipids. In the Turku sugar studies (Huttunen JK, et al., 1976), the effects of chronic consumption of sucrose, xylitol, and fructose was studied for 2 year in 127 healthy subjects. Substituting fructose or xylitol for sucrose did not influence plasma cholesterol
or triacylglycerol concentrations. Effects on body weight were reported. It is important to note, however, that an effect of fructose alone may have been obscured by comparing its effects with that of sucrose, which is composed of 50% fructose. In a review article on the effects of dietary fructose on lipid metabolism, Hollenbeck (Hollenbeck CB, et al., 1993) concluded that there is strong evidence that fructose consumed at 20% of total energy results in an increase in total and LDL-cholesterol concentrations but added that the effect of dietary fructose on triacylglycerol concentrations is less clear. Because most studies reported fasting plasma triacylglycerol concentrations, differences in postprandial triacylglycerol excursions in response to dietary changes may have been missed in some of the reported studies.

In a recent study in which 17% of energy was consumed as either crystalline fructose or glucose for 6 weeks, both fasting and postprandial triacylglycerol concentrations were measured (Bantle JP, et al., 2000). The fructose diet produced significantly higher fasting, postprandial, and daylong plasma triacylglycerol values in older men, although this effect of fructose was not seen in younger (less than 40 year of age) men or in the older (≥ 40 year of age) women included in the study. The fructose diet had no significant effects on fasting plasma cholesterol, HDL cholesterol, or LDL cholesterol in either men or women. In healthy persons, increases in triacylglycerol concentrations can decrease over time as a result of metabolic adaptation, but there does appear to be a subset of individuals who are particularly sensitive to dietary fructose, including those with hyperinsulinaemia (Glinsmann WH, Bowman BA, 1993). The effects of fructose- and glucose-sweetened beverages (providing 30% of total energy) consumed with 3 meals over 24 hour in 12 young, normal-weight women without hypertriacylglycerolaemia were compared recently (Teff K, et al., 2002). Plasma triacylglycerol concentrations increased more rapidly and peaked at higher concentrations after consumption of fructose-containing than after glucose-containing beverages. Plasma triacylglycerol concentrations remained elevated after fructose but declined to or below fasting concentrations several hours after glucose consumption. In addition, fasting triacylglycerol concentrations the morning after fructose consumption were increased above baseline concentrations and were elevated compared with fasting triacylglycerol concentrations after glucose consumption. Evidence exists that this effect of fructose (i.e.,
an increase in postprandial triacylglycerol concentrations) may be exacerbated in subjects with hypertriacylglycerolaemia (Jeppesen J, et al., 1995) or insulin resistance (Abraha A, et al., 1998).

In a comprehensive review of carbohydrate-induced hypertriacylglycerolaemia, Parks and Hellerstein (Parks EJ, Hellerstein MK, 2000) reviewed potential biological mechanisms for the phenomenon in humans. The authors concluded that elevated triacylglycerol concentrations observed with increased consumption of dietary carbohydrates result from elevated triacylglycerol synthesis and, in some persons, from reduced triacylglycerol clearance. The increased synthesis of triacylglycerol results primarily from both increases in the VLDL particle secretion rate by the liver and VLDL particle size. Reduction in triacylglycerol clearance may be due in part to reductions in lipoprotein lipase. Using a fructose-fed Syrian golden hamster animal model, Taghibiglou et al., (Taghibiglou C, et al., 2000) investigated mechanisms potentially responsible for the overproduction of VLDL in the insulin-resistance state. They found evidence for enhanced lipoprotein assembly, reduced intracellular apolipoprotein B degradation, and increased expression of microsomal triacylglycerol transfer protein. Together, these findings help to explain the increased assembly and secretion of apolipoprotein-B containing lipoprotein particles in a fructose-fed, insulin-resistant animal model (Taghibiglou C, et al., 2000).

Aloxan-induced type 2 diabetes animal models
Since the initial findings in 1943 of aloxan (ALX) induced β-cell necrosis in rabbits, this compound has long been used for inducing experimental diabetes. Aloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. ALX induce diabetes by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycaemia and ketosis (Rerup C, 1970). ALX causes diabetes in many rodent and non rodent animals and is most preferably used in case of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications (Rerup CC, 1970; Bell RH, Hye RJ, 1983; Ohno T, et al., 1998). Because of its low stability, relatively very short half-life (less than 1 min) and acidic nature of solution, intravenous route of
administration of ALX is preferred. The hypoglycaemic phase may be quite severe and therefore ALX should not be given to fasted animals. The ALX treated animals exhibit severe hyperglycaemia, glucosuria, hyperlipidaemia polydypsia, polyphagia and other symptoms of uncontrolled diabetes and do also develop complications such as neuropathy, cardiomyopathy, well marked retinopathy and others. ALX is disadvantageous because the percentage incidence of diabetes is quite variable and is not proportionately related to increasing doses of ALX (Battell ML, et al., 1999). Further, the incidence of ketosis and resulting mortality is high. However, there are evidences that the reversal of hyperglycaemia due to pancreatic regeneration is early and common in case of ALX treated animals (Srinivasan and Ramarao, 2007).

Mechanism of action of aloxan in diabetes. At least two models of the mechanism of Aloxan induction of β-cell damage have been proposed. Okamoto and co-workers have suggested that AXN/ STZ beak nuclear DNA strands of islet β-cells by generating free-radical oxygen (Yamamoto H, et al., 1981; Uchigata Y, et al., 1982). The breakage of the DNA strands activates nuclear poly adenosine diphosphate ribose (ADP-ribose) synthetase. This enzyme uses cellular nicotinamide adenine dinucleotide (NAD) as a source of ADP-ribose for DNA repair. The decline in cellular NAD concentration ultimately results in the death of beta cells. The administration of inhibitors of poly (ADP-ribose) synthetase inhibitors such as nicotinamide and 3-aminobenzamide suppresses the consumption of NAD and consequently prevents the development of STZ and ALX-induced diabetes (Uchigata Y, et al., 1983). A lethal concentration of STZ and a nonlethal concentration of its nitrosoamide moiety, methylnitrosourea alkylate the DNA of β-cells at the N7 position of Guanine to the same extent and cause comparable amounts of DNA strand breakage. This finding suggests that factors in addition to the activation of poly (ADP-ribose) synthetase contribute to the specific toxicity of STZ/ALX to β-cells.

Wilson and co-workers have proposed that ALX/STZ alkykates not only DNA but also other key cellular components, such as glycolytic or mitochondrial enzymes necessary for the generation of adenosine triphosphate (ATP) (Wilson GL, et al., 1988). This decline in the generation of ATP would impair the resynthesis of NAD, causing the
levels of this cellular component to drop below critical levels. In $\beta$-cells, which is very sensitive to the toxic effects of ALX/STZ, many more of the reactive carbonium ions bind to protein.

**Influence of ALX/STZ on the immune System of the treated animals.** In studies of ALX/STZ-induced diabetes, suppression of T-cell function associated with atrophy of the thymus and peripheral lymphoid tissues are universal observations. Direct toxic effects of ALX/STZ on the immune system have been reported. A large dose (250 mg) of STZ can induce a brief inhibition of DNA synthesis in bone marrow and thymus, a transient selective depletion of circulating thymocytes, and a transient selective depletion (CD8+) of cortical thymocytes. Even a single subdiabetogenic dose (50 mg/kg body weight) depletes circulating thymocytes (Rerup C, 1970; Uchigata Y, *et al.*, 1983; Wilson GL, *et al.*, 1988).

**2.6. Present concern**

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga and Rothman, 2006). According to the International Diabetes Federation (IDF), the total number of diabetic subjects is to be around 40.9 million in India at present which is expected to rise to 69.9 million by the year 2025 (Wild *et al.*, 2004; Sicree *et al.*, 2006). The National studies on prevalence of diabetes show a rising trend across different parts of India. Several barriers exist in the treatment of this challenging disease, including the presence of multiple defects and need for a multiple drug treatment approach, the management of postprandial blood glucose, increased rate of hypoglycaemia, treatment related weight gain and metabolic syndrome and drug tolerance. Despite numerous treatments, population studies show no improvement in glycaemic control (Mudaliar, 2007).

There are evidences that diabetes can now be controlled through improved medical care, monitoring, and lifestyle changes. However, the undesirable side effects of certain drugs and poor achievement of glycaemic and other goals have unnerved the patients. Furthermore, the population living below the poverty line is not able to afford the exorbitant cost of drugs and thus rely on herbal medicines. It is not too distant past, a
variety of small medical emergencies and ailments were surprisingly effectively controlled and cured by the elders in our families through application of home remedies. They had their own valued recipes passed down from one generation to another, for treating a wide array of health problems- from cough and cold to asthma, jaundice etc.

2.7. About the plant used in the present investigation

*Cissampelos pareira* (which has about 57 other synonyms), hirsuta (*Menispermaceae*) is a sub-erect or climbing herb, known as ambastha or laghupatha in Indian traditional medicine (*Vaidya GB, 1998*) and is called ‘sanmwblao’ by the Bodo people. The plant is common in orchards, hedges, parks and gardens on moist soils distributed throughout tropical and subtropical India, ascending up to an altitude of 2000m, either creeping or twinning around other plants, also commonly in the hilly tracts along water-courses.
Uses of *Cissampelos pareira* in folk medicine in India. The leaves of *Cissampelos* are eaten as potherb, and reported to be cooling. Crushed leaves are boiled with rice and given as tonic and in heart complaints; fresh juice is applied in eye diseases. The root posses astringent, mildly tonic, diuretic, stomachic, antilithic, analgesic, antipyretic activities and are prescribed for treating cough, dyspepsia, dropsy, urino-genital troubles such as uterine prolapses, cystitis, haemorrhage, menorrhagia and calcular nephritis (Kirtikar KR and Basu BD, 1933; Amaresh, et al., 2004).

Uses of *Cissampelos* by indigenous people in other countries. *Cissampelos pariera* is commonly referred to as the midwives herb throughout South America because of its long history of use for all types of women's ailments. The vine or root of abuta is used in tropical countries to prevent a threatened miscarriage and to stop uterine hemorrhages after childbirth. Midwives in the Amazon still carry abuta with them for menstrual cramps and pre- and postnatal pain, excessive menstrual bleeding, and uterine haemorrhage. Abuta is also believed to aid poor digestion, drowsiness after meals, and constipation (Bullough C, et al., 1982; Tiwari K C, et al., 1982). Virtually all parts of the plant have been used by indigenous peoples throughout the South American rainforest for thousands of years for other ailments and are still in use today. Members of the Palikur tribe in Guyana use a poultice of abuta leaves as a topical pain-reliever, and the Wayãpi Indians use a decoction of the leaf and stem as an oral analgesic. Ecuadorian Ketchwa tribes use the leaf decoction for eye infections and snakebite. The Créoles in Guyana soak the leaves, bark, and roots in rum and use it as an aphrodisiac. Indigenous tribes in Peru use the seeds of abuta for snakebite, fevers, venereal disease, and as a diuretic and expectorant. Amazonian herbal healers (called curanderos) toast the seeds of abuta and then brew them into a tea to treat internal haemorrhages and external bleeding. They also brew a leaf tea for rheumatism and a vine wood-and-bark tea to treat irregular heartbeat and excessive menstrual bleeding. In Brazil, abuta is widely employed in herbal medicine today as a diuretic and as a tonic (a general overall balancer), as well as to reduce fever and relieve pain. It is often employed for menstrual cramps, difficult menstruation, excessive bleeding and uterine hemorrhages, fibroid tumors, pre- and postnatal pain, colic,
constipation, poor digestion, and dyspepsia. In Mexico, abuta has a long history of use for muscle inflammation, snakebite, rheumatism, diarrohea, dysentery, and menstrual problems. In North American herbal medicine, abuta is used for many of the same conditions as in South America as well as for inflammation of the testicles and minor kidney problems (Bullough, et al., 1982). The common name of this plant has caused some confusion in herbal commerce today. In Brazil, this plant is well known as abutua, and in Peru it is known as abuta or barbasco. References to abuta in herbal commerce today may apply to either *Cissampelos pariera* or to a completely different plant, *Abuta grandiflora*. Another tropical vine, *Abuta grandiflora*, also has the common name of abuta in South America, but this is a very different plant with different chemicals and uses in herbal medicine. This plant is referred to in Peru as chiric sanago as well as abuta (hence the confusion). Abuta is a woody, climbing rainforest vine with leaves up to 30 cm long. It produces inedible, dark, grape-sized berries. It belongs to the genus *Cissampelos*, of which thirty to forty species are represented in the tropics. Abuta vine is blackish-brown and tough; when freshly cut it has a waxy luster. Abuta is found throughout the Amazon in Peru, Brazil, Ecuador, and Colombia, and it is cultivated by many to beautify their gardens.

**Chemical Constituents and reported biological activities.** The main chemicals in abuta are alkaloids, arachidic acid, bebeerine, berberine, bulbocapnine, cissamine, cissampareine, corytuberine, curine, 4-methylcurine, cyclanoline, ciscamine, dicentrine, dehydrodicentrine, dimethyltetrandrinium, essential oil, grandirubrine, hayatine, hayatinine, insularine, isochondodendrine, isomerubrine, laudanosine, linoleic acid, magnoflorine, menismine, norimeluteine, nor-ruffscine, nuciferine, pareirine, pareirubrine alkaloids, pareitropone, quercitol, stearic acid, and tetrandrine (George M and Pandalai KM, 1949; Roy PK, et al., 1952; Kupchan S M, et al., 1960; Bhatnagar AK, et al., 1967; Anwer F, et al., 1968; Morita H, et al., 1993; Ramirez I, et al., 2003; Zhu F, et al., 2006; Asai M, 2007). *Cissampelos* plants, including abuta, contain a group of plant chemicals called isoquinoline alkaloids. Since the late 1960s, these chemicals have received a great deal of attention and research. Out of thirty-eight alkaloids thus far discovered in abuta, one, called tetrandrine, (Wu SJ, et al., 2007; Hsu YC, et al., 2007) is
the most well documented. Clinical research over the years has found tetrandrine to have pain-relieving, anti-inflammatory, and fever-reducing properties. More than one hundred recent clinical studies also describe this chemical's promising actions against leukaemia and some other cancer cells, and research is ongoing. However, the therapeutic dosages of tetrandrine used in these animal studies are much higher than one can reasonably obtain from natural abuta root or vine. Other recently published studies examined tetrandrine's possible cardioactive and blood pressure-reducing (hypotensive) effects through numerous pathways and mechanisms of action at much smaller dosages (Floriani J, 1936; Feng PC, et al., 1962; Mokkhasmit M, et al., 1971; Mokkhasmit M, et al., 1971; Yao W. X. et al., 2002). Another well-known alkaloid chemical, berberine, (Jantova S, et al., 1997; Issat T, et al., 2006) has been documented to have hypotensive, antifungal, and antimicrobial actions. This chemical has been used for the treatment of irregular heartbeat, cancer, candida, diarrhoea, and irritable bowel syndrome (Kupchan, SM, et al., 1960; Amresh A, et al., 2004). Another alkaloid called cissampeline is sold as a skeletal muscle relaxant drug in Ecuador. The methiodide and methchloride derivatives of alkaloid hayatine were reported to be potent neuromuscular blocking agents that lower blood pressure (Jain SK, 1991). In 1962, researchers reported abuta demonstrated anti-inflammatory, smooth muscle relaxant, antispasmodic, and uterine relaxant actions in various laboratory animals (Feng PC, et al., 1962). Subsequent studies with animals confirmed the plant's antispasmodic and anti-inflammatory actions (Amresh G, et al., 2006; Choi BH, et al., 2006; Hsu Y C, et al., 2007; Wu SJ, 2007). These documented effects are quite similar to abuta's traditional uses for menstrual disorders (including cramping and pain). In other animal studies, a root extract was reported to have a diuretic effect (Caceres A, et al., 1982) a finding that confirms another of its traditional medicine uses. Other in vivo research on extracts of abuta indicated that the leaf has antiulcerous actions and that the root has a very mild hypoglycaemic action (only at high dosages). Studies have also shown that the abuta root has other possible therapeutic uses: it demonstrated anticonvulsant actions in mice (Adesina SK, 1982); and, in dogs, it was shown to significantly lower blood pressure. In addition, in vitro studies over the years has reported that abuta has antioxidant properties; antibacterial actions against Staphylococcus, Pseudomonas, Salmonella, and Klebsiella; and antimalarial effects.
(George M and Pandalai KM 1949; Bhatnagar AK, et al., 1967; Anwer F, et al., 1968; Gessler MC, et al., 1994; Sanchez Medina A, et al., 2001; Ramirez I, et al., 2003). One of these in vitro studies also reported that a root extract demonstrated a toxic effect against colon cancer cells.

2.8. Aim of the present study

It is clear from the above discourse that a holistic approach to the problem of diabetes is urgently needed, which could be accompanied by the combination of modern scientific knowledge and traditional folk knowledge. Medical treatment of diabetes can prosper with the practitioners of these two approaches working side by side.

Ethno-pharmacology and natural product drug discovery remains a significant hope in the improving the poor livelihoods of rural communities. Many modern pharmaceuticals have their origin in ethno-medicine, which relies upon a local pharmacopoeia (Singh HB et al., 2001). The ethno-pharmacology knowledge is a holistic system approach that can serve as an innovative and powerful discovery engines for newer, safer and affordable medicines (Tamboura HH et al., 2000). Plant based remedies constitute 90% of Indian system of medicine. Assam has diverse flora with about 4000 species of which 1200 species are known for their medicinal properties. About 700 species are used by different ethnic groups to treat various diseases (Borthakur SK, 2001).

Therefore, systematic scientific studies to validate the useful properties of these medicinal plants are the need of the hour. Ethno-botanical and ethno-pharmacological studies normally involve field explorations of indigenous medical knowledge and biodiversity (Pesek T, 2005; Cassandra LQ, et al., 2008).

The present study aims at realizing the goals stated above with the following objectives:

- Ethno-botanical explorations in the remote villages to gather indigenous knowledge on uses of medicinal plants in the symptoms of diabetes.

- Collection of the medicinal plants reported to have possible beneficial properties in diabetes and their cultivation.
• Preparation of extract and evaluation of anti-hyperglycaemic properties of the plants in fructose-induced hyperglycaemic rat model of diabetes mellitus.

• Evaluation of anti-diabetic properties of *Cissampelos pareira* leaf extract (CLE) in fructose-induced and fructose-alloxan-induced hyperglycaemic rats by studying its effect on serum glucose, triglyceride, cholesterol, and insulin level.

• Toxicity evaluation in the CLE-treated rats to observe whether the treated animals exhibit any toxic symptoms.

• Evaluation of effect of CLE on immune status of the normal and CLE-treated hyperglycaemic animals.

• Evaluation of glycaemic control in the CLE treated hyperglycaemic animals.

• Studies to investigate the changes in the islet morphology of CLE-treated hyperglycaemic animals.