CHAPTER-1
INTRODUCTION

1. Introduction

1.1. Historical background

Diabetes mellitus was known to mankind as —Madhumeha” from antiquity for more than 2500 years ago as can be seen from medical text such as Charaka Samhita. Charaka Samhita has described it as “madhumeha” or honey urine due to the phenomenon of attracting ants near the urine of a madhumeha patient (Chaturvedi and Shastri, 1980). Also in Egypt and Greece, knowledge about diabetes existed. The word diabetes has been derived from a Greek work “Dia” means through; “bêtes” means pass; referring to the cycle of heavy thirst and frequent urination. “Mellitus” is the Latin word for ‘sweetened with honey’, and refers to the presence of sugar in the urine. More appropriately defined as the secretion of an inordinate quantity of sweet tasting, urine with a peculiar smell, accompanied with great thirst, dryness of skin, extreme debility and general emaciation (Dijkstra, 2003).

1.2. Current concept of diabetes

Diabetes is recognized as a group of heterogeneous disorders with the common elements of hyperglycaemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both. The chronic hyperglycaemia is associated with long term damage, dysfunction and failure of various organs especially the eye, kidney, nerves, heart and blood vessels. Thus diabetes covers a wide range of heterogeneous diseases (International Diabetes Federation Atlas, 2009; Balkau and Eschwege, 2005). The complications of diabetes
mellitus include retinopathy, nephropathy and neuropathy (both peripheral and autonomic). The risk for atherosclerotic vascular disease is also increased in persons with diabetes mellitus. The risk for microvascular and neuropathic complications is related to both duration of diabetes and the severity of hyperglycaemia. The increased risk for vascular disease actually antedates the onset of hyperglycaemia to the degree associated with diabetes mellitus (Hoogwerf, 2005; Davis and Granner, 2001).

1.3. Classification of diabetes mellitus (DM)

Diabetes mellitus is classified into three types. The basis of classification is etiology and clinical presentation of the disorder.
1. Type 1 DM,
2. Type 2 DM,
3. Gestational diabetes mellitus (GDM)

1.3.1. Type 1 DM

Type 1 DM accounts about 5 to 10% of all diabetic patients. Type 1 DM is also called as insulin-dependent (IDDM), immune-mediated or juvenile-onset diabetes. It is caused by destruction of the insulin-producing cells of the pancreas, typically due to an auto-immune reaction, where they are attacked by the body’s defense system. The \( \beta \) cells of the pancreas therefore produce little or no insulin, the hormone that allows glucose to enter body cells. The reason why this occurs is not fully understood. The disease can affect people of any age, but usually occurs in children or young adults. Type 1 DM is one of the most common endocrine and metabolic conditions in childhood. People with type 1 DM need injections of insulin every day in order to control the levels of glucose in their blood. Without insulin, people with type 1 diabetes will die (International Diabetes Federation Atlas, 2009; Triplitt et al., 1999).
Pathogenesis of Type 1 DM

Type I DM may develop very abruptly, over a period of a few days or weeks, with three principal sequelae:

(1) increased blood glucose

(2) increased utilization of fats for energy and for formation of cholesterol by the liver and

(3) depletion of body proteins.

Blood glucose concentration rises to very high levels in DM

The lack of insulin decreases the efficiency of peripheral glucose utilization and augments glucose production, raising plasma glucose to 300 to 1200 mg/dl. The increased plasma glucose then has multiple effects throughout the body.

Increased blood glucose causes loss of glucose in the urine

The high blood glucose causes more glucose to filter into the renal tubules than can be reabsorbed and the excess glucose spills into the urine. This normally occurs when the blood glucose concentration rises above 180 mg/dl, a level that is called the blood “threshold” for the appearance of glucose in the urine. When the blood glucose level rises to 300 to 500 mg/dl common values in people with severe untreated diabetes 100 or more grams of glucose can be lost into the urine each day.

Increased blood glucose causes dehydration

The very high levels of blood glucose (sometimes as high as 8 to 10 times normal in severe untreated diabetes) can cause severe cell dehydration throughout the body. This occurs partly because glucose does not diffuse easily through the pores of the cell membrane, and the increased osmotic pressure in the extracellular fluids causes osmotic transfer of water out of the cells. In addition to the direct cellular dehydrating effect of excessive glucose, the loss of glucose in the urine causes osmotic diuresis. That is, the osmotic effect of glucose in the renal tubules greatly decreases tubular reabsorption of fluid. Thus, polyuria (excessive urine
excretion), intracellular and extracellular dehydration and increased thirst are classic symptoms of diabetes.

**Chronic high glucose concentration causes tissue injury**

When blood glucose is poorly controlled over long periods in DM, blood vessels in multiple tissues throughout the body begin to function abnormally and undergo structural changes that result in inadequate blood supply to the tissues. This in turn leads to increased risk for heart attack, stroke, end-stage kidney disease, retinopathy, blindness, ischemia and gangrene of the limbs. Chronic high glucose concentration causes damage to many other tissues. For example, peripheral neuropathy which is abnormal function of peripheral nerves and autonomic nervous system dysfunction are frequent complications of chronic, uncontrolled DM. These abnormalities can result in impaired cardiovascular reflexes, impaired bladder control, decreased sensation in the extremities and other symptoms of peripheral nerve damage. The precise mechanisms that cause tissue injury in diabetes are not well understood but probably involve multiple effects of high glucose concentrations and other metabolic abnormalities of proteins of endothelial and vascular smooth muscle cells, as well as other tissues. In addition, hypertension, secondary to renal injury and atherosclerosis, secondary to abnormal lipid metabolism, often develop in patients with diabetes and amplify the tissue damage caused by the elevated glucose.

**DM causes increased utilization of fats and metabolic acidosis**

The shift from carbohydrate to fat metabolism in diabetes increases the release of keto acids such as acetoacetic acid and $\beta$-hydroxybutyric acid into the plasma more rapidly than they can be taken up and oxidized by the tissue cells. As a result, the patient develops severe metabolic acidosis from the excess keto acids which in association with dehydration due to the excessive urine formation can cause severe acidosis. This leads rapidly to diabetic coma and death unless the condition is treated immediately with large amounts of insulin. All the usual physiologic compensations that occur in metabolic acidosis take place in diabetic acidosis. They include rapid and deep breathing which causes increased expiration
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of carbon dioxide; this buffers the acidosis but also depletes extracellular fluid bicarbonate stores. The kidneys compensate by decreasing bicarbonate excretion and generating new bicarbonate that is added back to the extracellular fluid. Although extreme acidosis occurs only in the most severe instances of uncontrolled diabetes when the pH of the blood falls below about 7.0, acidotic coma and death can occur within an hour. Excess fat utilization in the liver occurring over a long time causes large amounts of cholesterol in the circulating blood and increased deposition of cholesterol in the arterial walls. This leads to severe arteriosclerosis and other vascular lesions.

**Diabetes causes depletion of the body’s proteins**

Failure to use glucose for energy leads to increased utilization and decreased storage of proteins as well as fats. Therefore, a person with severe untreated DM suffers rapid weight loss and asthenia (lack of energy) despite eating large amounts of food (polyphagia). Without treatment, these metabolic abnormalities can cause severe wasting of the body tissues and death within a few weeks (Guyton and Hall, 2006).

### 1.3.2. Type 2 DM

The vast majority of diabetic patients have type 2 DM. Type 2 DM is far more common than type 1, accounting for about 90% of all cases of DM. In most cases, the onset of type 2 DM occurs after age 30, often between the ages of 50 and 60 years and the disease develops gradually. It is also called as non insulin dependent diabetes mellitus (NIDDM) or maturity onset or adult onset DM. This form of diabetes is characterized by insulin resistance and at least initially, a relative lack of insulin secretion. Most individuals with type 2 DM exhibit abdominal obesity which itself causes insulin resistance. In addition hypertension, dyslipidemia (high triglyceride levels and low HDL-cholesterol levels) and elevated inhibitor plasminogen activator-1 (PAI-1) levels are often present in these individuals. This clustering of abnormalities is referred to as the “insulin resistance syndrome” or the
“metabolic syndrome”. Because of these abnormalities, patients with type 2 DM are at increased risk of developing macrovascular complications (Triplitt et al., 1999).

**Pathogenesis of type 2 DM**

Type 2 DM is associated with increased plasma insulin concentration (hyperinsulinemia). This occurs as a compensatory response by the pancreatic β cells for diminished sensitivity of target tissues to the metabolic effects of insulin, a condition referred to as insulin resistance.

The decrease in insulin sensitivity impairs carbohydrate utilization and storage, raising blood glucose and stimulating a compensatory increase in insulin secretion. Development of insulin resistance and impaired glucose metabolism is usually a gradual process, beginning with excess weight gain and obesity. Some studies suggest that there are fewer insulin receptors, especially in the skeletal muscle, liver and adipose tissue in obese than in lean subjects. However, most of the insulin resistance appears to be caused by abnormalities of the signaling pathways that link receptor activation with multiple cellular effects. Impaired insulin signaling appears to be closely related to toxic effects of lipid accumulation in tissues such as skeletal muscle and liver secondary to excess weight gain. Insulin resistance is part of a cascade of disorders that is often called the “metabolic syndrome.” Some of the features of the metabolic syndrome include: (1) obesity especially accumulation of abdominal fat (2) insulin resistance (3) fasting hyperglycaemia (4) lipid abnormalities such as increased blood triglycerides and decreased blood high-density lipoprotein-cholesterol and (5) hypertension. All of the features of the metabolic syndrome are closely related to excess weight gain, especially when it is associated with accumulation of adipose tissue in the abdominal cavity around the visceral organs. The role of insulin resistance in contributing to some of the components of the metabolic syndrome is unclear although it is clear that insulin resistance is the primary cause of increased blood glucose concentration. The major adverse consequence of the metabolic syndrome is cardiovascular disease, including atherosclerosis and injury to various organs throughout the body. Several of the
metabolic abnormalities associated with the syndrome are risk factors for cardiovascular disease, and insulin resistance predisposes to the development of type 2 DM, also a major cause of cardiovascular disease.

*Other factors that can cause insulin resistance and type 2 DM*

Although most patients with type 2 diabetes are overweight or have substantial accumulation of visceral fat, severe insulin resistance and type 2 DM can also occur as a result of other acquired or genetic conditions that impair insulin signaling in peripheral tissues.

*Polycystic ovary syndrome (PCOS)* is associated with marked increases in ovarian androgen production and insulin resistance. It is one of the most common endocrine disorders in women affecting approximately 6% of all women during their reproductive life. Although the pathogenesis of PCOS remains uncertain, insulin resistance and hyperinsulinemia are found in approximately 80% of affected women. The long-term consequences include increased risk for diabetes mellitus, increased blood lipids, and cardiovascular disease.

*Excess formation of glucocorticoids (Cushing’s syndrome)* or *growth hormone (acromegaly)* also decreases the sensitivity of various tissues to the metabolic effects of insulin and can lead to development of DM. Genetic causes of obesity and insulin resistance if severe enough, also can lead to type 2 diabetes as well as many other features of the metabolic syndrome including cardiovascular disease.

*Development of type 2 DM during prolonged insulin resistance*

With prolonged and severe insulin resistance, even the increased levels of insulin are not sufficient to maintain normal glucose regulation. Moderate hyperglycaemia occurs after ingestion of carbohydrates in the early stages of the disease. In the later stages of type 2 diabetes, the pancreatic β cells become “exhausted” and are unable to produce enough insulin to prevent more severe hyperglycaemia especially after the person ingests a carbohydrate rich meal. Some
obese people, although having marked insulin resistance and greater than normal increases in blood glucose after a meal, never develop clinically significant DM. The pancreas gradually becomes exhausted from secreting large amounts of insulin and full blown DM occurs. Some studies suggest that genetic factors play an important role in determining whether an individual’s pancreas can sustain the high output of insulin over many years that is necessary to avoid the severe abnormalities of glucose metabolism in type 2 diabetes (Guyton and Hall, 2006).

1.3.3. Gestational DM

Gestational diabetes mellitus (GDM) is a glucose intolerance of varying degrees of severity which starts or is first recognized during pregnancy. The definition applies regardless of whether insulin is used for treatment or if the condition persists after pregnancy.

Maintaining control of blood glucose levels significantly reduces the risk to the baby as an increased maternal glucose level could result in complications in the baby including large size at birth, birth trauma, hypoglycaemia and jaundice. Women who have had GDM have an increased risk of developing type 2 diabetes in later years. GDM is also associated with increased risk of obesity and abnormal glucose metabolism during childhood and adult life in the offspring. GDM occurs in approximately 4% pregnancies in United States; most women revert to normal glucose tolerance post-partum but have a substantial risk (30-60%) of developing DM later life. Pregnant women commonly experience health issues that are either caused by or exacerbated by the pregnant state (International Diabetes Federation Atlas, 2009; Triplitt et al., 1999).

1.4. Diabetes complications

In virtually every high-income country, diabetes is ranked among the leading causes of blindness, renal failure and lower limb amputation. Diabetes is also now one of the leading causes of death, largely because of a markedly increased risk of coronary heart disease and stroke (cardiovascular disease).
In addition to the human suffering that diabetes-related complications cause, to those with diabetes but also to their careers, their economic costs are huge. Costs include those for healthcare, loss of earnings and economic costs to the wider society in loss of productivity and associated lost opportunities for economic development. Chronic elevation of blood glucose, even when no symptoms are present to alert the individual to the presence of diabetes will eventually lead to tissue damage with consequent and often serious disease. Whilst evidence of tissue damage can be found in many organ systems, it is the kidneys, eyes, peripheral nerves and vascular tree which manifest the most significant, and sometimes fatal, diabetes complications (Figure 1.1.).
Figure 1.1.: The major diabetes complications

(Source: International Diabetes Federation Atlas, 2009)
Unsatisfactory metabolic control in children can result in stunted growth and exposure to both severe hypoglycaemia and chronic hyperglycaemia can adversely affect neurological development. Children are more sensitive to a lack of insulin than adults and are at a higher risk of a rapid and dramatic development of diabetic ketoacidosis (diabetic coma). The mechanism by which diabetes leads to these complications is complex and not yet fully understood but involves the direct toxic effects of high glucose levels along with the impact of elevated blood pressure, abnormal lipid levels and both functional and structural abnormalities of small blood vessels. The major chronic complications of diabetes are:

1. Cardiovascular disease (CVD)
2. Nephropathy
3. Neuropathy
4. Amputation and
5. Retinopathy.

1. **Cardiovascular disease**

Cardiovascular disease is the major cause of death in diabetes accounting in most populations for 50% or more of all diabetes fatalities and much disability. The kinds of CVD that accompany diabetes include angina, myocardial infarction (heart attack), stroke, peripheral artery disease and congestive heart failure (CHF).

2. **Nephropathy**

Diabetes is an increasingly important cause of renal failure, and indeed has now become the single most common cause of end stage renal disease, i.e. that which requires either dialysis or kidney transplantation in the USA and in other countries.

3. **Neuropathy**

When blood glucose and blood pressure are not controlled, diabetes can harm the nerves. Problems with digestion, urination, impotence and many other functions can result but the most commonly affected area is the feet and legs. Nerve
damage in these areas is called peripheral neuropathy and could manifest in many ways including loss of feeling in the feet and toes. Loss of feeling is a particular risk because it can allow foot injuries to escape notice and treatment leading to major infections and amputation.

4. **Amputation**

Through effects on peripheral nerves and arteries diabetes can lead to foot ulceration, infection and the need for amputation. People with diabetes carry a risk of amputation that may be more than 25 times greater than that seen in those without diabetes.

5. **Retinopathy**

Diabetes can harm sight and cause blindness in several ways. The most common cause of blindness in diabetes is macular edema caused by fluid build-up behind the retina of the eye. A more common complication is background and proliferative retinopathy which can cause blindness as a result of repeated haemorrhages at the back of the eye. Diabetes also increases the risk of cataracts and glaucoma.

1.5. **Impaired glucose tolerance**

Impaired glucose tolerance (IGT) is an asymptomatic condition defined by elevated (though not diabetic) levels of blood glucose 2 hour after a 75 gram oral glucose challenge. Along with impaired fasting glucose (IFG), it is now recognized as being a stage in the transition from normality to diabetes. IGT shares many characteristics with type 2 diabetes being associated with obesity, advancing age, insulin resistance and an insulin secretory defect (International Diabetes Federation Atlas, 2009).
1.6. **Insulin**

Insulin is the internal secretion of the pancreas formed by groups of cells called the islets of Langerhans. In response to high levels of glucose in the blood, the insulin producing cells in the pancreas secrete the hormone insulin. Insulin is injected into the body by people with type 1 diabetes in whom the cells that produce insulin have been destroyed. This is the most common form of diabetes in children and young adults and they depend on insulin for survival. Insulin may also be used by people with type 2 diabetes. In type 2 diabetes, the body needs more insulin than it can produce (Figure 1.2.).

*Figure 1.2.:* **Insulin production and action**

(Source: International Diabetes Federation Atlas, 2009)
Since the landmark discovery of insulin by Frederick Banting and Charles Best in 1921, huge steps forward have been made in research and development in creating genetically engineered human insulin. Until relatively recently insulin was derived from a limited resource of the pancreas of cattle and pigs (International Diabetes Federation Atlas, 2009).

1.7. The global burden of diabetes in world

According to the Diabetes Atlas 2009 new data by the International Diabetes Federation (IDF) showed that a staggering 285 million people worldwide have diabetes. The latest figures from the IDF diabetes atlas indicate that people in low and middle-income countries (LMCs) are bearing the brunt of the epidemic, and that the disease is affecting far more people of working age than previously believed.

In 1985, the best data available suggested that 30 million people had diabetes worldwide. Fast-forward 15 years and the numbers were revised to just over 150 million. Less than 10 years on, the new figures launched at the 20th World Diabetes Congress in Montreal, Canada – put the number closer to 300 million, with more than half aged between 20 and 60. IDF predicts that, if the current rate of growth continues unchecked, the total number will exceed 435 million in 2030.

Diabetes claims four million lives every year and is a leading cause of blindness, kidney failure, heart attack, stroke and amputation. Both type 1 and type 2 diabetes represent a serious health threat (International Diabetes Federation Atlas, 2009).

1.7.1. Diabetes explodes worldwide

Diabetes now affects seven percent of the world’s adult population. The regions with the highest comparative prevalence rates are North America, where 10.2 % of the adult population have diabetes followed by the Middle East and North Africa Region with 9.3%. The regions with the highest number of people living with diabetes are Western Pacific, where some 77 million people have diabetes and South East Asia with 59 million.
India is the country with the most people with diabetes with a current figure of 50.8 million followed by China with 43.2 million. Behind them the United States (26.8 million); the Russian Federation (9.6 million); Brazil (7.6 million); Germany (7.5 million); Pakistan (7.1 million); Japan (7.1 million); Indonesia (7 million) and Mexico (6.8 million).

When it comes to the percentage of adult population living with diabetes, the new data reveal the devastating impact of diabetes across the Gulf Region, where five of the Gulf States are among the top ten countries affected. The Pacific island nation of Nauru has the world’s highest rate of diabetes, with almost a third of its adult population (30.9%) living with the disease. It is followed by the United Arab Emirates (18.7%); Saudi Arabia (16.8%); Mauritius (16.2%); Bahrain (15.4%); Reunion (15.3%); Kuwait (14.6%); Oman (13.4%); Tonga (13.4%) and Malaysia (11.6%).

Age distribution

The 40-59 age groups currently has the greatest number of people with diabetes with some 132 million in 2010, more than 75% of whom live in low- and middle-income countries.

By 2030, it is projected that there will be 188 million people with diabetes aged 40-59 years. More than 80% will be found in newly developed or developing countries. There will be even more people in the 60-79 age group, at some 196 million.

Gender distribution

The estimates for both 2010 and 2030 showed little gender difference in the number of people with diabetes. For 2010 there are expected to be about one million more women than men with diabetes (143 million women vs. 142 million men). However, this difference is expected to increase to six million by 2030 (222 million women vs. 216 million men).
Urban/rural distribution

There are more people with diabetes living in urban than in rural areas. In the LMCs, estimates put the number of people with diabetes in urban areas to be 113 million in 2010, compared to 78 million in rural areas. By 2030 it is expected that this discrepancy will increase to 228 million people with diabetes in urban areas and 99 million in rural communities (International Diabetes Federation Atlas, 2009).

1.7.2. Increasing economic burden

Diabetes has become a development issue. IDF 2009, predicts that diabetes will cost the world economy at least US$376 billion in 2010 or 11.6% of total world healthcare expenditure. By 2030, this number is projected to exceed US$490 billion. More than 80% of diabetes spending is in the world’s richest countries and not in the poorer countries where over 70% of people with diabetes now live. The United States accounts for US$198 billion or 52.7% of total diabetes spending worldwide. India which has the largest diabetes population spends US$2.8 billion or 1% of the global total. In most LMCs (low and middle cost countries), people with diabetes must pay for their care out of their own pocket because public medical services and insurance are lacking. The diagnosis of diabetes in a low or middle-income country can often drag entire families into poverty.

1.7.3. Burden of mortality due to diabetes

IDF Diabetes Atlas 2009, the prevalence of diabetes mellitus and IGT has been estimated for each country for the years 2010 and 2030. Data are provided for 216 countries and territories, which have been allocated into one of the seven IDF regions: Africa (AFR), Europe (EUR), Middle East and North Africa (MENA), North America and Caribbean (NAC), South and Central America (SACA), South-East Asia (SEA), and the Western Pacific (WP).

Close to four million deaths in the 20-79 age groups may be attributable to diabetes in 2010, accounting for 6.8% of global all-cause mortality in this age group. This estimated number of premature deaths is similar in magnitude to deaths in this age group from several infectious diseases. The highest number of deaths due to diabetes is expected to occur in countries with large populations as they have the largest numbers of people with diabetes—India, China, United States of America and the Russian Federation. More women than men are expected to die from diabetes-related deaths, and diabetes makes for a higher proportion of deaths in women than in men, reaching up to a quarter of all deaths in middle-aged women in some regions. In most age groups women with diabetes, compared to those without, have a higher relative risk of death than men with diabetes. It is this that accounts for diabetes making a proportionately greater contribution to female mortality.

The number of deaths attributable to diabetes in 2010 shows a 5.5% increase over the estimates for the year 2007. This increase is largely due to a 29% increase in the number of deaths due to diabetes in the NAC Region, a 12% increase in the SEA Region and an 11% increase in the WP Region. These increases can be explained by a rise in diabetes prevalence in some highly populated countries in each region, particularly in women (International Diabetes Federation Atlas, 2009).
1.8. Experimental models for DM

Animals exhibiting a syndrome of insulin resistance and type 2 diabetes with characteristics similar to humans comprise a wide range of species with genetic, experimental or nutritional causation. Diabetes can be induced by pharmacologic, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. The classical model employed by Banting and Best was pancreatectomy in dogs. Currently, the murine model is one of the most used model due to the availability of over 200 well characterized inbred strains and the ability to delete or over-express specific genes through knockout and transgenic technologies. Ideally, preclinical experiments should be initially carried out in vivo and be complemented when possible with in vitro studies to explore and advance in the mechanism of action of a natural product (Frode and Medeiros, 2008).

1.8.1. In vivo animal models of DM

1.8.1.1. Spontaneous type 2 diabetic models

Spontaneously diabetic animals of type 2 diabetes obtained from the animals with one or several genetic mutations transmitted from generation to generation (e.g., ob/ob, db/db mice) or by selected from non-diabetic outbreed animals by repeated breeding over several generation (e.g., (GK) rat, Tsumara Suzuki obese diabetes (TSOD) mouse). These animals generally inherited diabetes either as single or multigene defects. The metabolic peculiarities result from single gene defect (monogenic) which may be due to dominant gene (e.g., Yellow obese or KK/Ay mouse) or recessive gene (diabetic or db/db mouse, Zucker fatty rat) or it can be of polygenic origin (e.g., Kuo Kondo (KK) mouse, New Zealand obese (NZO) mouse). Type 2 diabetes occurring in majority of human being is a result of interaction between environmental and multiple gene defects though certain subtype of diabetes do also exist with well defined cause (i.e., maturity onset diabetes of youth (MODY) due to defect in glucokinase gene) and this single gene defects may cause type 2
diabetes only in few cases. Therefore polygenic animals represent the human condition more closely when compared to monogenic animals (Frode and Medeiros, 2008).

### 1.8.1.1. Spontaneous type 2 diabetic obese models

#### ob/ob mouse

*ob/ob* mouse (obese mouse) (also called as *Lepob*) is inherited as (monogenic) autosomal recessive mutation on chromosome 6 (obese) in C57BL/6J mouse strain, originating from the Bar Harbor, Jackson laboratory (Shafrir, 2003).

#### db/db mouse

The *db/db* (diabetic) mouse (now relabeled as *leprdb*) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine (Shafrir, 2003). The mutation in this diabetic animal was traced to *db* gene, which encodes for the leptin receptors.

#### KK mouse

KK (Kuo Kondo) mouse is polygenic model of obesity and type 2 diabetes produced by selective inbreeding for the large body size in Japan, also named as Japanese KK mouse (McIntosh and Pederson, 1999).

#### KK/Ay mouse

KK/Ay mouse (also called as Yellow KK obese mouse) carries lethal yellow obese (Ay) and diabetic gene unlike KK mouse. KK/Ay mouse is heterozygous which shows severe obesity, hyperglycaemia, hyperinsulinaemia and glucose intolerance after 8 week of age.

#### New Zealand Obese (NZO) mouse

The NZO strain is a polygenic model of obesity and diabetes obtained by selective inbreeding over several generations with the parents selected for their agouti coat colour. It exhibits a polygenic syndrome of hyperphagia, obesity, mild hyperglycaemia, hyperinsulinaemia, impaired glucose tolerance and insulin resistance.
NONcNZO10 mouse

It is a recombinant congenic new mouse strain model of type 2 diabetes developed by introgressing 5 genomic intervals containing NZO/H1Lt (NZO) diabetogenic quantitative trait loci onto the non obese non diabetic (NON/Lt or NON) genetic background at Jackson laboratory, Maine (Haskell, 2002).

TSOD mouse

Tsumara and Suzuki described the two inbred strains, one with obesity with increase in urinary glucose named TSOD (Tsumara Suzuki Obese Diabetes) and other without them (TSNO, Tsumara Suzuki Non Obese).

M16 mouse

M16 mouse is a new model for obesity and type 2 diabetes. M16 mice exhibit early onset of obesity and are larger at all ages characterized by increased body fat percentage, fat cell size, fat cell numbers, and organ weights.

Zucker fatty rat

The spontaneous mutation _obese_ (fatty) was found in the rat stock of Sherman and Merck, by Zucker, Harriet Bird Memorial Laboratory, Stow, Manssachusetts, USA in 1961. The Zucker (fa/fa) fatty or obese rat (now labeled as Leprfa) results from the simple autosomal recessive (fa) gene on chromosome 5.

Zucker diabetic fatty rat

ZFR selectively inbred for hyperglycaemia and is highly useful for the investigation of mechanism of type 2 diabetes. Male Zucker diabetic fatty (ZDF) rat progresses to frank diabetes due to failure to compensate adequately for insulin resistance.

SHR/N-cp rat

SHR/N-cp rat (spontaneously hypertensive rat/NIH corpulent) is derived by inbreeding of SHR/N strains at the National Institute of Health (NIH), Bathesda, Maryland, USA.
OLETF rat

OLETF (Otsuka Long Evans Tokushima Fatty) rat with mild obesity was obtained from the selective breeding of the spontaneous diabetic rats from the outbred colony of Long Evans rat maintained in Otsuka pharmaceuticals, Tokushima, Japan.

Obese rhesus monkey (Macaca mullata)

Obese rhesus monkey, an excellent non-rodent model develops obesity, hyperinsulinaemia and insulin resistance when maintained on ad libitum laboratory diet which gradually progresses to necrosis of β cells, severe fall in insulin levels and overt hyperglycaemia over a period of several years (Kim et al., 2005).

1.8.1.1.2. Spontaneous type 2 diabetic non obese models

Cohen diabetic rat

The Cohen diabetic rat is an exceptional genetically derived experimental model of diet induced type 2 diabetes that reproduces many features of the disease in humans. Cohen rat strain was newly inbred and metabolic phenotypes of this rebred colony of CDs (Cohen diabetic sensitive) and CDr (Cohen diabetic resistant) rats and their genetic makeup render the Cohen diabetic rat a useful experimental model that is highly suitable for studying the interaction between nutritional metabolic environmental factors and genetic susceptibility for the development of type 2 diabetes (Weksler-Zangen et al., 2001).

GK rat

The GK (Goto-Kakizaki) rat, a polygenic model of type 2 diabetes was established by Goto and his collaborators through selective inbreeding of Wistar rats with abnormal glucose tolerance repeated over several generations in Japan in 1973. It is characterized by non obesity, moderate but stable fasting hyperglycaemia,
Non obese mutant C57 BL/6 (Akita) mouse

The non obese mutant mouse (Akita mouse) has been derived from the colony of C57 BL/6 (B6) in Akita (Japan) and now commercially available for research at Jackson Laboratory, Bar Harbor. The $\text{Ins}_2$ gene is the mouse homologue of human preproinsulin gene. Mice possess another active insulin gene, $\text{Ins}_1$, which lacks an intron present in the C-polypeptide-encoding region. The Akita ($\text{Ins}_2\text{Akita}$) spontaneous mutation (commonly referred as Mody) is an autosomal dominant mutation in the insulin II gene ($\text{Ins}_2$) (Yoshioka, 1997).

ALS/Lt mouse

ALS/Lt (a substrain maintained at Jackson Laboratory, Bar Harbor) mice, hyperinsulinaemia and impaired glucose tolerance develop spontaneously between 6 and 8 week of age in alloxan-untreated males. This mouse model with reduced ability to diffuse free radical stress is of obvious interest because free radical mediated damage is implicated in the pathogenesis and complications of both type 1 and type 2 diabetes (Mathews et al., 2004).

1.8.1.2. Dietary or nutrition induced type 2 diabetic models

Sand rat, Tuco-Tuco and Spiny mouse are important models of nutritionally induced obesity and type 2 diabetes (Shafrir, 2003).

Sand rat

$\text{Psammomys obesus}$ ($P. \text{obesus;}$ Sand rat) remains normal in its natural habitat but develop obesity and diabetes in captivity when fed on standard laboratory chow (high energy diet) instead of its usual low energy vegetable diet (mainly of $\text{Atriplex}$) (Shafrir and Ziv, 1998). Sand rats develop hyperphagia, obesity, hyperinsulinaemia, glucose intolerance with pancreatic islet cells remain intact followed by $\beta$ cell degeneration and necrosis resulting in profound insulin deficiency and overt diabetes and ketosis ultimately leading to death of animal (Shafrir, 2001).
Type 2 diabetic model by simply feeding high fat feed to nonobese, non diabetic C57BL/6J mouse strain was initially developed in Japan. It is characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance (Surwit et al., 1988).

1.8.1.3. Chemically induced diabetic models

Chemically induced models of diabetes are common in elucidating the possible role of environmental factors involved in the endocrine pancreatic destructive processes and subsequent development of diabetes.

1.8.1.3.1. Obese models

1. Goldthioglucose obese diabetic mouse

Type 2 diabetes with obesity is induced in mice by goldthioglucose (GTG) (150-350 or 200 mg/kg, i.p.) injection. Mice gradually develop obesity, hyperinsulinaemia, hyperglycaemia, insulin resistance over a period of 16-20 week after GTG injection. The GTG is transported in particular to the cells of ventromedial hypothalamus (VMH) and causes a necrotic lesion which subsequently is responsible for the development of hyperphagia and obesity. It also shows increased body lipid and hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreased glucose metabolism in muscle, abnormalities that are qualitatively similar to genetically obese mice (ob/ob). It exhibits many molecular defects in relation to insulin signaling pathways (Le Marchand Brustel, 1999).

1.8.1.3.2. Non obese models

1. Alloxan induced diabetes in animals

Alloxan (31%) is by far the most frequently used chemical and this model has been useful for the study of multiple aspects of the disease. Alloxan is the most
prominent diabetogenic chemical in diabetes research and synthesized a pyrimidine derivative which they later called alloxan (Lenzen, 1988). In 1943, alloxan became of interest in diabetes research when Dunn and McLetchie reported that it could induce diabetes in animals (Dunn and McLetchie, 1943) as a result of the specific necrosis of the pancreatic $\beta$ cells (Jorns et al., 1997). The resulting insulinopenia causes a state of experimental diabetes mellitus called ‘alloxan diabetes’ (McLetchie, 1982). The chemistry of alloxan and its derivatives are as follows

**Figure 1.3. Structure of alloxan and its derivatives**

![Alloxan](image1.png)

![Dialuric acid](image2.png)

![Butylalloxan](image3.png)

![Alloxantin](image4.png)

**Alloxan**

Alloxan, a $\beta$-cytotoxic, chemically called mesoxalurea, mesoxalyurea, mesoxalycarbamide, and 2, 4, 5, 6-tetra-oxohexahye 4-pyrimide or pyrimidine
tetrone has been extensively used for in vivo induction of ‘chemical diabetes’ in animals (Figure 1.3. A). Alloxan causes diabetes in animals through its ability to destroy the insulin-producing β cells of the pancreas (Oberley, 1988). Alloxan is a hydrophilic compound, which readily decomposes at neutral pH (Lenzen and Munday, 1991). It inhibits thiol-dependent enzymes such as glucokinase and hexokinase and undergoes redox cycling in the presence of physiological reducing agents, generating ‘active oxygen’ species (Winterbourn and Munday, 1989). It is generally believed that the later species are involved in the initiation of the toxic changes that leads to pancreatic β cell death (Oberley, 1988). Alloxan and N substituted alloxan derivatives were selectively toxic to pancreatic β cells, with other endocrine cells and exocrine parenchymal cells being well preserved, even at high concentration.

**Alloxan derivatives**

Alloxan derivatives were much more toxic than alloxan itself. The toxicity increased with the lipophilicity. These are:

*Dialuric acid*

The 5 oxime of alloxan is violuric acid (Figure 1.3. B). When reduced with Zn/HCl alloxan forms dialuric acid. Dialuric acid was more toxic than alloxan.

*Butylalloxan*

N-substituted alloxan derivatives with a long carbon side chain, such as butylalloxan (Figure 1.3. C), differ chemically from alloxan in that they are lipophilic. Butylalloxan acts in a similar manner to alloxan and preferentially damages β cells.

*Alloxantin*

It can be obtained by reducing alloxan (one molecule) with half a molecule of hydrogen sulphide (Figure 1.3. D). It was even much more toxic than alloxan. Alloxantin dissociates in to alloxan and dialuric acid but the toxicity of alloxantin
was greater than that of an equimolar solution of the dissociation product (Lenzen, 2008). Administration of alloxan (i.v.) produced diabetes within 48h by selectively destroying β cells of islets of Langerhans in many species of experimental animals. It seems that intact pyridine is essential for diabetogenic activity. Activity is abolished by substitution at any position in the molecule other than one imino group in vitro.

**Mechanism of action**

Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β cells. The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals. This loss is likely to stem from kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered. The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher. For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species. In mice, doses vary among 100–200 mg/kg by intravenous route (i.v.). Guinea pigs, ducks, owl, chicks, frogs and toads are resistant to the diabetogenic action of alloxan (Frode and Mederos, 2008; Lenzen, 2008; Federiuk et al., 2004).

2. **Streptozotocin (STZ) induced diabetes in animals**

Streptozotocin (69%) is mostly useful for induction of diabetes in animals. Streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent (White, 1963). Streptozotocin is diabetogenic substance (Rakieten et al., 1963). Again, this insulinopenia syndrome, called ‘streptozotocin diabetes’ (Schein et al., 1967) is caused by the specific necrosis of the pancreatic β cells and streptozotocin has been the agent of choice for the induction of diabetes mellitus in animals (Lenzen, 2008). Streptozotocin exerts their diabetogenic action when administered parenterally, intravenously, intraperitoneally
or subcutaneously. The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. According to the administered dose of these agents, syndromes similar to either type 1, type 2 diabetes mellitus or glucose intolerance can be induced (Frode and Mederos, 2008; Mythili et al., 2004). In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes (Patel et al., 2006), but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single doses below 40 mg/kg may be ineffective (Katsumata et al., 1992).

In general, rats are considered diabetic if tail blood glucose concentrations in fed animals are greater than 200–300 mg/dl, 2 days after STZ injection. In adult mice, STZ given in multiple low doses (40 mg/kg, i.v. for 5 days) (Rees and Alcolado, 2005) induces an insulin dependent diabetes that is quite similar to the autoimmune forms (islet inflammation and β cell death) of type 1 diabetes. On the other hand, a single dose between 60 and 100 mg/kg of STZ (Sharma et al., 2006), administered systemically can also cause insulin dependent diabetes. The potential problem with STZ is that its toxic effects are not restricted to pancreatic β cells since it may cause renal injury (Valentovic et al., 2006). The destruction of pancreatic β cells by STZ is associated with a huge release of insulin which makes animals more susceptible to severe hypoglycaemia that may be lethal. Thus, following treatment with either STZ, animals are fed with glucose solution (5%) for 12–24 hour. Afterwards, an increase of glucose levels is observed in comparison to control animals due to insulin deficiency. In general experimental protocols recommend that administration of either STZ must be done in the fasting period (8–12h) followed by addition of glucose solution to avoid hypoglycemcia. Besides rats, dogs and mice other animal species such as rabbits and monkeys have been employed to induce diabetes by these protocols, but rabbits and pigs are more resistant to STZ (Rees and Alcolado, 2005).
Mechanism

a) \(\beta\) cell selectivity of streptozotocin

Streptozotocin is a nitrosourea analogue in which the N-methyl-N-nitrosourea (MNU) moiety (Figure 4.3) is linked to the carbon-2 of a hexose. The toxic action of streptozotocin and chemically related alkylating compounds requires their uptake into the cells. Nitrosoureas are usually lipophilic and tissue uptake through the plasma membrane is rapid; however, as a result of the hexose substitution, streptozotocin is less lipophilic. Streptozotocin is selectively accumulated in pancreatic \(\beta\) cells via the low-affinity GLUT2 glucose transporter in the plasma. Thus, insulin-producing cells that do not express this glucose transporter are resistant to streptozotocin. This observation also explains the greater toxicity of streptozotocin compared with N-methyl-N-nitrosourea in cells that express GLUT2, even though both substances alkylate DNA to a similar extent. The importance of the GLUT2 glucose transporter in this process is also shown by the observation that streptozotocin damages other organs expressing this transporter, particularly kidney and liver (Lenzen, 2008).

b) \(\beta\) cell toxicity of streptozotocin

It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnic. It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its
methylnitrosourea moiety (Murata et al., 1999). The transfer of the methyl group from streptozotocin to the DNA molecule causes damage, which along a defined chain of events results in the fragmentation of the DNA. Protein glycosylation may be an additional damaging factor. In the attempt to repair DNA, poly (ADP-ribose) polymerase (PARP) is over stimulated. This diminishes cellular NAD+, and subsequently ATP stores. The depletion of the cellular energy stores ultimately results in β cell necrosis. Although streptozotocin also methylates proteins, DNA methylation is ultimately responsible for β cell death, but it is likely that protein methylation contributes to the functional defects of the β cells after exposure to streptozotocin. Inhibitors of poly ADP-ribosylation suppress the process of DNA methylation. Thus, injection of nicotinamide and other PARP inhibitors in parallel with, or prior to the administration of streptozotocin is well known to protect β cells against the toxic action of streptozotocin and to prevent the development of a diabetic state. Also, mice deficient in PARP are resistant to β cell death mediated by streptozotocin, in spite of DNA fragmentation. The absence of PARP prevents the depletion of the cofactor NAD+ and the subsequent loss of ATP and thus cell death. Some minor generation of ROS, including superoxide and hydroxyl radicals originating from hydrogen peroxide dismutation during hypoxanthine metabolism may accompany the effect of streptozotocin and accelerate the process of β cell destruction but ROS do not play a crucial role. The effects of streptozotocin on glucose and insulin homeostasis reflect the toxin-induced abnormalities in β cell function (Lenzen, 2008).

3. **STZ-Nicotinamide (NTM) induced diabetes in animals**

Since injection of nicotinamide, a precursor to nicotinamide adenine nicodinamide adenine dinucleotide (NAD), immediately before, or soon after, the administration of streptozotocin completely protects against the development of diabetes. It was postulated that streptozotocin acts on the β cell by depletion of islet NAD (Gunnarsson et al., 1974). As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic β cell mass producing type 2 diabetes (Masiello et al., 1998).
4. *Neonatal STZ induced diabetes in animals*

Unlike the injection of single high dose of STZ, which can produce type 1 diabetes in adult rats, STZ when injected neonatally or immediately after birth, rats develop type 2 diabetes in the adult age. Single injection of STZ at the dose range of 80-100 mg/kg of STZ (i.v. or i.p. or s.c.) to one or two or five day old Wistar or Sprague-Dawley neonatal rats has been reported to produce type 2 diabetic conditions (Bonner-Weir et al., 1981). The neonatal STZ rats are considered to be better tools for the elucidation of the mechanisms associated with regeneration of the β cells, the functional exhaustion of the β cells and the emergence of defects in insulin action (Bonner-Weir et al., 1981). Some investigators have also developed neonatal type 2 diabetic models by injecting alloxan (200 mg/kg, i.p.) to male neonatal rats at age of 2, 4 or 6 day after birth and found to be much useful for the investigation of long term complication of type 2 diabetes.

5. *STZ with high fat or high fructose diet*

Feeding of high fat diet (HFD) or high fructose diet with STZ treatment produces hyperinsulinaemia and insulin resistance initially followed by treatment with STZ that causes the β cell damage and frank hyperglycaemia in the presence of almost absolute normal insulin circulating concentrations in nongenetic, outbreed animals such as rats (Reed et al., 2000) and mice (Manchem et al., 2001). Combination of short term HFD feeding followed by low dose of STZ (35 mg/kg, i.p.) treatment produced type 2 diabetes in rats (Srinivasan et al., 2005). It is unique and different from other combination rat models since the dose of STZ selected causes diabetes only in HFD-fed insulin resistant rats where as it fails to induce the same in normal control rats resembling the situation in humans with risk factors of obesity and insulin resistance to be more prone to develop type 2 diabetes than others without them. These rats are not insulinopenic and further responsive to the actions of both insulin sensitizing as well as insulinotropic agents. HFD-fed with low dose STZ treated rat model interestingly exhibits stable, long lasting hyperglycaemia and the symptoms of type 2 diabetes like polyuria, polydipsia and polyphagia and diabetic complications such as hypertension. These nongenetic type
2 diabetic rats may be good alternative and cost-effective as compared to genetic models for the investigation as well as regular screening experiments.

6. **Other chemicals used for induction of diabetes in animals**

Vacor, dithizone(diphenylthiocarbazone), and 8- hydroxyquinolone may also cause experimental diabetes, but their use in research is restricted due to their high level of toxicity (Clark *et al.*, 1994).

**1.8.1.4. Surgical models of diabetes**

Another technique used to induce diabetes is the complete or partial removal of the pancreas induction of type 1 or type 2 diabetes, respectively. Historically, the diabetic dog model discovered by Oskar Minkowski through surgical complete pancreatectomy has been considered to be the first animal model of diabetes and is rarely now used for the investigation (Ozturk *et al.*, 1996). Few researchers have employed this model in the last years to explore effects of natural products with animal species such as rats, pigs, dogs and primates. Limitations to this technique include (1) high level of technical expertise and adequate surgical room environment (2) major surgery and high risk of animal infection (3) adequate post-operative analgesia and antibiotic administration (4) supplementation with pancreatic enzymes to prevent malabsorption and (5) loss of pancreatic counter regulatory response to hypoglycemia. More recently, partial pancreatectomy has been employed, but large resection (more than 80% in rats) is required to obtain mild to moderate hyperglycaemia. In this case, small additional resection can result in significant hypoinsulinemia (Frode and Mederos, 2008).

**1.8.1.5. Genetic models of diabetes**

1. **Animal strains that spontaneously develop diabetes**

These models permit the evaluation of the effect of a natural product in an animal without the interference of side effects induced by chemical drugs like alloxan and STZ. Similar to the human condition, these strains display complex and heterogeneous characteristics. In some of these models, insulin resistance
predominates in association with obesity, dyslipidemia and hypertension, which provides valuable insights to study some events that are observed in human type 2 diabetes mellitus. Conversely, some strains like \textit{ob/ob} mouse may maintain euglycemia due to a robust and persistent compensatory pancreatic β cell response, matching the insulin resistance with hyperinsulinemia. On the other hand, the \textit{db/db} mouse rapidly develops hyperglycaemia since their pancreatic β cells are unable to maintain the high levels of insulin secretion required throughout life. Thus, food intake is important in determining the severity of the diabetic phenotype and restriction of energy intake reduces both the obesity and hyperglycaemia seen in this strain of mice. Another example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of type 2 diabetes originating from selective breeding over many generations of glucose-intolerant nondiabetic Wistar rats (Chen and Wang, 2005). Regarding type 1 diabetes models, the NOD mouse typically presents hyperglycaemia between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age. One great advantage of these models is that they can also be employed as model of atherosclerosis which represents the long-term complication of diabetes mellitus and tested against several natural products. The NOD mouse followed by the ZK rat are the most used to test natural products. Other prone strains to type 1 diabetes mellitus include New Zealand white rabbit, Kreesbond dog, Chinese hamster and Celebes black ape. However, they have not been employed in studies to evaluate natural products to treat diabetes except in preclinical trials of exenatide (incretin analog) (Frode and Mederos, 2008).

2. \textit{Genetically engineered diabetic mice}

Rodents may be produced to over (transgenic) or under (knockout)-express proteins thought to play a key part in glucose metabolism. Certainly, the high costs restrict their study in sophisticated protocols which explore mechanisms of potential therapeutic agents that either stimulate pancreatic β cell growth or inhibit pancreatic β cell death (Meiton, 2006).
1.8.1.6. Other models of type 2 diabetes to evaluate the reduction of pancreatic β cell mass

The importance of the progressive loss of pancreatic β cell reduction in the course of type 2 diabetes has been the focus of therapeutic targets in the development of novel and potential drugs acting by enhancing pancreatic β cell growth or survival (Masiello, 2006). Experimental reduction of pancreatic β cell mass by either surgical or chemical means has been reported above, but these models are limited due to the variety amount of residual pancreatic β cell content. On the other hand, experimental studies carried out predominantly in rodents have demonstrated that incretins, IGFs, hepatocyte growth factor, pituitary adenylate cyclase-activating polypeptide are capable of enhancing pancreatic β cell mass in the field of experimental diabetes. Hence, prolonged administration of these agents may expand pancreatic β cell mass, leading to increased insulin secretion and improved glycemic control (Hansotia and Drucker, 2005). A method to induce diabetes in adult rats is to mimic the unfavourable intrauterine environment, which in humans leads to low birth weight and is supposed to confer high risk for the development of diabetes in adult age. This model known as intrauterine growth retardation by uteroplacental insufficiency in the rat is based on the premise that uterine malnutrition may also increase the risk of diabetes amongst offspring in later life. This has been achieved by several means, including bilateral uterine artery ligation at 19 days of gestation, i.e. 3 days before term. The diabetogenic effects of manipulating the intrauterine environment are probably mediated by a permanent programming of the developing offspring, e.g. by the mechanism of imprinting. It should also be pointed out that the increased risk of diabetes continues into subsequent generations, which in turn, suggests that changes also affect the germ cell line. Finally, animal models with increased pancreatic β cell apoptosis have also been developed (Frode and Mederos, 2008).
1.8.2. *In vitro* models of DM

1.8.2.1. *In vitro* studies on insulin secretion

Conventional antidiabetic agents can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption. Incretins and transcription factors such as peroxisome proliferator-activated receptors-PPAR are targets of modern therapy. Insulin receptor, glucose transporters, however, has not been yet the focus of antidiabetic therapy. Although few studies using natural products have been published (Frode and Mederos, 2008). These methodologies may serve as complementary tools to explore findings obtained in *vivo* models.

1. Studies using isolated pancreatic islet cell lines

Several *in vitro* assays are available to study different steps of insulin secretion. It is known that insulin secretion occurs when pancreatic β cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage-dependent Ca2+ channels. This results in elevation of the intracellular Ca2+ concentration which triggers insulin secretion. In type 2 diabetes, pancreatic β cells exhibit atypical ion channel activity and an abnormal pattern of insulin secretion (Ashcroft and Rorsman, 2004). These pathways can be studied with isolated pancreatic β cells from either control or diabetic rat or mouse that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium (Storling *et al*., 2005).

2. Studies using insulin-secreting cell lines

Bioengineered technologies have provided new opportunities to improve and establish more appropriate cultured cell lines to help to facilitate studies of mechanisms of both insulin secretion and β cell dysfunction being also the target to the study of natural products. The most widely used insulin secreting cell lines are RIN, HIT, β-TC, MIN6 and INS-1 cells. These cell lines release mainly insulin and
small amounts of glucagon and somatostatin. Although the behaviour of none of these cell lines perfectly mimics primary β cell physiology, they are extremely valuable tools for the study of molecular events underlying β cell function (Poitout et al., 1996).

1.8.2.2. In vitro studies on glucose uptake

Adipose tissue is considered a key link between obesity and type 2 diabetes by promoting the development of lipotoxicity, i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance. Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycaemia. However, adipocytes from different sites of the body may have different biological or pathological effects. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as murine 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4 and may be employed as tools to evaluate the effects of natural products upon glucose uptake (Frode and Mederos, 2008).

1.9. Herbal medicines in India

Traditional medicines are used by about 60% of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used (Kamboj, 2000). There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs. Since herbal medicines are prepared from materials of plant origin they are prone to contamination, deterioration and variation in composition. This gives rise to inferior quality of herbal products with little or no therapeutic efficacy. Most often the desired biological response is due to not one but a mixture of bioactive constituents and the relative proportion of active constituents can vary from plant to plant of the same species and also in different plant parts. Hence before proceeding to clinical studies, scientists need a tool to authenticate plants and also to detect their potency.
Current estimates indicate that about 80% of people in developing countries still rely on traditional medicine based largely on various species of plant and animals for their primary healthcare. 30% of the world-wide sales of drugs are based on natural products (Grabley and Thiericke, 1999). Opportunities for multidisciplinary research are immense the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, and pharmacology are combined to exploit the vast diversity of chemical structures and biological activities of natural products (Clark, 1996). Ayurveda is the most ancient health care system and is practiced widely in India, Srilanka and other countries (Chopra and Doiphode, 2002). Atharveda (around 1200 BC), Charak Samhita and Sushrut Samhita (100-500 BC) (Dash and Sharma, 2001) are the main classics that given detailed descriptions of over 700 herbs. Researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on ayurvedic medicinal plants and many of the major pharmaceutical corporations have renewed their strategies in favour of natural products drug discovery. Numerous drugs have entered the international pharmacopoeia through the study of ethnopharmacology and traditional medicine (Patwardhan et al., 2004).

There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. There are currently about 250 000 registered medical practitioners of the Ayurvedic system (total for all traditional systems: approximately 291 000) as compared to about 700,000 of the modern medicine system. In rural India, 70 per cent of the population is dependent on the traditional system of medicine, the Ayurveda (Seth and Sharma, 2004). Efficacy testing of the traditional and new herbal products in experimental screening method is important to establish the active component and appropriate extract of the plant (Chakravarty, 1993).
1.10. Indian medicinal plants with hypoglycaemic activity

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Since time immemorial, various plants and plant derived compounds have been used in the treatment of diabetes to control the blood sugar of the patients. The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time. Several medicinal plants have reported to possess potential hypoglycaemic activity in Indian system of medicines. There have been several reviews on the hypoglycaemic medicinal plants, more particularly use of Indian botanical for hypoglycaemic activity. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. Herbal drugs are prescribed widely because their effectiveness, less side effects and relatively low cost. Therefore investigation on such agents from traditional medicinal plants has become more important.

India has a rich history of using various potent herbs using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals. Many herbal drugs are used in the Indian systems of Medicine as well as in folk and tribal medicine for common ailments. The disease was most often treated with diet control, herbs and herbo-mineral drugs. In India from ancient times, the herbalists treated the disease with indigenous herbs which were free from side effects. Many tribal and non-tribal herbalists keep this information as patent medicine (Rana et al., 1999). The ethno botanical information reports about 800 plants that many possess antidiabetic potential. Several such herbs have shown antidiabetic activity when assessed using presently available experimental techniques. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes. Among these are alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans, hypoglycans, guanidine,