CHAPTER 1

Introduction

Diabetes mellitus (DM), commonly known as diabetes, is one of the world’s oldest known disorders characterized by elevated blood glucose concentrations. It is a chronic disorder, causing disturbances in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance (Devlin, 1997). When fully expressed, diabetes is characterized by fasting hyperglycemia, but the disease can also be recognized during less overt stages, most usually by the presence of glucose intolerance. Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, weight loss, and polyphagia. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, heart, and blood vessels (Peter and William, 2005).

1.1 Epidemiology of Diabetes

The number of people with diabetes is increasing worldwide due to population growth, ageing, urbanization, and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources (King and Rewers, 1993). There have been several previous estimates of the number of persons with diabetes. The World Health Organization (WHO) published estimates for the years 2000 and 2030, using data from 40 countries but extrapolated to the 191 WHO member countries. The WHO has predicted that the major burden will occur in the developing countries and there will be a 42% increase (from 51 to 72 million) in the developed countries and 170% increase (from 84 to 228 million) in the developing countries. The countries with the largest number of diabetes patients are, and will be in the year 2025, India, China and United States. The number of diabetes patients in India currently around 40.9 million is expected to rise to 80 million by 2030 (Wild et al., 2004).
Table 1.1 Countries with the highest numbers of estimated cases of diabetes for 2010 and 2030*

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>PERSONS (MILLIONS)</th>
<th>COUNTRY</th>
<th>PERSONS (MILLIONS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 India</td>
<td>50.8</td>
<td>1 India</td>
<td>87.0</td>
</tr>
<tr>
<td>2 China</td>
<td>43.2</td>
<td>2 China</td>
<td>62.6</td>
</tr>
<tr>
<td>3 United States of America</td>
<td>26.8</td>
<td>3 United States of America</td>
<td>36.0</td>
</tr>
<tr>
<td>4 Russian Federation</td>
<td>9.6</td>
<td>4 Pakistan</td>
<td>13.8</td>
</tr>
<tr>
<td>5 Brazil</td>
<td>7.6</td>
<td>5 Brazil</td>
<td>12.7</td>
</tr>
<tr>
<td>6 Germany</td>
<td>7.5</td>
<td>6 Indonesia</td>
<td>12.0</td>
</tr>
<tr>
<td>7 Pakistan</td>
<td>7.1</td>
<td>7 Mexico</td>
<td>11.9</td>
</tr>
<tr>
<td>8 Japan</td>
<td>7.1</td>
<td>8 Bangladesh</td>
<td>10.3</td>
</tr>
<tr>
<td>9 Indonesia</td>
<td>7.0</td>
<td>9 Russian Federation</td>
<td>10.3</td>
</tr>
<tr>
<td>10 Mexico</td>
<td>6.8</td>
<td>10 Egypt</td>
<td>8.6</td>
</tr>
</tbody>
</table>


1.2 Types of Diabetes

The vast majority of cases of diabetes fall into three broad etiopathogenetic categories. In one category, i.e., type 1 diabetes (IDDM), the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, i.e., type 2 diabetes (NIDDM), the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. The third category is gestational diabetes and is the carbohydrate intolerance associated with hyperglycemia of variable severity with the onset or first recognition during pregnancy (American Diabetes Association, 2011).

1.2.1 Type 1 Diabetes

Type 1 diabetes is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases) which is
thought to be due to immunological destruction of pancreatic β-cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylase (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with β-cell destruction (Atkinson and Maclaren, 1994). There is no known etiological basis for type 1b diabetes mellitus. Some of these patients have permanent insulinopaenia and are prone to ketoacidosis, but have no evidence of autoimmunity (McLarty et al., 1990). This form is more prevalent among individuals of African and Asian origin (Ahrén and Corrigan, 1984).

Table 1.2 Clinical stages and aetiologic types of diabetes*

<table>
<thead>
<tr>
<th>Stages</th>
<th>Normoglycaemia</th>
<th>Hyperglycaemia</th>
<th>Diabetes Mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aetiological process</td>
<td>Normal glucose tolerance (achieved without pharmacological agents)</td>
<td>IGT and/or fasting hyperglycaemia</td>
<td>Not insulin requiring</td>
</tr>
<tr>
<td>Islet cell destruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Autoimmune</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Idiopathic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Predominantly insulin resistance</td>
<td>Predominantly insulin secretory defects</td>
<td>Type 1 diabetes</td>
<td></td>
</tr>
<tr>
<td>Other specific disorders (eg MODY, endocrinopathies)</td>
<td></td>
<td>Other specific types of diabetes</td>
<td></td>
</tr>
<tr>
<td>Pregnancy related defects</td>
<td></td>
<td>Gestational diabetes</td>
<td></td>
</tr>
</tbody>
</table>

*Source: Diabetes Care, 2000; 23 (Suppl. 2): B5-B10.

1.2.2 Type 2 Diabetes

Type 2 diabetes is the most common form of diabetes and is characterized by disorders of insulin secretion and insulin resistance. Traditionally, type 2 diabetes is common in individuals over the age of 40. It is often associated with obesity, decreased physical activity and heredity (Zimmet et al., 1990). Type 2 diabetes shows strong
familial aggregation, so that persons with a parent or sibling with the disease are at increased risk, as are individuals with obesity, hypertension, or dyslipidemia and women with a history of gestational diabetes. The frequency of type 2 diabetes varies considerably among different racial or ethnic subgroups. Persons of Native American, Polynesian or Micronesian, Asian-Indian, Hispanic, or African-American descent are at higher risk than persons of European origin. Although the disease is most commonly seen in adults, the age of onset tends to be earlier in persons of non-European origin. The disease can occur at any age and is now seen in children and adolescents (Dabelea et al., 1999; Kaufman, 2002).

Table 1.3 Diagnostic Criteria for Diabetes Mellitus and Related Stages of Glycemia*

<table>
<thead>
<tr>
<th>Glucose concentration, mg/dL (mmol/L)</th>
<th>Capillary whole bloodb</th>
<th>Venous plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting or 2-hour postglucose</td>
<td>≥110 (≥6.1)</td>
<td>≥126 (≥7.0)</td>
</tr>
<tr>
<td><strong>Impaired glucose tolerance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (if measured) and 2-hour postglucose</td>
<td>&lt;110 (&lt;6.1)</td>
<td>&lt;126 (&lt;7.0)</td>
</tr>
<tr>
<td><strong>Impaired fasting glycaemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting and 2-hour postglucose</td>
<td>140–199 (7.8–11.0)</td>
<td>140–199 (7.8–11.0)</td>
</tr>
<tr>
<td>Capillary whole bloodb</td>
<td>100–125 (5.6–6.9)c</td>
<td></td>
</tr>
<tr>
<td>Venous plasma</td>
<td>&lt;140 (&lt;7.8)d</td>
<td>&lt;140 (&lt;7.8)d</td>
</tr>
<tr>
<td></td>
<td>&lt;200 (&lt;11.1)e</td>
<td>&lt;200 (&lt;11.1)e</td>
</tr>
</tbody>
</table>

NA, not applicable.


1.2.3 Gestational Diabetes (GD)

GD refers to the onset or initial recognition of glucose intolerance during pregnancy, usually in the second or third trimester. It occurs in about 4% of all pregnancies. Patients with GD have a 30% to 50% chance of developing DM, usually
type 2 DM. (American Diabetes Association, 2001). Gestational diabetes can have deleterious consequences for both the fetus and mother. Diabetes occurring before or recognized during pregnancy with elevated fasting glucose concentrations is associated with an increased risk of intrauterine fetal death during the last 4 to 8 weeks of gestation and other complications, including congenital abnormalities (Comess et al., 1969).

Other types of DM include genetic defects of the pancreatic β-cell or in insulin action pathways (insulin receptor mutations or post-receptor defects) as well as disease of the exocrine pancreas (pancreatitis, pancreatic reaction, or cystic fibrosis), are less common causes of DM. Endocrinopathies producing insulin counter regulatory hormones in excess (e.g., Cushing’s syndrome, acromegaly) may result in DM. Certain drugs like glucocorticoids, pentamidine, niacin, and α-interferon may also lead to DM (Raffel et al., 1997).

1.3 Pathophysiology of Diabetes

The pathogenesis of type 2 diabetes is multifactorial and includes both genetic and environmental elements that affect β-cell function and tissue (muscle, liver, adipose and pancreas) insulin sensitivity. Thus the main pathophysiological features of type 2 diabetes are impaired insulin secretion and increased insulin resistance. The impairment of pancreatic cell function notably shows progression over time (Kahn, 2003). The pathogenesis has been assumed to involve genetic abnormality in the molecules related to the regulatory system of glucose metabolism. The analyses of candidate genes targeted at glucose-stimulated insulin secretion of pancreatic β-cells and the molecules comprising the molecular mechanism for insulin action have identified genetic abnormalities that can be independent causes of pathogenesis, including those in glucokinase genes, mitochondrial genes, and insulin receptor genes (Pyke, 1979).

A majority of individuals suffering from type 2 diabetes are obese, with central visceral adiposity. Therefore, the adipose tissue should play a crucial role in the pathophysiology of type 2 diabetes. Its role was highlighted, especially the interactions of non-esterified fatty acids (NEFA) with glucose metabolism (Montague and O’Rahilly, 2000). The deleterious role of ectopic triglyceride storage in the development of defective insulin action and insulin secretion has been emphasized
leading to the concept of lipotoxicity. The adipose tissue can secrete various molecules that may interfere with glucose metabolism and insulin sensitivity, such as leptin, tumor necrosis factor (TNF)-α, resistin and adiponectin (Greenberg and McDaniel, 2002).

**Figure 1.1 Contribution of genetic and environment factors in the pathophysiology of Type 2 Diabetes**

It is clear that hyperglycemia is associated with both insulin resistance and β-cell dysfunction. Once hyperglycemia exists, impaired insulin secretion is clearly present in subjects with type 2 diabetes. This change manifests in a number of different ways including decreases in the early insulin response to intravenous or oral glucose and a decline in the ability of glucose to potentiate the insulin response to non-glucose secretagogues (Polonsky, 1995). Three main mechanisms have been proposed to explain the β-cell deficiency observed in subjects prone to develop type 2 diabetes: first, a genetic defect may be present, although such a defect has not been detected yet in subjects with common type 2 diabetes associated with obesity, in contrast to what was reported in some particular forms of “Maturity-Onset Diabetes of the Youth” (MODY). Second, in utero malnutrition may lead to insufficient β-cell development and later partial insulin secretory defect. This hypothesis has been called the “thrifty phenotype
hypothesis” (Hales and Ozanne, 2003). And third, unfavorable metabolic environment may play a deleterious role, especially increased glucose levels that may induce glucotoxicity and a chronic increase in NEFA levels that may induce lipotoxicity, both processes contributing to alter insulin secretion (Yki-Jarvinen, 1992). Defects in insulin signaling pathways associated with insulin resistance in peripheral tissues have been found to disrupt insulin secretion by pancreatic β-cells, suggesting that insulin resistance in the β-cells may be responsible for the β-cell dysfunction and the development of type 2 diabetes. In this case, insulin resistance develops and expands prior to the disease onset (Scheen and Lefèvre, 2000).

1.4 Complications of Diabetes

Figure 1.2 Glycemic profiles in normal (A) and diabetic (B) subjects.

Diabetes mellitus is associated with long term damage, dysfunction and failure of various organs, and its complications are mainly a consequence of macro-vascular and micro-vascular damage. It is widely accepted that chronic hyperglycemia causes the development of micro and macroangiopathic complications in diabetes mellitus (Heine et al., 2004).

The complications of diabetes mellitus include cardiovascular disease, nephropathy, diabetic retinopathy, neuropathy and respiratory failure. DM increases the expression of
adhesion molecules through hyperglycemia; these molecules play an important role in
the pathophysiological dysfunction of the vasculature (Boulbou et al., 2003).

1.4.1 Acute Complications

These include diabetic ketoacidosis (DKA) and non-ketotic hyper-osmolar state
(NKHS). Diabetic ketoacidosis primarily results from insulin deficiency and
hyperglycemic hyperosmolar state (HHS) from severe insulin resistance. Both these
crisis result in subsequent glucagon and counter-regulatory hormone excess from lack
of suppression from insulin (Chiasson et al., 2003). Both disorders are associated with
absolute or relative insulin deficiency, volume depletion, and altered mental state
(Schade and Eaton, 1979). In diabetic DKA, the balance between catabolism and
anabolism is broken. With the lack of insulin, there is decreased storage of glucose,
increased breakdown of glycogen stores, and increased synthesis of glucose in both the
liver and kidney. To add to the overall hyperglycemic state, there is also a concomitant
decreased utilization of glucose in peripheral tissues (Klatt and Kumar, 2004). In HHS
there is usually enough insulin to suppress ketogenesis, but not control blood sugars,
and are usually higher as ketoacidosis produces more severe symptoms and presentation
is usually earlier (Chupin et al., 1981).

1.4.2 Chronic Complications

The chronic complications of diabetes mellitus affect many organ systems and
are responsible for the majority of morbidity and mortality. Chronic complications can
be divided into vascular and nonvascular complications. The vascular complications are
further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and
macrovascular complications (coronary artery disease, peripheral vascular disease, and
cerebrovascular disease). Nonvascular complications include problems such as
gastroparesis, sexual dysfunction (erectile dysfunction), and skin changes (DCCT,
1993).

1.4.2.1 Diabetic Retinopathy

Diabetic retinopathy (DR) can be defined as damage to microvascular system in
the retina due to prolonged hyperglycemia. Diabetic retinopathy is primarily classified
into non proliferative diabetic retinopathy (NPDR), or background retinopathy, and proliferative diabetic retinopathy (PDR). Progression from mild form characterized by increased vascular permeability, to moderate, and then to severe NPDR characterized by vascular closure and an increased risk for the development of PDR distinguished by the growth of new blood vessels on the retina and posterior surface of the vitreous. Visual impairment in diabetic retinopathy occurs due to diabetic macular edema (DME) and PDR (Rema and Pradeepa, 2007).

1.4.2.2 Diabetic Neuropathy

Diabetic neuropathies are a family of nerve disorders caused by diabetes. It can be classified as peripheral, autonomic, proximal and focal. Each affects different parts of the body in different ways. Diabetic foot ulcers may develop, mainly because of the abnormal distribution of pressure. The early detection of diabetic neuropathy results in less hospitalization of patients with foot ulcers and fewer lower-extremity amputations. Screening for neuropathy can be done reliably by using the 10-g Semmes-Weinstein monofilament over 10 areas of the feet, ankle reflexes and vibration perception over the great toe and ankle. A standard neuropathy disability score (NDS) will be measured and a score of over 6 shows the presence of significant neuropathy (Leung and Lam, 2000).

1.4.2.3 Diabetic Nephropathy

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. This is a major cause of chronic end-stage renal disease worldwide and is responsible for renal failure in about one third of patients who undergo dialysis. There are glomerular hemodynamic abnormalities resulting in glomerular hyper-filtration, leading to glomerular damage as evidenced by microalbuminurea, one of the initial markers of this condition. There is overt proteinuria, decreased glomerular filtration rate, and end-stage renal failure. Dysfunction of the glomerular filtration apparatus is manifested by microalbuminurea and is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening. Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal vascular endothelial growth factor (VEGF) levels observed in preclinical models of
diabetes, since VEGF is both an angiogenic and a permeability factor (Ritz and Orth, 1999).

1.4.2.4 Macrovascular Complications

Macrovascular complications include coronary artery disease (CAD), peripheral vascular disease (PVD), and cerebrovascular disease. PVD is the disease of any blood vessel that is not part of heart or brain. The more common form of PVD is observed in lower extremity which is termed as the Lower extremity arterial disease (LEAD). The simplest screening test for PVD is palpitation of peripheral pulses and this is the usual clinical tool to assess the occlusive arteries in peripheries. (Beach et al., 1988). In diabetes mellitus there is marked increase in several cardiovascular diseases (CVDs), including congestive heart failure, coronary artery disease, and myocardial infarction, and a one to five fold increase in sudden death. In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus (threelfold increase in stroke). The absence of chest pain (silent ischemia) is common in individuals with diabetes, and a thorough cardiac evaluation is indicated in individuals undergoing major surgical procedures. Individuals with DM have increased incidence of congestive heart failure (diabetic cardiomyopathy). The etiology of this abnormality is probably multifactorial and includes factors such as myocardiac ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycemia (Grundy et al., 1999).

1.5 Risk Factors of Diabetes

The important risk factors for the high prevalence of diabetes include: (1) Family history, (2) Obesity especially central obesity, (3) Hypertension, (4) Life style changes and physical inactivity. Several studies in India and abroad have shown that nearly 75% of the type 2 diabetes patients have first degree family history of diabetes; this indicates a strong familial aggregation in the Indian diabetic patient. Insulin resistance has been demonstrated to be a characteristic feature of Asian Indians. A comparative study of Asian Indians, Europeans and other ethnic groups has shown that the Asian Indians have higher insulin response than others at fasting and in response to glucose (Chowdhury and Lasker, 2002).
1.5.1 Obesity

Obesity indicates deposition of large quantities of visceral and subcutaneous fat. Visceral fat increases the risk of diabetes and hyperlipidemia by favoring insulin resistance. In several ethnic populations including the relatively non-obese Asian population, the android pattern of body fat, typified by more upper body adiposity measured as waist: hip ratio was found to be a greater risk factor for type 2 DM than general obesity (Gupta et al., 2007). Adipose tissue secretes many factors that regulate metabolic and vascular biology, collectively called adipokines, which include adiponectin, leptin, tumor necrosis factor-alpha (TNF-α), resistin, angiotensinogen, interleukin-6 (IL-6), plasminogen activator inhibitor-1(PAI-1) and C-reactive protein (CRP). Dysregulation of these adipokines may participate in the pathogenesis of metabolic syndrome (Hutley & Prins, 2005). Studies have suggested that central obesity precedes the development of the other components of metabolic syndrome (MetS) and that, weight reduction at that point could be the best way to prevent it (Plandevall et al., 2006).

1.5.2 Hypertension

Hypertension is a very common comorbid condition in diabetes and accounts for up to 85% of excess cardiovascular disease risk. Conversely, patients with hypertension are more prone to diabetes than are normotensive patients. Hypertension substantially increases the risk for CHD, stroke, retinopathy, and nephropathy. When hypertension coexists with diabetes, the risk of stroke or cardiovascular disease is doubled and the risk for developing end-stage renal disease is increased five to six times compared with the risk for hypertensive patients without diabetes (Sowers et al., 2001). In type 2 diabetes, hypertension usually clusters with the other components of the cardiometabolic syndrome, such as microalbuminuria, central obesity, insulin resistance, dyslipidemia, hypercoagulation, increased inflammation, left ventricular hypertrophy (LVH), and hyperuricemia. In type 1 diabetes, hypertension is usually a manifestation of diabetic nephropathy, and hypertension and nephropathy appear to exacerbate each other (Gress et al., 2000).
1.5.3 Life Style Factors

Susceptibility to "Western" diet and lifestyle is strongly associated with an increased risk of developing diabetes, for people in developing countries (Omran, 1971). Due to rapid changes in lifestyle, risk factors such as obesity, smoking, chronic alcoholism and physical inactivity have become widespread throughout these regions. Urbanization has brought several changes in the life style in most urban areas in India and it is associated with greater prevalence of diabetes (Chowdhury and Lasker, 2002).

1.6 Experimental Diabetes

Approximate experimental models are essential tools for understanding the pathogenesis, complications and genetic or environmental influences that increase the risk of diabetes and testing of various therapeutic agents. For the demonstration of biochemical effects of naturally occurring products or drugs, various workers have used animal models. Drugs which lower glucose concentration in non-diabetic states may not be effective in diabetic states. Since DM is a heterogeneous condition, no single animal model reflects the diversity of lesion in DM (Srinivasan and Ramarao, 2007). Small laboratory rodents have mostly been preferred for testing hypoglycemic agents for reasons of cost, convenience and greater characterization of diabetic condition. Diabetes, both in humans and animals may be initiated by stress, infection, or toxins. Certain other manipulations, including pancractectomy and lesioning of the central nervous system also cause diabetes (Bell and Hye, 1983; Matteucci and Giampietro, 2008).

Over the years, several animal models have been developed for studying diabetes mellitus or testing anti-diabetic agents. These include surgical (pancreatectomy) and chemical models in several animal species to induce DM. Among them, chemical induction of diabetes is widely used. The major diabetogenic drugs used include: alloxan monohydrate and streptozotocin with or without nicotinamide. The selection of these models to use for investigating the antidiabetic properties of a new compound may be a very difficult task (Thatte, 2009).
1.6.1 Chemical Induction of Diabetes Mellitus

The majority of studies published in the field of ethnopharmacology employed this model. Streptozotocin (STZ, 69%) and alloxan (ALX, 31%) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are 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 (Federiuk et al., 2004).

1.6.1.1 Streptozotocin model of diabetes mellitus

Streptozotocin (STZ) or streptozocin or izostazin or zanosar is a synthetic nitrosouridoglucopyranose derivative isolated from fermentations of Streptomyces achromogenes. It has been used as a chemotherapeutic alkylating agent in the treatment of metastasizing pancreatic islets cell tumors and in other malignancies (Schein et al., 1974). The induction of diabetes mellitus with STZ takes some time, and experiment carried out at suitable time intervals after administration of the agent will give additional information into mechanism of action of the tested compound. Single dose of STZ in sterile citrate buffer (pH 4.5, 0.1M) may be used: mice 150 mg/kg; rats 80 mg/kg, administered intraperitoneally. Diabetes develops gradually and may be assessed after a few days, usually four days for mice and seven days for rats. A serum glucose level of about 180 - 500 mg/dL indicates the induction of diabetes mellitus. Sometimes diabetic animals are maintained on insulin if the experiments are not to commence immediately to prevent the animals’ death (Williamson et al., 1996). Type 2 DM was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) and nicotinamide (120 mg/kg) to rats (Pellegrino et al., 1998).

1.6.1.2 Alloxan model of diabetes mellitus

Alloxan is the next most commonly used chemical for induction of diabetes mellitus. Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. For all animals a single dose of alloxan, 150 mg/kg, is administered as a 5% w/v in distilled water injected intravenously into the marginal ear vein of rabbit or
intraperitoneally in case of mice and rats (Macedo et al., 2005). A rest period of seven days for rabbits, 12 days for rats and mice is allowed during which the animals have free access to food and water. Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells (Szkudelski, 2001).

1.7 Metabolic Derangements in Diabetes Mellitus

1.7.1 Hyperglycemia

Hyperglycemia is a phenomenon that has been observed in essentially all individuals with diabetes and may be due to dysregulation of the normal circadian hormonal patterns resulting in increased hepatic glucose output. Fasting hyperglycemia generally can be attributed to inadequate or inappropriate hepatic insulinization or the dawn phenomenon, which is the fasting hyperglycemia occurring in the absence of antecedent hypoglycemia (Sheehan, 2004). Fasting plasma glucose (FPG) levels are determined primarily by hepatic and, to a lesser degree, by renal glucose production. As the plasma glucose levels decrease during fasting, plasma insulin levels decrease proportionately. The decrease in plasma insulin causes an increase in adipose tissue lipolysis and skeletal muscle proteolysis and a decrease in uptake of glucose by peripheral tissue. Fasting hyperglycemia occurs when glucose production exceeds glucose utilization, as occurs with absolute or relative insulin deficiency at the level of the liver. Controlling hepatic glucose output and disposal is essential for effectively managing fasting hyperglycemia. (Dostou and Gerich, 2001).

Postprandial plasma levels of glucose are determined in the initial phase by meal-mediated suppression of hepatic production of glucose and throughout the postprandial period by hepatic and muscle uptake of glucose. The regulation of postprandial plasma hyperglycemia therefore is highly dependent on the qualitative and quantitative aspects of meal-mediated insulin secretion, as well as by the sensitivity of muscle to insulin action (van Haeften et al., 2000). Postprandial hyperglycemia occurs
several years before fasting hyperglycemia and is the initial phase of glucose intolerance. Contributions of postprandial hyperglycemia to the overall glycemic control, as estimated by the HbA1c, will vary with the individual and the stage of glucose intolerance (Harris et al., 1992). Pharmacologic agents may differentially affect fasting and postprandial hyperglycemia. Near-normal glycemia cannot be achieved unless both fasting and postprandial hyperglycemia are controlled (Hanefeld et al., 2000; Ratner, 2001).

1.7.2 Insulin resistance

Insulin resistance occurs when cells in the body (liver, skeletal muscle and adipose/fat tissue) become less sensitive and eventually resistant to insulin. Glucose can no longer be absorbed by the cells but remains in the blood, triggering the need for more and more insulin (hyperinsulinemia) to be produced in an attempt to process the glucose. The production of ever-increasing amounts of insulin weakens and may eventually wear out the β-cells. Once the pancreas is no longer able to produce enough insulin then a person becomes hyperglycemic and will be diagnosed with type 2 DM. Even before this happens, damage is occurring to the body, including a build-up of triglycerides which further impairs insulin sensitivity (Hu et al., 2004). Several possible mechanisms of insulin resistance have been proposed: pre-receptor, receptor and post-receptor mechanism. The most studied pathway that appears to be absolutely necessary for mediating metabolic effects of insulin involves the phosphorylation of the insulin receptor substrate (IRS) 1 and 2 and activation of phosphatidylinosital (PI) 3-kinase (Wang et al., 2004).

1.7.3 Dyslipidemia

Dyslipidemia constitutes elevated triglycerides and low levels of HDL cholesterol. Increased triglycerides in the presence of insulin resistance and hyperinsulinemia results from increased circulating free fatty acids. As insulin resistance increases, the lipolysis inhibitory mechanism of insulin on adipose tissue diminishes and more free fatty acids are produced. Also, with more insulin circulating in the periphery, lipoprotein lipase is stimulated and increases the release of triglycerides. Hypertriglyceridermia is associated with alterations, both in structure and metabolism of LDL and HDL (Menuet et al., 2005). LDL cholesterol levels are often
normal, but a common finding is that LDL particles are smaller and denser than normal, which is associated with increased cardiovascular risk. Small dense LDL (sdLDL) particles contain higher amount of polyunsaturated fatty acids. They have lower binding affinity for LDL receptors, but a greater affinity for intimal proteoglycans and are better able to penetrate the intima and taken up by macrophages, eventually leading to atherosclerosis (Renjith and Jayakumari, 2011). The smaller HDL particle is metabolized and cleared at an abnormally high rate, resulting in low HDL levels. This shift to smaller particles makes them less antiatherogenic since the larger and more buoyant they are the more free cholesterol they can remove from cells and atherosclerotic plaque (Grundy, 2004).

1.8 Oxidative Stress and Diabetes

Oxidative stress was defined as the imbalance between antioxidants and prooxidants in favor of the latter, potentially leading to damage. However, a broader concept was recently defined, including the disruption of redox signaling and control and/or molecular damage (Bandeira, 2013). Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and a biological system’s ability to readily detoxify ROS or easily repair the resulting damage. ROS are free radicals that contain the oxygen atom and the biologically important ROS are superoxide (O2\(^{-}\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radical (OH\(^{-}\)). Oxidative stress can damage all components of the cell, including cellular proteins, membrane lipids and nucleic acids (Sies, 1997). In patients with diabetes, especially in those with poor glycemic control, oxidative stress is increased. In addition, it was reported that in aortic endothelial cells incubated with high glucose, ROS formation was increased by 250% within 24 h (Giugliano et al., 1996).

Various mechanisms have been suggested to contribute to diabetes-induced oxidative stress. Glucose oxidation is one of the sources of ROS. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to superoxide anion radicals, which eventually form extremely reactive hydroxyl radicals (Hunt et al., 1990). The cytosolic enzyme aldose reductase converts high intracellular glucose concentrations to sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) derived from the pentose phosphate pathway as a cofactor. It is likely that during
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Hyperglycemia, consumption of NADPH by this reaction inhibits replenishment of reduced glutathione, which is required to maintain glutathione peroxidase activity (Williamson et al., 1993). Hyperglycemia activates protein kinase-C (PKC) in membrane fraction through an increase in de novo diacylglycerol synthesis in vascular cells, and hyperglycemia is reported to stimulate ROS production through PKC-dependent activation of NADPH oxidase (Inoguchi et al., 2000). Hyperglycemia increases non-enzymatic glycation, characterized by the binding of reactive dicarbonyls and amino groups of proteins. This reaction leads to advanced glycation end products (AGEs). Glycation and oxidative stress are closely linked, and both phenomena are referred to as glycoxidation. All steps of glycoxidation generate ROS. In addition, plasma proteins modified by AGE precursors activate AGE receptors on macrophages and induce an intracellular oxidative stress by activating NADPH oxidase (Yan et al., 1994).

1.9 Advanced Glycation End products in Diabetes Mellitus

Glycation is a non-enzymatic reaction between free amino groups of proteins and reducing sugars. This reaction is known as Maillard reaction. Glycation is closely associated with the pathogenesis of diabetes-related complications like neuropathy, angiopathy and nephropathy (Monnier et al., 1992). AGEs are formed from covalent reactions between free amino groups of amino acids and the oxo groups of sugars; glucose, fructose, ribose. One of the most well-known AGEs is hemoglobin A1c (HbA1c). Chronic hyperglycemia contributes to diabetic complications through the formation of advanced glycation end products, which are irreversibly formed biochemical end products of non-enzymatic glycosylation (Brownlee et al., 1988). It has been shown that chronic diabetic complications are caused by the crosslinking of AGEs with long-lived proteins such as collagen and hemoglobin, so that protein structure and function are altered (Jakus et al., 2004).

Using radiolabeled AGE proteins, it has been shown that several cells, such as human and mouse monocyte, macrophage and lymphocyte, bind these types of glycated compounds in a relatively selective way. Receptor for advanced glycation end products (RAGE) is a central signal transduction receptor for these species. RAGE function is inextricably linked to its capacity to activate an array of signal transduction cascades,
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strongly suggesting that RAGE transduces the effects of AGEs by its capacity to engage such signaling cascades, rather than simply mediating AGE removal/detoxification (Imani et al., 1993). The engagement of RAGE by AGEs in a variety of settings triggers rapid generation of reactive oxygen species (ROS) and the up-regulation of inflammatory pathways, mechanisms dependent on RAGE signal transduction. Once set in motion, this ligand/RAGE axis markedly perturbs cellular properties and sets the stage for the sequelae of AGE generation/accumulation, such as diabetic complications, amplification of inflammation and tissue injury/breakdown, and the myriad consequences of natural aging (Ramasamy et al., 2005).

1.10 Antioxidant Components in Diabetes

Figure 1.3 Mechanisms of Antioxidants in Diabetes Mellitus

In the pathogenesis of type 2 DM, a relationship between glycemic control, oxidative stress, and vascular complications has long been recognized. Hyperglycemia by multiple independent mechanisms, including increased oxidative stress, worsens both endothelial dysfunction and insulin resistance (Neri et al., 1994). Oxidative stress may also be an initial step in the development of diabetic complications, promoting the activation of many stress-sensitive pathways, such as NF-kB and others, leading to cellular dysfunction and damage (Evans et al., 2002). In light of these observations, a
role for antioxidants in the prevention or treatment of type 2 DM has been proposed (Jha et al., 1995).

Antioxidant defense mechanisms involve both enzymatic and nonenzymatic strategies. Common non-enzymatic antioxidants include the vitamins A, C, and E, glutathione, α-lipoic acid, mixed carotenoids, coenzyme Q10 (CoQ10), antioxidant minerals (copper, zinc, manganese and selenium), and cofactors like folic acid, uric acid, albumin, and vitamins B1, B2, B6, and B12. Enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Maritim, 2003).

### Table 1.4 Antioxidant efficacies of vitamins and supplements in diabetes*

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Target</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>Patients with type 2 Diabetes</td>
<td>Decreases fasting plasma insulin level, decreases HbA1c level, improves insulin action</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>STZ-induced diabetic rats</td>
<td>Reduces LP, reduces the activity of GSH-Px and GST</td>
</tr>
<tr>
<td>(alpha tocopherol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td></td>
<td>Reduces oxidative stress indicators, reduce protein glycosylation, reduces insulin resistance.</td>
</tr>
<tr>
<td>Patients with type 2 Diabetes</td>
<td></td>
<td>Reduces retinal hemodynamic abnormalities, normalizes creatinine clearance</td>
</tr>
<tr>
<td>Vanadium</td>
<td>Patients with type 1 Diabetes</td>
<td>Scavengers of free radical, Reduces LP in pancreas</td>
</tr>
<tr>
<td>Zinc</td>
<td>STZ-induced diabetic rats</td>
<td>Induces synthesis of metallothionein, Reduces retinal LP</td>
</tr>
<tr>
<td>Selenium</td>
<td>Alloxane-induced diabetic rats</td>
<td>Increases GSH in liver and brain</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>Alloxane-induced diabetic rats</td>
<td>Reduces oxidative LDL</td>
</tr>
<tr>
<td></td>
<td>Patients with type 2 Diabetes</td>
<td></td>
</tr>
</tbody>
</table>


Retinol and carotenoids (pro-vitamin A compounds) may play a role in the prevention of type 2 DM due to their antioxidant properties. (Akbaraly et al., 2008). Tocopherols are called primary antioxidants because they interrupt oxidation directly, converting free radicals into more stable species. The antioxidation action of vitamin E (tocopherol-OH) involves the donation of a hydrogen atom to a lipid peroxyl radical to form lipid peroxide, thus stopping oxidative damage (Nwose et al., 2008). Ascorbic acid could act as a preventive antioxidant since it reacts with oxygen before the start of oxidation. Moreover, it is able to restore oxidized vitamin E and this synergistic effect may be important in normalizing levels of antioxidants (Halliwell, 1995).
1.11 Therapeutics of Diabetes Mellitus

The aim of therapy in diabetes is to maintain blood glucose at normal levels and to treat or prevent the secondary complications of the disease. This involves pharmacological and non-pharmacological management of the disease.

1.11.1 Pharmacological Management of Diabetes Mellitus

Hyperglycemia in patients with diabetes is always the result of a mismatch between the quantity of insulin necessary to regulate the person’s metabolic processes and the amount of insulin being secreted by the person’s β-cells. Oral antihyperglycemic agents such as thiazolidinediones or metformin decrease insulin resistance, α-glucosidase inhibitors decrease postprandial insulin needs, insulin secretagogues improve and increase endogenous insulin secretion, and insulin and its analogues replace endogenous insulin secretion by exogenous insulin administration (Tripathi and Srivastava, 2006).

1.11.2 Drug Targets for Diabetes Mellitus and Insulin Resistance

The current therapeutic approaches were largely developed in the absence of fine molecular targets or understanding of the pathogenesis of the diseases. In last few years a large number of molecular drug targets involving various biochemical pathways have been worked out. These are based on the predicted roles in modulating one or more key aspects of the pathogenesis of the diabetes and metabolic syndrome. These are: 1) reducing excessive glucose production by liver, 2) targeting β-cells, 3) targeting insulin-signaling pathways, and 4) targeting lipid metabolism (Goldstein et al., 1998; Drucker, 2001).

1.11.3 Oral Antidiabetic Drugs

For type 2 DM, it is clearly a priority to provide effective control of the hyperglycemia to reduce macro and microvascular complications. Oral agents, notably sulphonylureas, metformin, thiazolidinediones and acarbose, are instituted as monotherapy. If adequate glycemic control is not achieved with oral monotherapy, then two different classes of oral drugs are used in combination (Bailey, 1996).
1.11.3.1 Sulphonylureas

Sulphonylureas are widely considered as fine-line drug treatment in type 2 DM patients who are not grossly obese. Sulphonylureas act directly on the islet β-cells to close ATP-sensitive K⁺ channels, which stimulate insulin secretion. The efficacy of these agents depends on the presence of enough β-cells with sufficient functional reserve. The major acute problem associated with sulphonylureas is hypoglycemia, the risk of which markedly increased in the elderly and patients with renal insufficiency. Sulphonylurea induced hypoglycemia can be exacerbated by interaction with numerous drugs, including alcohol (ethanol), aspirin, phenylbutazone, and oxidase inhibitors (Groop, 1992; Bailey, 1998).

1.11.3.2 Biguanides

Metformin is the only established antidiabetic drug that deals with insulin resistance. Its glucose-lowering effect is mainly a consequence of reduced hepatic glucose output (gluconeogenesis and glycogenolysis) and increased insulin-stimulated glucose uptake and glycogenesis in skeletal muscle. Another action of metformin is to reduce fatty acid oxidation in an apparently insulin-independent manner, which serves to redress the imbalance in the glucose-fatty acid cycle. Thus, metformin improves insulin sensitivity in skeletal muscle without raising insulin concentrations. Metformin also improves insulin action in adipose tissue, but obesity is offset by increased glucose turnover and lower insulin concentration. Metformin offers a range of benefits that combat insulin resistance and various aspects of metabolic syndrome consistent with the treatment regimens for type 2 DM that are initiated with metformin show a particularly favorable long-term reduction in morbidity and mortality from micro and macrovascular complications (Bailey and Turner, 1996; Stith et al., 1996).

1.11.3.3 Thiazolidinediones (TZDs)

PPARs (peroxisome proliferator-activated receptors) are ligand-activated transcription factors (members of the nuclear receptor family) which offer a promising therapeutic approach to the metabolic syndrome. The known beneficial effects of PPAR ligands are largely consistent with the mechanism that can ameliorate lipotoxicity. PPAR-γ is the predominant molecular target for insulin-sensitizing thiazolidinedione
drugs. This class of oral antidiabetic agents targets the nuclear PPAR-γ, which increases transcription of certain insulin-sensitive genes. TZDs include troglitazone, rosiglitazone, and pioglitazone. Troglitazone was effective in type 2 diabetes, but it has been withdrawn from the market as a result of idiosyncratic hepatotoxicity; however, this has not been observed with rosiglitazone or pioglitazone (Saleh et al., 2000; Willson et al., 2000).

1.11.3.4 α-glucosidase Inhibitors

Acarbose and related compounds delay the intraluminal production of monosaccharide, particularly glucose. Acarbose competitively inhibits α-glucosidases that are associated with the brush border membrane of the small intestine and are responsible for the digestion of complex polysaccharides and sucrose. This slows carbohydrates digestion and lowers post-prandial hyperglycemia. Although insulin resistance is not addressed directly, the blood glucose lowering effect with reduced glucotoxicity without increasing and possibly decreasing, insulin concentrations thereby reduce at least one part of insulin resistance (Creutzfeldt, 1988; Lebovitz, 1997).

1.11.3.5 Insulin Therapy

The discovery of insulin in 1922 by Banting and Best was a breakthrough in the treatment of diabetes. Insulin produces a remarkable life expectancy for diabetics, whether of type 1 or type 2. Insulin therapy, however, should be reserved to patients who have failed on an adequate trial of diet, exercise, and oral antidiabetics. Insulin therapy can improve or correct many of the metabolic abnormalities present in patients with type 2 DM. Insulin administration significantly reduces glucose concentrations by suppressing hepatic glucose production, increasing postprandial glucose utilization, and improving the abnormal lipoprotein composition commonly seen in patients with insulin resistance. Insulin therapy may also decrease or eliminate the effects of glucose toxicity by reducing hyperglycemia to improve insulin sensitivity and β-cell secretory function. It suppresses ketosis (Bolli, 1997).

The advent of recombinant DNA technology provided an opportunity to design insulin analogues in an attempt to overcome these limitations. The subsequent availability of rapid-acting (insulin lispro, insulin aspart) and long-acting (insulin
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glargine and detemir insulin) insulin analogues for meal and basal requirements offer both individual and collective advantages. The subsequent developments towards insulin delivery led to external continuous subcutaneous insulin infusion pumps, capable of achieving excellent metabolic control and reduced risk of hypoglycemia (Hamaty, 2011).

1.11.4 Non-Pharmacological Management of Diabetes Mellitus

This involves lifestyle management including diet control and adequate exercise. Dietary recommendations may be developed based on the individual’s requirements and treatment goals. Most patients with type 2 DM are overweight or obese, and it is now well recognized that this is a major factor in insulin resistance. Consequently, reduction of excess weight is a primary component in the management of type 2 DM. When extreme caloric restriction and/or rapid weight loss seem desirable, a very low caloric diet or protein-sparing modified fast may be considered (Kosaka, 2005). The ideal balance of carbohydrate, protein, or fat intake in patients with type 2 DM is still a matter of discussion. It has recently been recognized that a diet containing 60% carbohydrates, even if not including sugar, may predispose to the development of dyslipidemia. Carbohydrates should be predominantly complex and high in soluble fiber; foods with an aglycemic index are preferred, although moderate intake of simple sugar such as sucrose does not seem to be detrimental. Protein intake should not exceed the daily requirement, since high protein intake appears to have a detrimental effect on renal function. Addition of certain types of soluble fiber, particularly guar gum and pectin, may result in significant reduction of postprandial glucose and insulin levels in patients with type 2 DM (Jenkins et al., 1989; Steyn et al., 2004).

There is evidence that fish oils or fish-derived omega-3 fatty acids may play some role in preventing atherosclerotic vascular disease by reducing plasma triglyceride and lipoprotein levels. However, there is also evidence that in type 2 DM the decrease in plasma triglyceride levels is counterbalanced by adverse effects on blood glucose or low density lipoprotein (LDL)-cholesterol (Axelrod, 1989). There is good evidence that regular exercise has a positive influence via various cardiovascular risk factors that worsen diagnostics in patients with type 2 DM. Regular exercise improves insulin sensitivity and, as a consequence, may improve glucose tolerance. Such effects result
partly from enzymatic adaptation in skeletal muscles, considered to be responsible for improvement in maximal oxygen uptake, and partly from a decrease in body weight, body fat and, possibly, also cell size. Such effects are beneficial in patients with type 2 DM since they enhance work capacity and quality of life and may also help to reduce the requirement for insulin or oral hypoglycemic agents (American Diabetes Association, 1990; Horton, 1986).

1.12 Phytonutrients in the Prevention of Diabetes

Phytonutrients play an important role in the development and control of type 2 DM. For proper management of diabetic individuals, the diet must be designed to supply adequate amount of nutrients namely carbohydrate, fat, protein, vitamins and minerals.

1.12.1 Effects of Dietary Protein on Diabetes

DM is basically a disorder of carbohydrate metabolism, but with progression of the disease, protein metabolism is also affected. Gluconeogenesis, a major biochemical process that produces glucose from protein, is accelerated in diabetes mellitus. So the type and amount of protein may affect diabetic conditions. It was recommended that diabetic individuals should include 15-20% protein in their diet (Jenkins and Josse, 1985). Turnbull and Ward in 1995 studied the effect of mycoprotein on acute glycemia and insulinaemia in normal healthy individuals. Mycoprotein reduced glycemia and insulinemia. They reported that the role of mycoprotein was significant in the dietary treatment of diabetics.

Proteins in legume seeds represent from about 20% (dry weight) in pea and beans, up to 38–40% in soybean and lupin. Therefore legume seeds are among the richest food sources of proteins and amino acids for human and animal nutrition (Guéguen and Cerletti, 1994). Plant-based diets may be superior to traditional animal protein diets for prevention and treatment of diabetes. Intake of meals rich in animal protein increases renal blood flow and glomerular filtration rates (GFR) in the order: beef, chicken, and fish. Substitution of soy protein for animal protein in individuals with diabetic nephropathy would decrease hyperfiltration and glomerular hypertension.
with resultant protection from diabetic nephropathy (Anderson et al., 1998). Most soy protein products provide these components: soy protein with its unique amino acid profile, soy peptides, and isoflavones. Each of these components appears to have specific and unique effects on renal function. Soy peptides may affect renal function in many different ways and may be the most active component of the soy protein package. However, soy isoflavones also have a wide range of activities that probably act synergistically with the soy peptides to mediate favorable effects on renal function (Anderson and Fanti, 2005).

1.12.2 Effects of Dietary Fiber on Diabetes

High fiber diets are effective in diminishing insulin secretion and lowering blood glucose and are thus beneficial for treating diabetics, particularly those with type 2 DM. Fibers of soybean and fenugreek seed are beneficial in reduction of plasma glucose in patients with type 2 DM and in pregnant diabetic women. It has been reported that 15 g daily of soluble dietary fiber in the average type 2 diabetic patient, has produced 10% improvement in fasting plasma glucose glycosylated hemoglobin and in total and LDL cholesterol (Fuessl et al., 1987).

Different fibers have different effect on diabetic patients. The high soluble fiber meal produced significantly lower glucose and insulin responses than diet containing low fiber or high insoluble fiber. Dietary fiber promotes satiety and assists in weight loss in diabetic patients and hence helps in controlling diabetes (Toma, et al. 1988; Garrow and Owens, 1990). Pectin is a water soluble dietary fiber. Inside the gastrointestinal tract, pectin maintains the ability to form a gel or thicken a solution. This is thought to be the likely mechanism behind its beneficial effects on health including diabetes prevention (Jenkins et al., 1977). It was suggested that combination of fibers from different sources might be more effective than a single fiber type and further noted that increased intake of dietary fiber was associated with significant decrease in total serum protein and increase in serum albumin in all subjects (Vorster et al., 1988).
1.13 Phytochemicals with Antidiabetic Activity

Phytochemicals can be defined as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may affect health, but are not essential nutrients. Plant based foods are complex mixtures of bioactive compounds. Information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods that contain those phytochemicals. Since ancient times, a number of phyto medicines have been used in the treatment of diseases like diabetes. There is increasing demand by patients to use the natural products with antidiabetic activity. Herbal medicines for diabetes can be divided into four categories according to their mode of action: drugs acting like insulin, drugs acting on insulin secreting β-cells, drugs acting by modifying glucose uptake and utilization, drugs acting by miscellaneous mechanisms (Wadkar, 2008).

1.13.1 Polyphenols

Polyphenols are secondary metabolite widely distributed in the plant kingdom. They are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanones, isoflavones, proanthocyanidins), stilbenes and lignans, which are distributed in plants and food of plant origin (Manach et al., 2004). The different types and distribution of polyphenols in plant foods are as follows:

1.13.2 Flavonoids

Flavonoids are molecules with a phenolic benzopyran structure and occur only in plants where they are present predominantly as glycosides. The flavonoids may themselves be divided into six subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (Kahkonen et al., 1999).

More than 4000 flavonoids have been identified in plants, and the list is constantly growing (Harborne & Williams, 2000). Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are kaempferol and quercetin. Considered as the most abundant dietary flavonol, quercetin is a potent antioxidant
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Flavones consist chiefly of glycosides of luteolin and apigenin (Neo et al., 2008). In human foods, flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present in high concentrations only in citrus fruit. The main aglycones (the non-sugar component that results from hydrolysis of glycoside flavanones) are naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons (Hertog et al., 1993).

Proanthocyanidins, which are also known as condensed tannins, are the major polyphenols in grapes, where they are localized mostly in skins and seeds (Wittig et al., 2001). Supplementation of diabetic mice with hesperidin and naringin, two main citrus bioflavonoids, was accompanied with increased hepatic glucokinase activity and glycogen content, attenuated hepatic gluconeogenesis via decrease in the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK), and subsequent improvement of glycemic control (Jung et al., 2006). Isoflavones, particularly genistein, have amazing effects on pancreatic β-cells. Genistein acts as an agonist of cyclic AMP/protein kinas A signaling, an important physiological amplifier of glucose-induced insulin secretion by the pancreatic β-cells (Liu et al., 2006). Green tea flavanols, mainly catechins and epicatechins have been shown to attenuate hyperglycemia and hepatic glucose output via downregulation of the expression of liver glucokinase and upregulation of PEPCK (Waltner-Law et al., 2002). The results from in vitro studies showed that some compounds such as quercetin and epigallocatechin gallate improved insulin-dependent glucose uptake in muscle cells and adipocytes by translocation of glucose transporter, GLUT 4, to plasma membrane mainly through induction of the AMP-activated protein kinase (AMPK) pathway (Zhang et al., 2011).

1.13.3 Lignans

Lignans are formed of two phenylpropane units. The richest dietary source is linseed (flaxseed), which contains secoisolariciresinol (up to 3.7 g/kg dry wt) and low quantities of matairesinol. Lignans are metabolized to enterodiol and enterolactone by the intestinal microflora. Thompson et al., in 1991 used an in vitro technique involving the fermentation of foods by human colonic microflora to quantitatively evaluate precursors of enterodiol and enterolactone. They confirmed that oleaginous seeds (linseed) are the richest source and identified algae, leguminous plants (lentils), cereals
(triticale and wheat), vegetables (garlic, asparagus, carrots), and fruit (pears, prunes) as minor sources. Cunnane et al. (1993) carried out studies where muffins containing 25 g flaxseed each, two consumed daily for 4 weeks, resulted in 27% lower postprandial glucose values than control muffins.

Figure 1.4 Beneficial effects of polyphenols on management of blood glucose in diabetes

1.13.4 Stilbenes

Stilbenes are found in only low quantities in the human diet. One of these, resveratrol, for which antidiabetic effect has been shown during screening of medicinal plants. Resveratrol treatment during 15 weeks increased both insulin-stimulated whole-body and steady-state glucose uptake of both soleus muscle and liver in high
cholesterol-fructose-fed rats. In the presence of insulin, resveratrol also potentiated the effect of insulin on glucose uptake via AMPK activation, but leading to activation of the PI3K-Akt signal pathway (Deng et al., 2008). The heartwood of the plant Pterocarpus marsupium (PM) has been shown to exhibit antihyperglycemic properties due to the presence of pterostilbene (Grover, 2005). Studies were conducted by Pari and Satheesh (2006) evaluating the antiglycemic effects of pterostilbene in rodent models of STZ induced DM. They found that pterostilbene treatment reduced glycosylated hemoglobin (HbA1c), decreased the expression of gluconeogenic enzymes and increased the expression of glycolytic enzymes and the effects of pterostilbene were comparable to the experimental effects of 500 mg/kg oral metformin.

1.13.5 Phenolic acids

Two classes of phenolic acids can be distinguished: derivatives of benzoic acid and derivatives of cinnamic acid. Hydroxybenzoic acids are components of complex structures such as hydrolyzable tannins (gallotannins in mangoes and ellagitannins in red fruit such as strawberries, raspberries, and blackberries). The hydroxycinnamic acids are more common than the hydroxybenzoic acids and consist chiefly of p-coumaric, caffeic, ferulic, and sinapic acids. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilisation, or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid, and tartaric acid. Caffeic and quinic acid combine to form chlorogenic acid, which is found in many types of fruit and in high concentrations in coffee. Caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represents between 75% and 100% of the total hydroxycinnamic acid content of most fruit. Hydroxycinnamic acids are found in all parts of fruit, although the highest concentrations are seen in the outer parts of ripe fruit. Concentrations generally decrease during the course of ripening, but total quantities increase as the fruit increases in size (Clifford, 1999; Macheix et al., 1990).

Caffeic acids, chlorogenic acids, ferulic and tannic acids could interact with absorption of glucose from intestine via inhibition of Na\(^+\)-dependent glucose transporters, SGLT-1 and SGLT-2 (Johnston et al., 2005). Ferulic acid, a hydroxycinnamic acid derivate, effectively suppresses blood glucose by elevating
glucokinase activity and production of glycogen in the liver and increased plasma insulin levels in diabetic rats (Jung et al., 2007). In a study, rice derived ferulic acid was administered to diabetic mice for 17 days and results showed an increase in plasma insulin level while blood sugar level decreased significantly compared to control (Jung et al., 2007). Oral administration of sinapic acid for a period of 35 days significantly decreased lipid peroxidation markers and increased the antioxidants in diabetic rats (Kanchana et al., 2011). Studies reported that in 3T3 L1 cells, tannic acid induced phosphorylation of the insulin receptor (IR) and Akt, as well as translocation of GLUT 4, the protein factors involved in the signaling pathway of insulin-mediated glucose transport (Liu et al., 2005).

1.14 Research Studies on Coconut and Coconut Products

As mentioned before, the plants provide a potential source of natural therapeutic agents, because many plants and plant derived compounds have been used in the treatment of several degenerative diseases including diabetes. Several indigenous plants in India have been investigated for their beneficial use in different types of diseases. Coconut (Cocos nucifera L.) is a large palm belongs to the family Arecaceae or Palmae. From time immemorial, coconut has been used as a vital source of food and as natural remedy for curing some ailments in many tropical regions of the world. The nut of the coconut provides a nutritious source of meat (coconut kernel), juice (coconut water), and oil. Coconut kernel is the major culinary item in the population of Kerala along with coconut oil. In view of its importance in Kerala diet, several studies have been carried out in the past using coconut and its products, namely coconut oil, kernel, protein, fiber and coconut water, to find out their biochemical effects in health and disease.

Studies carried out using virgin coconut oil (VCO) extracted from fresh coconut kernel by wet processing under mild temperature revealed that it posses significant hypolipidemic, antioxidant and antithrombotic effects compared to copra oil and sunflower oil in normal and cholesterol fed rats (Nevin and Rajamohan, 2004, 2006; Arunima and Rajamohan, 2013). The beneficial effects of virgin coconut oil over other oils are mainly due to the presence of unsaponifiable components. Apart from coconut oil, coconut kernel is a rich source of protein and fiber. Fresh coconut kernel contains
5% protein. Experiments revealed that coconut kernel protein (CKP) has cardioprotective property against isoproterenol induced myocardial infarction in rats (Mini and Rajamohan, 2002). Studies also indicated that CKP has potential antidiabetic activity and potential effect in lowering oxidative stress associated with diabetes (Salil et al., 2011). Amino acid analysis of coconut kernel protein revealed that it has a much higher amount of L-arginine. A previous study showed that the health benefits of coconut kernel protein are mainly due to the presence of L-arginine (Salil et al., 2012).

The coconut kernel contains 7% dietary fiber. Sindhurani and Rajamohan in 2000 reported that dietary supplementation of coconut fiber exerted significant hypoglycemic action in normal rats. The composition of neutral detergent fiber (NDF) isolated from coconut kernel contains hemicellulose, cellulose, lignin, cutin and silica.

The water of tender coconut, technically the liquid endosperm, is the most nutritious wholesome beverage which is largely consumed all over the world. Research revealed that tender coconut water (TCW) has significant hepatoprotective property on carbon tetra chloride induced hepatic injury in rats (Loki and Rajamohan, 2003). The antioxidant, antithrombotic and cardioprotective effects of TCW were also reported in rats induced myocardial infarction (Anurag and Rajamohan, 2003; Prathapan and Rajamohan, 2010). Moreover, TCW was found to exhibit significant blood pressure lowering potential (Bhagya et al., 2012). Recent studies carried out using mature coconut water (MCW) showed that it significantly attenuated hyperglycemia and oxidative stress in alloxan induced diabetic rats (Preetha et al., 2012).

1.15 *Cocos nucifera* Inflorescence (CnI)

In coconut palm, the flowers and the flower bearing ramification are collectively called inflorescence. The inflorescence is covered by a sheath called spathe. Botanically, this type of inflorescence is referred to as spadix. In immature CnI, the male and female florets lie very close to the main axis (peduncle) and the whole is so tightly packed. The watery sap that drips from young inflorescence is used as a natural drink in tropical countries. Coconut sap contains sugars, essential vitamins, amino acids, macro and micronutrients. The fermented sap is the common alcoholic drink in
the tropical coconut region. Coconut inflorescence is also used for the production of organic sugar and vinegar.

CnI sap sugar is a low glycemic index (GI) sugar. The higher the GI number, the greater the blood sugar response. A low GI food will cause a small rise in blood glucose level, while a high GI food will trigger a dramatic spike. Thus diabetics can also effectively use the sap sugar. In Ayurveda, CnI is often used to cure back ache. The fresh juice is useful in dyspepsia, diarrhea, dysentery, diabetes, hemoptysis, strangury and leprosy. But, scientific evidence in terms of modern medicine is lacking in most of these cases.

**Figure 1.5 Opened Inflorescence**  
**Figure 1.6 Inflorescence with Spathe**

**Figure 1.7 Young Inflorescence with Compact Arrangements of Florets**
Introduction

Objectives of the Study

Diabetes has become a major health problem in India and around the globe. Presently, the treatment of DM in clinical practice has been confined to the use of oral hypoglycemic agents and insulin. These agents utilize different mechanisms for regulating the blood glucose levels in normal or glycemic conditions. But, oral hypoglycemic drugs have been reported to be endowed with serious side effects due to their continuous intake. Hence there is need for effective, safe and better antidiabetic agents. Thus the present interests are being geared towards phytotherapeutics. The inflorescence of *Cocos nucifera* is a rich source of proteins, amino acids and dietary fibers, micronutrients like vitamins and minerals, and secondary plant metabolites like polyphenols, flavanoids and tannins. These phytoconstituents are reported to have antidiabetic and antioxidant properties (Jadhav and Puchchakayala, 2012; Karau *et al.*, 2013). However, it is necessary to provide scientific proof as whether to justify the use of CnI or its active principles in the treatment of diabetes and related complications. In view of this, a systematic study was carried out to investigate the effects of CnI in experimental diabetes.

The main objectives focused in the present study are; 1) *in vivo* experiments using young inflorescence of *Cocos nucifera* in normoglycemic and hyperglycemic male albino rats. 2) *In vitro* qualitative and quantitative phytochemical analyses of young CnI. 3) Extraction of active fraction from CnI using solvents such as ethyl acetate, ethanol and methanol and *in vivo* screening experiments using these solvent fractions in experimental diabetes. 4) To carry out dose dependent and acute toxicity studies of the solvent fraction of CnI. 5) To find out the active components present in the solvent fraction and also to find out the mechanisms of action of the bioactive principles offering antidiabetic action.

The results of these studies are discussed in this thesis.