CHAPTER I

GENERAL BACKGROUND

1. Introduction

At present, diabetes is possibly the world fastest growing metabolic disease (Abdel-Barry *et al.*, 1997a). At least 30 million populations in the world are diabetic patients and increasing rate is 6% per year. Furthermore, diabetic patients don't know when the symptom appears so it's the reason why mortality rate is increasing especially in developed countries. Despite having used different methodologies, authors who have estimated the future global trends in the frequency of diabetes mellitus generally agree that Asia is the major site of a rapidly emerging epidemic of diabetes (Amos *et al.*, 1997; King *et al.*, 1998; Roglic and King, 2000). In Asia, there are diabetic people around 2% of overall, it may exceed 5% in some region resulting in a total of 7.5 million patients. In Thailand, diabetics are quite a problem of national health. Prevalence rate of diabetics is 2.5% (male 2.6% and female 2.4%), and those who lived in urban area with age more than 20 years is expected to be 3.4-4.0%. Mortality rate increases to 1.8 persons per 100,000. It showed that the number diabetic patients is increasing very fast. Approximately 10 % of the diabetic population are of Type 1 diabetes or insulin-dependent diabetes, whereas the remainders of diabetics are Type 2 diabetes or non-insulin-dependent. It is estimated that a third of the non-insulin dependent diabetics are unaware of their disease (ADA, 1997)

Diabetes mellitus, both Type 1 and Type 2 diabetes are a metabolic disorder characterized by disturbance in carbohydrate, lipid and protein metabolism. These metabolic derangement result from a combination of insulin deficiency and/or insulin resistance and lead to a variety of acute and chronic complications (DeFronzo, 1998). Type 1 diabetes is characterized by an immune-mediated, selective destruction of 90% of insulin secreting β-cell. Individuals with Type 1 diabetes therefore require regular insulin injections to control blood sugar levels. The hypoglycemia that is associated with Type 2 diabetes results from both an impaired insulin secretory

response to glucose and decreased insulin effectiveness (insulin resistance) (Berkow, 1992). A third, less common form of diabetes is gestational diabetes. Although this form usually disappears following delivery, 40% of woman with gestational diabetes will go on to develop Type 2 diabetes later in life.

Diabetic as groups are increased risk for a number of disease states such as, the acute complications include symptomatic hyperglycemia, hypoglycemic, hyperosmolar non-ketotic coma and diabetic ketoacidosis. Chronic complications usually occur 10 to 15 years after the onset of diabetes and include microvascular (nephropathy, retinopathy, neuropathy); macrovascular (stroke, myocardial infarction); and peripheral vascular (amputation) disease (DeFronzo, 1998). What is more, diabetes is currently the fifth leading cause of death in the United Stated and the third if complications of the condition are included. While the exact cause or causes of diabetic complications are not fully understood, the underlying factor that appears to make those with diabetes more prone to so many health problems is prolonged and frequent elevation of blood sugar. Some of the excess sugar or glucose accumulates in various tissues, thereby reducing the tissue function. Excess glucose in the blood damages the walls of the capillaries, the smallest type of blood vessel in the body. The passage of nutrients such as glucose, fats and amino acids as well as oxygen is impaired with such damage. The immune system, including the white blood cells that fight against infection, does not work at optimal levels when blood sugar is elevated, when combined with reduced oxygen being provided from the blood and damaged blood vessels, it is no wonder that many diabetics experience a reduced healing rate from infections (Kris *et al.*, 1986). While the tendency to develop diabetes is strongly hereditary and as knowledge of the heterogeneity of this disorder increases. Furthermore, there is believed to be an environmental component yet to be fully identified that triggers its development in susceptible individuals (Tim, 1998).

Obviously, diabetes mellitus requires close control of the blood sugar in order to minimize or avoid adverse outcome (Miller *et al.*, 1998). The management objectives for both IDDM and NIDDM patients are similar and include the following: (1) prevention of acute complication; (2) prevention of chronic microvascular and neuropathic complications; (3) prevention of premature atherosclerotic cardiovascular and peripheral vascular complications; and (4) attainment of normal quality of life

without symptoms referable to diabetes (DeFronzo, 1998). So, it does the need for more appropriate therapies.

In many countries, some of the plants used by the population, as anti-diabetic remedies are edible plants. These have added interest because they join two basic diabetes mellitus control factors: food and medication. A menu including these types of plants could potentially reduce the dose of the hypoglycemic agents. Mild non-insulin dependent diabetic patients could possibly even avoid the use of these hypoglycemic agents. (Roman-Ramos *et al.*, 1995).

This study is on one of such medicinal plants, locally known as "HAMM" (*Coscinium fenestratum*). Recently, HAMM is widely used as traditional medicine in the north-eastern part of Thailand, particularly along the border of Lao people's Democratic Republic. It is claimed as a Laos traditional medicine and used for balancing blood pressure, detoxifying agent, for the treatment of high cholesterol and antihyperglycemic, etc. Since it is imported from Laos and widely used as medicinal plant among Thai people, the information concerning the use of this plant has been frequently requested (Dechwisissakul *et al.*, 2000). HAMM has been recognized as effective remedy for anti-hyperglycemic effect. Therefore, it is interesting to examine anti-hyperglycemic activity of HAMM.

The aim of this study was to investigate

(1) The hypoglycemic effect of crude water extract from *C. fenestratum* in normal and streptozotocin-induced diabetic male Wistar rats.
(2) The acute toxicity test from crude water extract of *C. fenestratum* in normal male Wistar rats.

2. Review of literature

Diabetes mellitus is a chronic systemic disease (Waife, 1967). It is one of the most common diseases associated with disorder of the metabolism of carbohydrate, fat and protein (El-Fiky *et al.*, 1996). This disease is associated with a relative or absolute insufficiency of insulin and with varying degrees of insulin resistance, as a result of which the blood sugar level is abnormally high and sugar appears in the urine. The most common symptoms of the disease are polyuria, polydypsia, fatigue and loss of weight (Wilson and Schold, 1968).

The way our bodies use digested food for growth and energy. Most of the food we eat is broken down into glucose, the form of sugar in the blood. Glucose is the main source of fuel for the body. After digestion, glucose passes into the blood stream, where it is used by cells for growth and energy. For glucose to get into cells, insulin must be present. When we eat, the pancreas is supposed to automatically produce the right amount of insulin to move glucose from blood into our cells. However, in people with diabetes, their pancreas produces either little or no insulin, or their cells do not respond appropriately to the insulin that is produced. So, glucose builds in the blood will overflow into the urine and passes out of the body (NIH, 2002).

Diabetes, therefore, is not a single disease but a syndrome (Fajans *et al.*, 1991 cited in Rifkin and Porte, 1991). Ideally a classification of diabetes should be based on etiology and pathogenesis only.

2.1 Causes of diabetes mellitus

2.1.1 Heredity

Many unusual causes of diabetes result from generation of determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of insulin receptor (Taylor, 1992). A point mutation in mitochondrial DNA (which is therefore maternally transmitted) has been found to be associated with diabetes and deafness (Gerbitz *et al.*, 1996; Maassen and Kacowaki, 1996). Patients with these syndromes and other patients with alterations in insulin receptor function

may have defects in (1) receptor synthesis, (2) transport of the receptor to the plasma membrane, (3) binding of the receptor to the insulin molecule, (4) transmembrane signaling, or (5) endocytosis recycling-degradation of the receptor. However, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion.

2.1.2 Environments

(1) Foods

Sugars do not cause diabetes. All sugars including common sugar, fruit sugars and starch are decomposed into glucose for utilization. Glucose is there to feed your brain and your muscles but if without glucose, you can't live for a second. Merely the utilization of sugars can never cause diseases. But consuming too much sugar does. Only consuming too much protein and/or sugars combined with consuming too little fat, can cause diabetes. Sugar and fats are the only sources of energy in your blood (redundant protein is converted into sugars and fats). Sugars supply when with "fast" energy, but this source is exhausted pretty fast too. Fats keep on going all day, and while sleeping (NLM, 2003).

When a meal mainly consists of protein* or carbohydrate, the blood glucose level increases much more than when much fat is also absorbed. If there is less fatty acid available, then more glucose is utilized for energy. Because of this, the blood glucose level decreases sooner too. So, there is a much stronger fluctuation of the blood-glucose level. Consuming too little fat causes your blood glucose level to fluctuate too much, exhausting the insulin-energy system, which cause diabetes.

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* Protein consists of animo acids. Amino acids that can only be transformed into glucose-like substances are: methionine, cysteine, valine, threonine, alanine, aspartic acid, glutamic acid, glycine, histidline, proline, serine, and arginine. Leucine and lysine can only be converted into fat-like substances. Phenylalanine, tyrosine, isoleucine, and troptophan can both be converted in fat- or glucose-like substances. Amino acids like arginine increase insuline secretion (Hostens *et al.*, 1999). All food-proteins contain arginine.

(2) Obesity and lack of exercise

Diabetes often comes with obesity. But obese people can only become diabetic if excessive protein and carbohydrate consumption regularly comes without excessive fat consumption (or when one has to deal with too much stress of course). Furthermore, genetic maybe a cause of obesity. Obesity tends to run in families, suggesting a genetic link. Some illness can lead to obesity or a tendency to gain weight. These include hypothyroidism, Cushing's syndrome, depression, and certain neurological problems that can lead to overeating. Also, drugs such as steroids and some antidepressants may cause weight gain (ACD, 2003).

Exercise can lower the blood sugar and improve the body's ability to use glucose with regular exercise, the amount of insulin needed decreases. Exercise can also help reverse the resistance to insulin that occurs as a result of being overweight. Exercises are important for everyone's health if lack of exercise, it can be increasingly risk to health (HCO, 2003).

(3) Infectious disease

The role of viruses in causing diabetes is controversial (Yoon, 1995). They may be involved in the pathogenesis of diabetes in one of two ways: either by directly infecting and destroying pancreatic β-cell or by precipitation or contributing to the autoimmune process that underlies immune-mediated Type 1 diabetes. The virus are most likely to be involved in the pathogenesis of diabetes such as *Congenital rubella*, Cytomegalovirus, Coxsackie B, Mumps and Adenovirus by participating somehow in the autoimmune process, because circulating autoantibodies are found in the majority of patients whose diabetes has been linked to viruses (Davidson, 1998).

(4) Toxicity drug for β-cell

Drug can cause diabetes by either impairing insulin secretion or enhancing insulin resistance. Those that affect insulin secretion are intravenous (not inhaled) pentamidine (Bouchard *et al.*, 1982), vacor (a rat poison, not a drug) (Davison, 1998), phenytoin (dilantin), interferon-α (probably by an autoimmune mechanism) (Fabris, *et al.*, 1992), diazoxide and thiazides (secondary to potassium

deficiency), streptoxotocin (STZ) and alloxan (ALX) damaging islet cells. Those that affect insulin action are nicotinic acid (niacin), glucocorticoids, β-adrenergic agonists, thyroid hormone, and estrogens. Estrogens and thyroid hormones usually precipitate diabetes only in those who have impaired β-cell reserves, who in the absence of these two drugs are able to maintain normoglycemia.

(5) Abnormal hormone

Hormonal secretion by some endocrine tumors can cause diabetes. Excess secretion of glucocorticoids (Cushing's syndrome, in which Cushing's disease is one cause), excess growth hormone (acromegaly), and excess catecholamines (pheochromocytoma) impairs insulin action (Shen *et al.*, 1988), excess glucagon (glucagonoma) and excess somatoststion (Somatostatinoma) inhibiting islet cell secretion of cell (Konomi *et al.*, 1990), and aldosteronomas (via hypokalemia) (Conn, 1965) impair insulin secretion. Glucagonomas cause mild diabetes by increasing hepatic glucose production. Diabetes generally disappears with successful treatment of the endocrinopathies, although it may persist even after resolution of Cushing's syndrome and acromegaly. (Davidson, 1998).

(6) Stress

Not exactly, Stress can cause diabetes but it can cause symptoms to appear more quickly. Stress increases epinephrine (adrenaline) secretion. This hormone stimulates transformation of glycogen into directly available glucose, to enable you to fight, or flee from danger. If, however, this glucose is not directly used for physical action, it increases insulin secretion, what eventually can lead to insulin exhaustion and diabetes.

(7) Pregnant

Refers to the onset or first recognition of diabetes mellitus during pregnancy, most commonly during the third trimester, women are characterized by insulin resistance and impaired insulin secretion, and approximately three-fourths have a family history of Type 2 diabetes mellitus. Most of these women probably have typical Type 2 diabetes mellitus that was unmasked by the stress of pregnancy.

They are at very high risk to develop overt Type 2 diabetes mellitus within a 10-year period and require frequent long-term follow-up (DeFronzo, 1998).

2.1.3 Abnormal β-cell (Khalid, 1991)

(1) Pancreatic disease from Malnutrition-Related Diabetes Mellitus (MRDM) may be insulin dependent, e.g., after total pancreatectomy, chronic pancreatitis, damage to exocrine and endocrine pancreas, e.g., chronic alcholism, muco-viscidosis (cysticfibrosis), and furthermore, deposition disease, e.g., haemochromatosis, Wilson's disease.

(2) Abnormalities of insulin and receptors had been reported in rare cases, abnormal insulin and receptors cause deficient insulin hormone action. Moreover, insulin receptor antibodies seen in acanthosis nigricans.

2.2 Symptoms of diabetes mellitus

2.2.1 Major symptoms

- polydypsia (increased thirst)
- polyuria (increased urination)
- polyphagia (increased appetite and food intake)
- weight losses
- fatigue
- weakness

2.2.2 Minor symptoms

- cramps
- constipation
- blurred vision
- candidiasis
- skin sepsis.
- poor wound healing

Assessment of the state of carbohydrate production, metabolism, and excretion primarily involves the measurement of glucose in body fluids. The normal blood glucose level is approximately 70-110 mg/dl, depending on the assay method. Variations in glucose metabolism result in levels as low as 20-30 mg/dl (in hypoglycemia) or as high as 800 mg/dl (in diabetes mellitus). Therefore, any method used to quantitate glucose in serum or plasma must be able to measure this material accurately throughout a wide range of values.

2.3 Type of diagnostic tests

2.3.1 Qualitative determination of reducing substance in urine

The nonspecific approach (which measures "total reducing substances") uses copper sulfate, generally packaged in the form of a tablet (Figure 1). This tablet is placed in the bottom of a test tube and several drops of urine are carefully layered on top of the tablet. The reaction involves the interaction of the Cu^{2+} ion with the carbohydrate. The sugar is oxidized and the Cu^{2+} ion is reduced to Cu^{1+} (sugars which produce this reaction are called reducing sugars. All these compounds contain a carbonyl group, which can be oxidized in this reaction. When the copper is in the +2 state, it gives a blue color in solution. The change to Cu^{1+} results in the formation of an orange-red color. The tablet also contains sodium carbonate, citric acid, and sodium hydroxide. When water comes into contact with the tablet, carbon dioxide is released and heat is generated. The CO_2 provides a "blanket" which keeps out oxygen and hinders the reoxidation of the Cu^{1+} ion. The heat produced makes the reaction between the copper and the sugars take place more rapidly (Sharon, 1980; Brownlee and Cerami, 1981).

There are two major limitations to the reducing sugar approach to urine carbohydrate screening. First, the test is not very sensitive. Second, the test lacks specificity. A wide variety of substances also give a positive reaction if present in the sample.

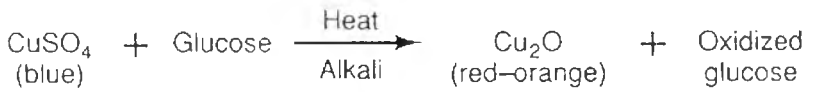


Figure 1. Qualitative test for urinary reducing substances (Sharon, 1980)

2.3.2 Qualitative assessment of urine glucose

The qualitative (or semiquantitative) measurement of urine glucose relies on a more specific test than that for reducing substances (Figure 2). The enzyme glucose oxidase reacts only with glucose, not with any other carbohydrate. Products of the reaction are gluconic acid and hydrogen peroxide. When a chromogen (a material capable of forming a colored product) comes in contact with the hydrogen peroxide, a color change results. The intensity of the color is proportional to the amount of glucose present (Knowles, 1975).

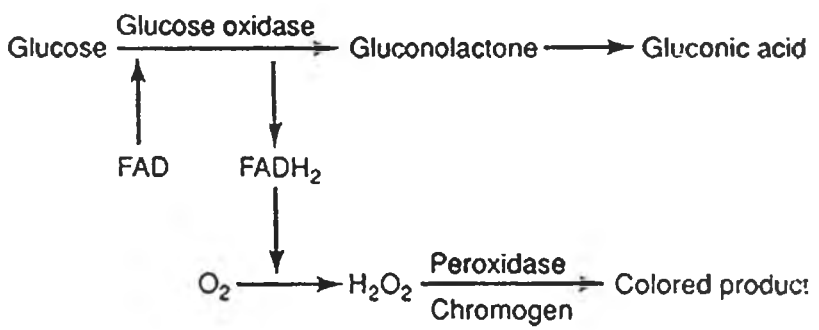


Figure 2. Glucose analysis with glucose oxidase/peroxidase (Knowles, 1975)

Commercial dipsticks are available which have glucose oxidase and the chromogen incorporated into a reaction package at the end of the stick. After the stick is dipped into a urine sample, the reagents are allowed to react with the glucose in the specimen for 1 min. Then the color is observed and compared with a standard color chart to determine how much glucose is in the sample.

There are three major advantages to the dipstick method compared with reducing-sugar approach (Table 1). First, the reaction is specific for glucose; no other carbohydrate gives a positive test. In addition, the problem of drug interference is eliminated. Since the reaction uses a specific enzyme, there are no false positive

results. However, the presence of ascorbic acid in the urine can interfere with the peroxidase reaction, producing false negative values. Finally, use of the dipstick allows the detection of lower levels of glucose in urine. Whereas the reducing-sugar test only detects carbohydrates at 250 mg/dl or higher concentration, the glucose oxidase reaction has a lower limit of detection of approximately 100 mg/dl.

Table 1. Comparison of methods for assaying urine carbohydrate

Method	Reducing Substances	Glucose Oxidase
Specificity	Nonspecific	Glucose only
Lower limit	250 mg/dl	100 mg/dl
Interferences	Many drugs	Negligible
Cost	Very inexpensive	More expensive
Automation	No	Yes

(Knowles, 1975)

2.3.3 Quantitation of blood glucose

The measurement of glucose in blood is one of the most commonly performed laboratory tests. A wide variety of methodologies and instrumentation which make it possible to assay glucose accurately in a sample as small as 10 ml (or less) and in a time as short as 1 min.

When a blood sample is collected for glucose analysis, some of the components involved in the conversion of glucose to other biochemical compounds are still present. In particular, red blood cells have all the enzymes necessary to metabolize glucose. Therefore, it is important to separate the cells from the liquid portion of the blood as soon as possible. If anticoagulants are used, the sample should be centrifuged as soon as possible (preferably within an hour after collection) and the plasma removed. Serum samples can be obtained after the blood clots; centrifugation again should not be delayed unduly. The use of collection tubes containing a gel-like material as a separation barrier between the cells and the serum minimizes the loss of glucose through cellular metabolism. Fluoride or iodoacetate is sometimes used in

sample collection, since these materials inhibit the glycolytic process and prevent most glucose consumption by the erythrocytes (Passey, 1974).

After separation of either plasma or serum from the cells, glucose remains stable at room temperature for several hours in serum samples; glucose is somewhat less stable in plasma. If samples are not to be assayed soon after collection, glucose stability may be enhanced by cold storage of the samples.

A wide variety of methods for colorimetric measurement of glucose have been used over the years with varying degrees of success. Early methods involved an adaptation of the copper reduction technique previously described for qualitative determination of urine glucose. Both manual and automated procedures employed measurement of the color change from blue to orange-red as copper was reduced.

2.3.3.1 Spectrophotometric assay of glucose

Once colorimetric method used today involves the reaction of glucose in strong acid with o-toluidine (Figure 3). When heated at 100 °C in glacial acetic acid, glucose forms a bluish-green derivative with o-toluidine, with an absorbance maximum of approximately 620 nm. The reaction is reasonably specific for glucose. Other carbohydrates, which give this color, are galactose, mannose, and lcactose, all of which are present in extremely low concentrations in blood. No significant drug interferences are known. The major drawbacks to the o-toluidine method are the harsh chemicals and strong reaction conditions required. Heat and concentrated acid produce hazardous conditions and are very destructive to instrumentation over the long term (Sharon, 1980).

2.3.3.2 Enzymatic assays

A higher degree of specificity can be achieved using an enzymative method; enzymatic methods for glucose analysis are more popular than colorimetric assays. The glucose oxidase assay has already been discussed briefly in our consideration of urine glucose measurements. For serum or plasma, a coupled assay involving both glucose oxidase and peroxidase is frequently employed. The reaction principle is the same as that used for urine glucose (Figure 4).

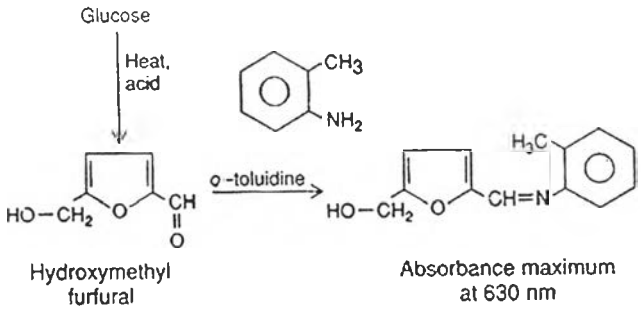


Figure 3. Colorimetric assay of glucose using 0-toluidine (Sharon, 1980)

In the glucose oxidase methods, for each molecule of glucose reacted there is consumption of one molecule of oxygen (Figure 4A). Glucose is converted by glucose oxidase in the usual fashion. The oxygen concentration decreases as the glucose reacts with the enzyme. The rate of decrease is proportional to the concentration of glucose present. It left to itself, the hydrogen peroxide breaks down again, regenerating oxygen. This would obviously create a problem in measuring the rate of oxygen utilization. Therefore, two other reactions are incorporated into the system to take care of this difficulty. Hydrogen peroxide is either reacted with ethanol to form acetaldehyde (Figure 4B) or with iodide to form molecular iodine (Figure 4C). In either case, the hydrogen peroxide is destroyed and cannot regenerate oxygen. Although the glucose oxidase method is reasonably free of interferences, it appears that acetaminophen and a few related compounds interfere with the reaction, probably by interacting with the enzyme system themselves. Other medications do not seem to present a problem with the assay (Passey, 1974).

2.3.4 Oral glucose tolerance test

To facilitate the diagnosis of diabetes, several laboratory tests are needed. The initial clue usually comes from the routine urinalysis. The urine glucose level is elevated, sometimes quite markedly. Fasting blood glucose would then be ordered for follow-up. If this test shows a blood glucose level greater than 140 mg/dl, the patient is presumed to have diabetes mellitus. If the fasting glucose is elevated, but not over 140 mg/dl, further testing is necessary and on oral glucose tolerance test (OGTT) is ordered.

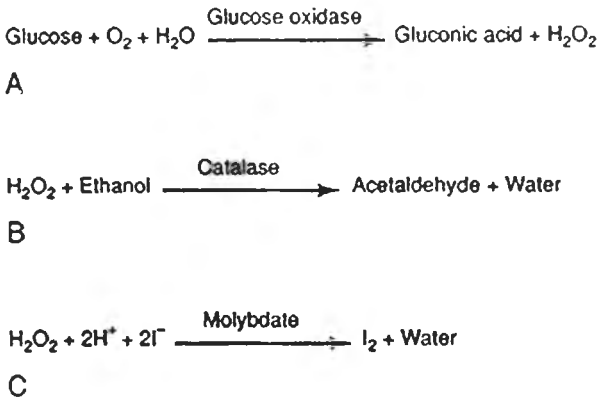


Figure 4. Oxygen rate method for glucose analysis. A. For each molecule of glucose that reacts, one molecule of oxygen is consumed. B. Hydrogen peroxide reacts with ethanol to produce acetaldehyde. C. Hydrogen peroxide reacts with iodide to form molecular iodine

The OGTT is designed to determine how well the body utilizes glucose after it has been absorbed into the circulation. That the patient is not to smoke, eat, or drink during the test (although a few sips of water occasionally are permitted). A glucose load is given to the patient to drink and the time noted. For adults, a solution containing 75g of glucose is the standard amount administered although pregnant women should receive 100g. If the test is being administered to a child, use a load based on body weight. Give glucose 1.75g/kg of body weight up to a maximum of 75g of glucose. After giving the glucose load, blood samples are collected every 30 min for 2 hr. The diagnosis of diabetes mellitus is made on the basis of criteria published in 1979 by an international committee of scientists and physicians (Table 2) (Watts, 1981).

For a classification to be useful (1) for the clinician in categorizing patients for purpose of treatment and diagnosis, (2) for purposes of research, and (3) to serve as a framework for collection of clinical and epidemiological data in diverse population groups (Rifkin *et al.*, 1991).

Table 2. Criteria for diagnosis of diabetes using glucose tolerance test

1. Diabetes mellitus is considered to be present if:
   Fasting glucose > 140 mg/dl (no glucose tolerance test required)
   Two-hour value and one other value both > 200 mg/dl
2. Impaired glucose tolerance is consider to be present if:
   Two-hour value 140-200 mg/dl and one other value>200 mg/dl
3. Gestational diabetes is consider present if two or more values exceed:
   105 mg/dl fasting
   190 mg/dl at 1 hr
   165 mg/dl at 2 hr
   145 mg/dl at 3 hr

(Burke, 1979; Stolk *et al.*, 1995)

2.4 The criteria for hyperglycemia in the diagnosis and prediction of diabetes

The diagnosis of diabetes mellitus in non-pregnant adults should be made by the criteria in Table 3. If either the fasting or random criteria are not met, an oral glucose tolerance test (OGTT) should not be performed since not only does a diagnosis by that means usually not result in different clinical advice, but the diagnoses of diabetes may have negative insurance and employment ramifications for the patient. So, subjects should be active and should have ingested at least 150 g of carbohydrate during each of the 3 days prior to the test (Davidson, 1991).

The first systematic classification of diabetes was published by the National Diabetes Data Group (NDDG) in 1979 in an attempt to eliminate confusion stemming from the use of many often quite different criteria for the diagnosis of diabetes (NDDG, 1979 cite in Harris *et al.*, 1979). And then, the World Health Organization (WHO) endorsed the substantive recommendation of the NDDG and published its diagnostic criteria in 1985 (WHO, 1980 cite in Lee *et al.*, 2000; WHO, 1985 cite in Gabir *et al.*, 2000). The 1985 WHO diagnostic criteria for diabetes have been used in most epidemiological and clinical studies since then. These criteria were

based on clinical manifestations or treatment and pathogenesis and required measures of fasting plasma glucose (FPG) and plasma glucose 2 h after an oral glucose tolerance test (OGTT). WHO criteria involve three broad diagnostic classifications: diabetes impaired glucose tolerance (IGT) and normal glucose tolerance (NGT).

In 1997, the American Diabetes Association (ADA) Expert Committee on the diagnosis and classification of diabetes mellitus recommended a set of revised diagnostic criteria on the basis of the 1979 accepted standards and new research findings since that time (ADA, 1997). The revised criteria suggest three possible ways to diagnose diabetes. One of the new criteria allows the diagnosis of diabetes to be made by using only an FPG measurement without requiring a 2-h OGTT. They also recommended that, for epidemiological studies, diabetes prevalence and incidence should be estimated on the basis of FPG. Instead of using IGT, which requires a 2-h OGTT, 1997 ADA criteria introduced a new category based only on FPG called "impaired fasting glucose" (IFG).

2.5 Classification of diabetes mellitus and other categories of glucose intolerance

The present classification (Table 4) includes three clinical classes.

(1) Diabetes mellitus is characterized either by fasting hyperglycemia, or by levels of plasma glucose above defined limits during a glucose tolerance test.

(2) Impaired glucose tolerance is characterized by plasma glucose levels during a glucose tolerance test that lie above normal but below those defined as diabetes. The levels of plasma glucose in the fasting state or during a glucose tolerance test that are defined as normal, impaired, or diabetes are also compromises and not subscribed to as ideal by all investigators.

(3) The third clinical class is gestational diabetes. The classification also includes two statistical risk classes in the natural history of diabetes for research purposes only in which there are no abnormalities of carbohydrate metabolism. These are previous abnormality of glucose tolerance, and potential abnormality of glucose tolerance.

In a comparison of the FPG-based NDDG criteria, WHO criteria and ADA criteria showed according to Table 3.

Table 3. Comparison of NDDG, WHO and ADA diagnostic categories for undiagnosed diabetes

	NDDG criteria	WHO criteria	ADA criteria*
FPG (mg/dl)	<115 (NGT) 116-140 (IGT) >140 (Diabetes)	<140 + 2-h NGT (NGT) <140 + 2-h IGT (IGT) >140 + 2-h Diabetes (Diabetes)	<110 (NFG) 110-125 (IFG) >126 (Diabetes)
2-h PG (mg/dl)	<140 (NGT) 140-199 (IGT) 200 (Diabetes)	<140 (NGT) 140-199 (IGT) 200 (Diabetes)	-- -- --

* classification by ADA (1997) criteria for fasting plasma glucose only

(Adapted from NDDG, 1979; WHO, 1985; ADA, 1997)

Diabetes mellitus is subdivided into four different types that appear to differ in etiology and pathogenesis. Type 1 and type 2 diabetes are the major clinical forms of diabetes in the world, while malnutrition-related diabetes is a major clinical form in parts of the world. Under the fourth category, other types are classified various entities that, in contradistinction to primary or essential diabetes (Type 1 and Type 2), formerly were classified under secondary diabetes or diabetes associated with certain rare genetic syndromes.

2.5.1 Diabetes Mellitus (DM)

2.5.1.1 Type 1 diabetes or Insulin-Dependent Diabetes Mellitus (IDDM)

It occurs in approximately 10% of all diabetes in the world. In the most common type of IDDM, it has been postulated that environmental (acquired) factors such as certain viral infection and possibly chemical agents superimposed on genetic factors may lead to cell-mediated autoimmune destruction of beta cells. They are probably the result of an autoimmune process, rather than the other cause which in

a very small subset of patients an overwhelming viral infection or chemical insult may lead to destruction of beta cells without a genetic predisposition. This type of disease occurs most commonly in childhood and adolescence; however, it can be recognized and may become symptomatic for the first time at any age. The possible presence or development of other autoimmune disease, such patients have associated autoimmune endocrine disease, such as Hashimoto's thyroiditis, Graves'disease, Addison's disease, primary gonadal failure and associated nonendocrine autoimmune disease, such as pernicious anemia, connective tissue disease, celiac disease and myasthenia gravis.

Usually, there is an abrupt symptomatic onset secondary to severe insulin insufficiency (polyuria, polydypsia, polyphagia, weight loss, fatigue) a proneness to ketosis and the patient is thin. People with Type 1 diabetes must take daily insulin injections that administration of insulin is essential to prevent spontaneous ketosis, coma and death. By prospective testing in asymptomatic siblings of insulin dependent diabetes, one can even discover patients with diabetes glucose tolerance tests and the other with normal fasting plasma glucose levels (Fajans *et al.*, 1978: Vranic, 1985).

2.5.1.2 Type 2 diabetes or Non-Insulin-Dependent Diabetes Mellitus (NIDDM)

It presents in approximately 90% of diabetics in the world, and also has a genetic basis that is commonly expressed by a more frequent familial pattern of occurrence than is seen in IDDM. Environmental factors superimposed on genetic susceptibility are undoubtedly involved in the evolution of NIDDM as well. Patients with Type 2 diabetes may have a body weight that ranges from normal to excessive, which has association with obesity. The intake of excessive calories leading to weight gain and obesity and resulting in insulin resistance are important factors in the pathogenesis of NIDDM in the majority (60% to 80%) of patients in societies (Milton, 1976).

Obesity and pathological insulin resistance are by no means essential in the evolution of NIDDM. In NIDDM patients who are not overweight, even small increases in body weight (including normal growth in childhood and

adolescence) can exacerbate glucose intolerance and precipitate fasting hyperglycemia (Fajans *et al.*, 1978: Köbberling and Tattersall, 1982). In an analysis of non-insulin-dependent diabetes found that the prevalence of diabetes in siblings was higher in non-obese than in obese human diabetes subjects (Köbberling, 1971). In the majority of patients with Type 2 diabetes a diagnosis in made in middle age. A subclass of NIDDM includes families in which diabetes can be recognized in children, adolescents and young adults, which is referred to as maturity onset type diabetes of the young or MODY (Fajans *et al.*, 1978: Tattersall and Fajans, 1975).

Patients with NIDDM are non-insulin-dependent for prevention of ketosis (i.e., they may require insulin for correction of symptomatic or non-symptomatic persistent fasting hyperglycemia if this cannot be achieved with the use of diet or oral agents. Thus, therapeutic administration of insulin does not distinguish between IDDM and NIDDM but patients with NIDDM may even develop ketosis under circumstances of severe stress precipitated by infections or trauma. Usually other factors, such as age of onset, family history of diabetes, clinical course, or natural history (rapidity of progression in severity, fluctuations in plasma glucose levels, frequency of reactions, frequency of ketonuria) will aid in proper classification (DeFronzo and Ferrannini, 1982). However, in patients with NIDDM or MODY with similar abnormalities of glucose tolerance and mild fasting hyperglycemia.

2.5.1.3 Malnutrition-Related Diabetes Mellitus (MRDM)

It occurs in certain parts of the world far more frequently than IDDM and may approximate the frequency of NIDDM. It is usually found in young people, and is characterized by severe protein malnutrition and emaciation. The diabetes of these patients is characterized by severe hyperglycemia unaccompanied by ketosis. These individuals require insulin and they are dependent on insulin for preservation of health and life although they are not dependent on insulin for prevention of ketosis (Mohan *et al.*, 1983 cite in Rifkin *et al.*, 1991).

2.5.1.4 Gestational Diabetes Mellitus (GDM)

Patients with GDM have detection or onset of glucose intolerance during pregnancy. A known diabetic who becomes pregnant is not classified as GDM. Impaired glucose tolerance during pregnancy is similar but not

identical to IGT. Gestational diabetes occurs in approximately 2% of all pregnancies and is associated with increased perinatal morbidity and mortality and an increased frequency of loss of a viable fetus. Gestational diabetes usually returns to a state of normal glucose tolerance after parturition; even so, 60% of such women develop diabetes within 15 years after parturition. Thus, after termination of pregnancy, patients with gestational diabetes should be reclassified as patients with impaired glucose tolerance, diabetes mellitus, or previous abnormality of glucose tolerance (Rifkin *et al.*, 1991).

2.5.1.5 Other types of diabetes mellitus

It includes entities secondary to or associated with certain other conditions or syndromes. This subclass can be divided according to the known or suspected etiological relations. Diabetes may be secondary to pancreatic disease or removal of pancreatic tissue; secondary to endocrine disease, such as acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, somatostatinoma and primary aldosteronism; secondary to the administration of hormones causing hyperglycemia; and secondary to the administration of certain drugs. Furthermore, Diabetes may be associated with a large number of genetic syndrome (Harris *et al.*, 1979), genetic defects of insulin receptors or the result of either abnormalities in number or affinity of insulin receptors or due to antibodies to insulin receptors with or without associated immune disorders.

2.5.2 Impaired Glucose Tolerance (IGT)

The NIDDM workgroup recommended that a category be established for individuals who have fasting plasma glucose level and levels during the glucose tolerance test that lie between normal and diabetes. In some subjects impaired glucose tolerance (IGT) may represent a stage in the natural history of IDDM (Fajans *et al.*, 1978; Vranic, 1985), and much more frequently of NIDDM (Fajans *et al.*, 1978; Harris *et al.*, 1979). In such patients, conversion of IGT to NIDDM, and particularly to NIDDM with fasting hyperglycemia, has taken years or decades (Fajans *et al.*, 1978). It has been found to occur in 10% of 505 of patients with IGT followed for a period of 10 years.

Thus, impaired glucose tolerance, particularly in otherwise healthy and ambulatory individuals under the age of 50 years (condition appropriate for using the oral glucose tolerance test) may have prognostic implications and should not be ignored or taken lightly. Impaired glucose tolerance may be associated also with the conditions and syndromes listed under the section other type of diabetes (Table 4).

2.6 Complication of Diabetes Mellitus

2.6.1 Acute Complications

2.6.1.1 Diabetes ketoacidosis

Diabetic ketoacidosis continues to be a prominent cause of morbidity and mortality in IDDM patients. Ketoacidosis is precipitated by an absolute or relative insulin deficiency and an increase in catabolic hormones, leading to hepatic overproduction of glucose and ketone bodies. Although it principally affects younger patients with IDDM, ketoacidosis may occasionally be precipitated in patients with non – insulin – dependent diabetes mellitus (NIDDM) during severe intercurrent illness. Symptoms of ketoacidosis include increasing polyuria and polydypsia, weight loss, weakness, drowsiness and eventually coma (in 10% of case); abdominal pain may be present, particularly in the young (Krentz, 1997).

2.6.1.2 Hyperosmolar non-ketotic syndrome

The diabetic hyperosmolar non-ketotic syndrome is defined as marked hyperglycemia (plasma glucose usually exceed > 50 mmol/l), without significant hyperketonaemia, ketonuria and acidosis. There is some biochemical and clinical overlap between diabetic ketoacidosis and the hyperosmolar state. Other features include profound dehydration, prenatal uremia and some depression of the level of consciousness (Arieff and Carroll, 1972). Characteristic symptoms are polyuria, intense thirst and gradual clouding of consciousness. These may develop over several weeks. Vomiting is less prominent than in diabetic ketoacidosis, and kussmaul respiration is not a feature of the hyperosmolar non-ketotic state, because significant acidosis is absent.

Table 4. Classification of diabetes mellitus and other categories of glucose intolerance

Clinical Classes	Subclasses
A. Diabetes mellitus (DM)	
I. Type 1 diabetes or Insulin-Dependent Diabetes Mellitus (IDDM)	a. Type 1A: Classical b. Type 1B: Primary autoimmune
II. Type 2 diabetes or Non-insulin-Dependent Diabetes Mellitus (NIDDM)	a. NIDDM in obese b. NIDDM in non-obese c. MODY-NIDDM in young plus autosomal dominant inheritance
III. Malnutrition-related Diabetes Mellitus (MRDM)	a. Fibrocalculous pancreatic diabetes b. Protein-deficient diabetes
IV. Gestational Diabetes Mellitus (GDM)	
V. Other types, including Diabetes Mellitus associated with certain conditions any syndromes:	1. Pancreatic disease 2. Hormonal etiology 3. Drug or chemically induced 4. Certain genetic syndromes 5. Insulin receptor abnormalities 6. Other miscellaneous conditions
B. Impaired Glucose Tolerance (IGT)	a. IGT in obese b. IGT in non-obese c. IGT in MODY d. IGT associated with certain conditions and syndromes: 1. Pancreatic disease 2. Hormonal etiology 3. Drug or chemically induced 4. Certain genetic syndromes 5. Insulin receptor abnormalities 6. Other miscellaneous conditions

(From Rifkin *et al.*, 1991)

2.6.1.3 Lactic acidosis

Although diabetes causes macrovascular and microvascular complications that favor tissue hypoxia, it is only rarely associated with severe lactic acidosis (Kreisberg, 1984). Severe lactic acidosis (type B) (Table 5) can occur in diabetic patients, either as a feature of ketoacidosis (in about 10-15% of cases) or as a rare complication of biguanide therapy. Lactic acidosis associated with ketoacidosis resolves with standard insulin and fluid replacement.

Table 5. Classification and causes of lactic acidosis

Type A (primarily associated with tissue hypoxia)
Shock
- Cardiogenic
- Endotoxic
- Hypovolaemic
Cardiac failure
Asphyxia
Carbon monoxide poisoning
Type B
Sytemic disorders
- Diabetes mellitus
- Neoplasia
- Liver disease
- Convulsion
Drugs and toxins
- Biguanides
- Ethanol
- Methanol
- Salicylate
Inborn errors of metabolism
- Type 1 glycogen storage disease
D-Lactic acidosis due to abnormal gut flora

(Krentz, 1997)

2.6.1.4 Hypoglycaemia in diabetes mellitus

Hypoglycaemia has many possible causes, but in the diabetic population is almost invariably a side – effect of therapy. It occurs most commonly during treatments with insulin or sulphonylureas. Hypoglycaemia is also a side – effect of insulin, which despite refinements in the formulation and delivery of the drug, is unable to match the versatility of the homeostatic mechanisms that normally regulate endogenous insulin secretion and maintain blood glucose levels within their narrow physiological range. Hypoglycaemia is also a side – effect of the sulphonylureas, which act primarily by stimulating insulin secretion, but is not caused by metformin, α - glucosidase inhibitors or the diets conventionally prescribed for diabetic patients. Symptoms are sweating, palpitations, tremor, hunger, behavioral changes, nausea and headache. Symptoms vary considerably between and within individuals (Frier, 1997).

2.6.2 Chronic Complications

2.6.2.1 Microvascular disease

It was notably retinopathy and nephropathy, is frequently seen in patients with long-standing IDDM and may affect NIDDM subjects of shorter disease duration. The abnormalities associated with diabetic microangiopathy are both structural and functional. Structural changes include thickening of the capillary basement membrane, throughout the body together with mesangial expansion in the glomerulus, while functional, haemodynamic alterations include increased blood flow, raised intravascular pressure and enhanced vascular leakiness. The relationship between the structural and functional abnormalities, and whether either or both are the cause or consequence of diabetic microangiopathy, are still matters for investigation and debate (Pick up and Williams, 1997).

2.6.2.2 Diabetic retinopathy

It is a long-term sequel or complication of diabetes. The prevalence of retinopathy (of any degree) is highest in young-onset, insulin-treated diabetic patients and lowest in older-onset diabetic patients not taking insulin. The exact cause of the abnormalities seen is not known, a considerable amount of work

has been done to unravel the sequence of events which finally leant to sight threatening forms of the disease. The three principal abnormalities are capillary occlusion, leakage (usually associated with vascular dilation) and finally new vessel formation. This last event only occurs when, in addition to the capillaries, large vessels, both arteries and veins, are also occluded (Pick up and Williams, 1997).

2.6.2.3 Diabetic neuropathy

It is an important complication of diabetes for a number of reasons. Firstly, it is relatively common, affecting about one in three of patients with IDDM. Secondary, the proteinuria, which is its hallmark, is only one consequence of widespread damage to small and large blood vessels, and is a marker for the cardiovascular disease, which is a common cause of death in these patients. Thirdly, there is increasingly convincing and optimistic evidence that the progression of nephropathy and its associated mortality can be ameliorated by anti-hypertensive and other treatments if started at an early stage (Clements, 1979).

2.6.2.4 The heart and macrovacular disease in diabetes mellitus (Cardiovascular disease)

They were predominating in diabetes patients of over 30 years duration and in those diagnosed after 40 years of age. General risk factors for cardiovascular disease include smoking, obesity, hyperlipidaemia, hypertension, insulin resistance, haemostatic and platelet abnormalities, specific diabetes-related risk factors may include hyperglycaemia (especially for peripheral vascular disease) and hyperinsulinanemia (Pick up and Williams, 1997).

Hypertension in diabetes represents an important health problem, as the combination of the two diseases is common and will frequently be associated by chance alone, but diabetes apparently predisposes to hypertension, conversely and hypertensive people are more likely to develop diabetes.

2.6.2.5 The foot in diabetes mellitus

Diabetes is the commonest cause of amputation of the foot in civilian life. A background to such a foot management program, the physician needs to understand the pathology and sequence of events that commonly lead to

amputation. There are five factors in such a sequence: vascular, neuropahtic, mechanical, infective and metabolic. The common sequence of events:

1. The diabetic may have a reduced peripheral circulation, adequate for regular use but inadequate for the extra blood supply needed to combat gross infection.
2. The patient has diminished sensation – not total anesthesia, but a change threshold of perception of pain and pressure.
3. He or she the suffers a break in the skin from external mechanical force, often associated with improper footwear.
4. In the absence of pain, the patient continues to walk on this open wound. pressing on the infected tissues and spreading the infection until it becomes a gross cellulitis and osteomyelitis.
5. Now feeling ill, and with pus in his shoe, the patient goes to a surgeon or to an emergency room. The surgeon finds that he is a diabetic, check his glucose level, and finds him out of control (perhaps due to the infection). He assumes that such a foot in a danger to the limb or even to the life of the patient, and amputates below or above the knee it is at stage 3 and 4 that early intervention can often prevent the need for amputation and restore the patient to a normal life (Brand, 1981).

2.6.2.6 Gastrointestinal manifestations of diabetes mellitus

To a large extent, gastrointestinal abnormalities of diabetes are the result of structural, hormonal and neural disorders, which interact in complex ways, particularly in patients with long-standing diabetes. The physiological interdependence of the pancreas and the gut in normal digestive process is underlined by the manifestations of diabetes in all parts of the gut (McBride and Spiro, 1981).

2.6.2.7 Skin manifestations of diabetes mellitus

Thirty percent of patients with diabetes mellitus (DM) develop a skin disorder which may act as an indicator for the development of DM or which may complicate the course of the diabetes. Furthermore, certain skin problems are far more threatening to the diabetic than non-diabetic patient and these problems require prompt, aggressive management to prevent serious complications (Gilgor and Lazarus, 1981).

2.6.2.8 Dental aspects of diabetes mellitus

Diabetes mellitus has been implicated in a variety of pathological conditions of the oral cavity and dental structures, ranging from dry mouth and increased incidence of moniliasis to loss of all the teeth because of aggressive periodontitis. Symptoms related to the oral and dental structure frequently furnish valuable clues to the possible presence of diabetes (Gottsegen, 1981).

2.6.2.9 Other chronic complications

Such as, Diabetic renal disease, Renal failure in diabetes mellitus and Erectile dysfunction in diabetes mellitus (Shaw, 1996)

2.7 Major factors regulate blood glucose in diabetes

2.7.1 Diet

The vast majority of people with diabetes are Type 2 (about 85% to 90%) and about 75% of these people are overweight. But people with Type 1 diabetes are typically lean or have average body fatness. In the world, obesity is incredible problem. They eat a diet high in carbohydrates such as bread and rice and relatively low in protein and fat. Foods are available in untold quantity and in concentrated caloric form. So, weight gain and weight losses are more complex than simply computing the balance between caloric intake and caloric expenditure.

If we can be control glucose or blood glucose does not move into the cells. Other physical disadvantages of obesity include risk of heart disease, high blood pressure, varicose vein, hemorrhoids, hiatal hernia, arthritis, gout, low backache and cancer decreased (Milton, 1976).

2.7.2 Physical activity

Exercise of the proper type and amount can be very effective in reducing body fat, adding muscle, and improving physique. Because the loss of weight in Type 2 diabetes, along with exercise, reduces the amount of oral medication needed, exercise is of particularly great value to persons with this type of diabetes. Good blood sugar control helps the process because when the blood sugar is elevated, protein and fat are broken down in larger than normal quantities to provide energy for

the body. By reducing the extent of protein and fat breakdown, body weight can be added.

Endurance exercise, a basic component of the overall diabetic management program, reduces cholesterol (total cholesterol and LDL-VLDL cholesterol). Adequate exercise also reduces a number of other cardiovascular disease risk factors. As body fat is reduced, blood pressure tends to drop, the tendency for the blood to clot is reduced, uric acid level in the bloods drops (elevated uric acid tends to crystallize in the joints to produce gout), and stress can be reduced as well as tolerated more effectively. When exercise is used as part of the overall diabetes management program it often enhances the ability to maintain good blood sugar control. Exercise burns up glucose and glycogen within the skeletal muscles. During and after exercise, glucose from the blood enters the muscle to build the glucose and glycogen levels back to normal levels. Endurance training may reduce the likelihood of severe insulin reaction because it allows trained muscles and the liver to store more glycogen. Furthermore, trained muscle uses more fat and less glucose/glycogen during exercise, which reduces the breakdown of muscle and liver glycogen (Milton, 1976).

Good diabetic management will reduce the tendency toward ketosis. Ketosis promotes the breakdown of protein while good glucose control saves protein for more normal use (e.g., maintenance of muscle mass, white blood cells, enzymes and hormones).

2.7.3 Insulin or oral blood sugar-lowering medication

Insulin is synthesized in the beta cells of the pancreatic islets of Langerhans. The secretion of insulin is controlled by the concentration of glucose in the blood stream. Insulin concentrations increase as the level of glucose increases which usually follows eating a meal. Insulin plays a major part in the uptake of glucose by the cells of the body. It stimulates the formation of glycogen in the muscles and in the liver, while suppressing gluconeogenesis by the liver. The increase of glycolysis in the liver helps to increase synthesis of fatty acid. Insulin also controls the uptake of valine, leucine and isoleucine by the muscles, which in turn helps to increase the synthesis of muscle proteins (Stryer, 1995)

Insulin induces its effects by binding to specific tyrosine kinase receptors in the plasma membrane of its target cells. This in turn causes the glucose transporters in the membranes of the cells to fuse with the plasma membrane. This causes the concentration of glucose to increase inside the cells. An increase in the plasma glucose concentration causes the release of insulin into the blood stream, while a decrease causes the suppression of the release of glucose. The uptake of glucose by the cells causes the concentration of glucose in the blood stream to decrease which ultimately leads to the suppression of insulin release (Vander, 1998). Insulin stimulates the synthesis of glycogen by triggering a pathway that dephosphorylates glycogen synthase. The dephosphorylation activates the synthase. This also leads to the dephosphorylation of phosphorylase kinase, an enzyme needed in the breakdown of glucose. When insulin binds to its receptors, a cascade effect occurs that leads to the phosphorylation of protein phosphotase 1, which is the enzyme that dephosphorylates both glycogen synthase and phosphorylase a (Stryer, 1995). The control mechanism for insulin is located in the liver. The liver measures the amount of glucose in the blood stream and takes up or releases glucose as necessary. Phosphorylase a is this receptor for the measurement of the glucose. When an influx of glucose occurs, glucose binds to the phosphorylase a receptor, which alters the shape so that it can be dephosphorylated. This causes the release of insulin while stimulating the formation of glycogen in the liver (Figure 5A-5B) (Stryer, 1995).

Modern insulin therapy can take a variety of forms ranging from a single daily insulin injection to intensive therapy utilizing multiple insulin injections self-monitoring and diet therapy should be recommended in a way that is consistent with the insulin injection regimen and overall treatment goals. Furthermore, oral hypoglycemic agents are able to modify metabolic processes so that plasma glucose level is reduced. However, remedies have been proven toxic, and others are controversial as to whether they are sufficiently effective and safe enough to warrant their widespread use. Oral hypoglycemic agents have two groups as sulfonylurea and biguanide (Strowing and Raskin, 1991; Lebovitz, 1991).

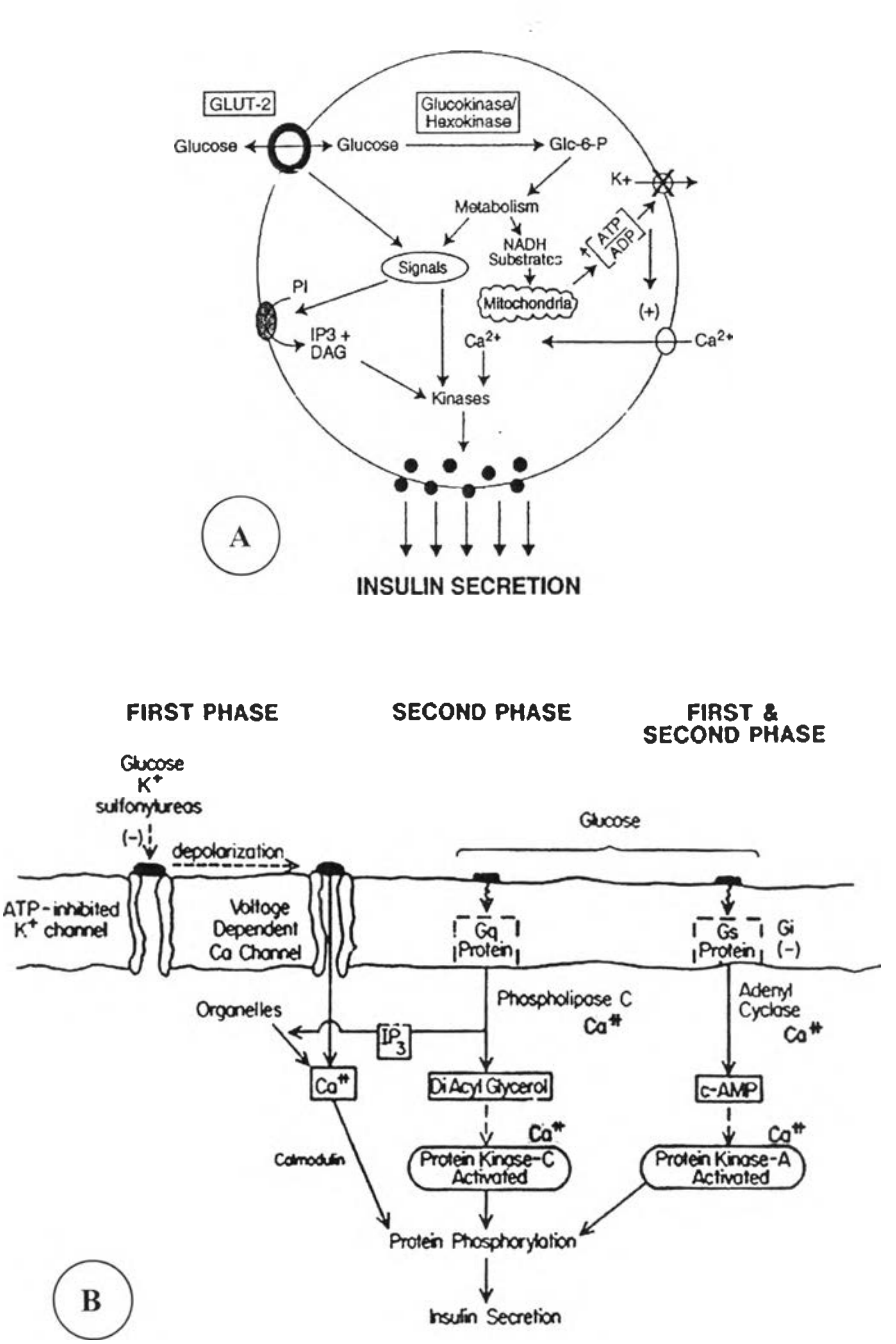


Figure 5. Pathways affecting insulin secretion. A. Schematic summary of biochemical events in the glucose-stimulated insulin secretion pathway. B. Metabolic functions in the β-cell and the phase of insulin secretion that they regulate (LeRoith *et al.*, 1996)

The sum of the effects of each factor determines blood sugar level. The interactions of each factor exert an effect on blood sugar. Exercise will lower blood sugar if there is adequate insulin in the blood, eating obviously will raise the

blood sugar while insulin or oral medication will reduce it. Blood sugar measurement therefore provides an index to the overall balance of the three factors.

2.8 Diabetic animal model

The induction of diabetes in experimental rats by using the most common chemicals of alloxan (ALX) or streptozotocin (STZ), which selectively destroy pancreatic β cells, is very convenient and simple ones. The understanding of changes in pancreatic β cells as well as in other organism post ALX or STZ treatment is an essential data for using these compounds as diabetogenic agents.

Szkudelski (2001) studied the mechanism of ALX and STZ action in β cells of the rat pancreas. ALX and STZ are widely used to induce an experimental diabetes in animals. The mechanism of their action in β cells of the pancreas has been intensively investigated. The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, however the source of their generation is different in the case of ALX and STZ. ALX 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. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells. STZ enters the β cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of STZ than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, STZ liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, β cells undergo the destruction by necrosis.

Many investigators suggested that the selectivity of ALX action is not quite satisfactory. Using ALX to evoke diabetes, animals should be examined after proper period of time to minimize side effect of ALX action. It should also be emphasized that the range of the diabetogenic dose of ALX is quite narrow and even light overdosing may be generally toxic causing the loss of many animals. This loss is most likely due to kidney tubular cell necrotic toxicity, in particular when too high does of ALX are administered (Lenzen, 1996).

STZ is used to induce both IDDM and NIDDM. The range of the STZ dose is not as narrow as in the case of ALX and may also be given in multiple low doses. Furthermore, calcium which may induce necrosis, does not seem to play a significant role in the necrosis evoked by STZ since calcium channel antagonists do not protect β cells against STZ, as they do in the case of ALX (Katsumata *et al.*, 1992)

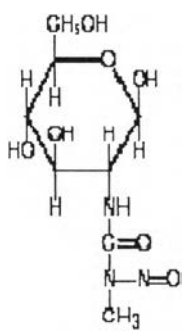


Figure 6. Structure of streptozotocin (Weiss, 1982)

In lots of literature expressed STZ action that is safe and useful variety. So, in the present investigation, STZ is used to produce hyperglycemia in rats.

Streptozotocin (STZ) is a naturally occurring broad-spectrum antibiotic produced from a fermentation broth of *Streptomyces achromogenes* (Lewis and Barbiers, 1960; Svendsen and Hau, 1994). It is a glucosamine-nitrosourea compound, has a chemical name of 2-deoxy-2- (3-methyl-3-nitrosoureido)-D-glucopyranose (CHNO). The structure is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other as shown in Figure 6. The

molecular weight is 265 g/mol (Weiss, 1982) and it is rapidly cleared from the bloodstream with a serum half-life of 15 minutes (Svendsen and Hau, 1994).

STZ structure has been determined to be the nitrosamide methylnitrosourea (MNU) linked to the C2 position of D-glucose. The nitrosamide MNU contributed to its alkylation properties and the glucose moiety directs it to the β cell specifically. Once inside the cell, STZ is metabolized to cut apart between the 2′-carbon and the methyl nitrogen, the N-nitrosoureido moiety can do further damage in cells (Figure 7).

The mechanisms of STZ-induced hyperglycemia are considered as follows: (1) STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP-ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibit proinsulin synthesis and induces diabetes (Wilson *et al.*, 1988). (2) Activated oxygen species, such as superoxide ($O_2^{\bullet-}$) hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), have been implicated to play important roles in diabetes, especially diabetic angiopathy (Figure 8) (Sato *et al.*, 1979).

One of the most possible mechanisms, that STZ causes animal diabetes, is that STZ administration damages pancreatic β cells and result in diabetes. The study showed that free radicals generated by STZ might be involved in the toxic action of STZ. STZ is a chemically unstable molecule that accumulates in pancreatic β cells and produces toxic radicals during its decay. The results showed that STZ enhanced generation of the DMPO-OH radical adduct, which is a degradation product of the $O2^{\bullet-}$ both in the presence and in the absence of cellular components in a hypoxanthine-xanthine oxidase (XOD) system with a homogenate of β cells (Nukatsuka *et al.*, 1988). It is proposed that the cytotoxic effect of STZ be closely related to free radical generation in pancreatic β cells. These findings may support a proposal that STZ induces diabetes through the following biochemical events: STZ→ H_2O_2 generation→DNA fragmentation→B-cell destruction (Takasu *et al.*, 1991)

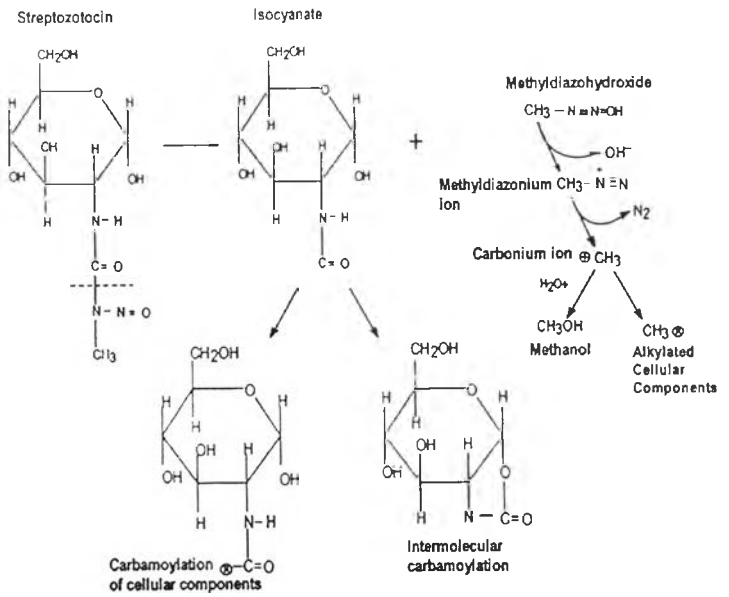


Figure 7. Spontaneous decomposition of STZ to form carbamoylation and alkylating species. The isocyanate component is able to either carbamoylate various cellular components or undergo intramolecular carbamoylation. The methlyldiazohydroxide decomposes further to form a highly reactive carbonium ion or methyl radical, which is able to alkylate various cellular components such as DNA, protein or to react with H_2O to form methanol which can subsequently enter the 1-carbon pool. ® can be biological molecules (Adapted from Wilson and Letter, 1990)

Nitric oxide generated by STZ has been proposed to be involved in the damage of pancreatic β cells. STZ consists of a 2-deoxyglucose substituted by N-methyl-N-nitrosourea, which can decompose to generate $^{\bullet}NO$. Research showed that STZ could produce $^{\bullet}NO$ by photodecomposition or in acidic conditions. Nitric oxide synthase (NOS) inhibitors (Kwon *et al.*, 1994), such as L-N^G-monomethyl-arginine (NMMA) and aminoguanidline reduce STZ-induced islet destruction and hyperglycemia in mice (Lukic *et al.*, 1981). STZ-induced double-strand DNA breaks in rat pancreatic islets have been demonstrated to be inhibited by NMMA and nicotinamide, which suggest the involvement of STZ in NOS expression during β cell injury (Bedoya *et al.*, 1996 cite in Li, 2001).

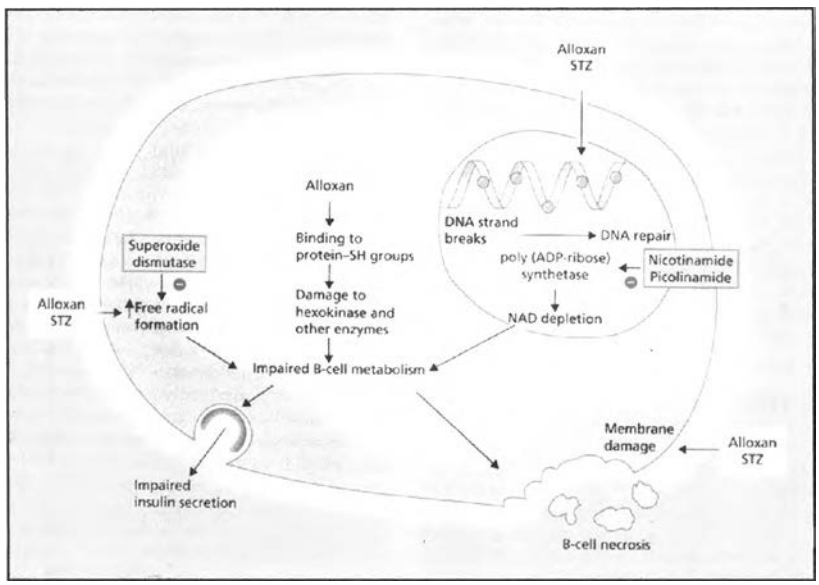


Figure 8. Suggested mechanisms of alloxan and streptozotocin toxicity on the β cell. Inhibitors of poly (ADP-ribose) synthetase such as nicotinamide and superoxide dismutase, a free-radical scavenger, can protect against the diabetogenic effects of these agents. Multiple low doses of streptozotocin can also induce autoimmune β cell damage (Bone and Gwilliam, 1997)

In recent years, several plant extracts have been examined for anti-diabetic activity in an effort to identify alternative treatment strategies that pose less of a risk for diabetics. It is important to note that as with any change of diet, medication, or lifestyle with the diabetic, the administration of herbal supplements requires close monitoring of blood glucose levels, as these agents may reduce requirement for insulin or oral hypoglycemic drugs, and may cause hypoglycemia in some individuals.

In traditional practices, medicinal plants are used to control diabetes mellitus in many countries. This has caused an increase in the number of experimental and clinical investigations directed toward the validation of the anti-diabetic properties, which are empirically attributed to these remedies (Roman-Romos *et al.*, 1995)

Recently it has been reported that *Gymnema sylvestre* show to exert a hypoglycemic effect. In the diabetic rabbit model, administration of *G. sylvestre* was shown to not only bring about blood glucose homeostasis, but also increase the

activities of enzyme involved in glucose utilization (Shanmugasundaram *et al.*, 1983). Furthermore, the oral administration of *G. sylvestre* to diabetic rats increased the number of pancreatic islet and beta cells, as well as insulin levels, suggesting a possible repair or regeneration of the endocrine pancreas (Shanmugasundaram *et al.*, 1990a). Extracts of *Gymnema* have been shown to effectively lower blood glucose levels in both Type 1 and Type 2 diabetic patients. In study, Type 2 diabetic patients were treated with aqueous extract of *G. sylvestre* 400 mg/day demonstrated a significant reduction in blood glucose and *G. sylvestre* was shown to reduce fasting blood glucose and insulin requirement in patients with insulin-dependent diabetes (Type 1 diabetes) undergoing insulin therapy. The investigators speculate that *G. sylvestre* may help to increase levels of insulin and infer from animal studies that this mechanism may be as a result of the repair or regeneration of the residual beta cells in the islet of Langerhans in diabetic patients (Baskaran *et al.*, 1990; Shanmugasundaram *et al.*, 1990b).

Bitter Gourd (*Momordica charantia*), also known as balsam pear, is a tropical vegetable widely cultivated in part of Asia, Africa and South America, which has been extensively used in folk medicine as a remedy for diabetes (Welihinda *et al.*, 1982). Srivastava *et al.* (1988) showed that an aqueous extract of 100 g reduced to a 100 ml volume dose given once per day was found to be highly effective in lowering blood sugar level in Type 2 diabetics over a period of 7 weeks.

Gomes *et al.* (1995) investigated the effect of the hot water extract of black tea (Camellia sinenesis (L.) O.kuntze (Theaceae)) on streptozotocin (STZ)-induced diabetes in rats. The extract significantly reduced the blood glucose levels and was found to possess both preventives and curative effects on experimentally produced diabetes in rats. The study reveals that, like green tea, black tea also possesses antidiabetic activity.

However, there are widely investigation about hypoglycemic effect and anti-hyperglycemic effect such as hypoglycemic effect of water-soluble extract from *Rhizoma Polygonati Odorati* in diabetic mice and rats (Chen *et al.*, 2001), Effect of epicoprostanal (3-α-hydroxy-5β- cholestanol) from *Physter catodon* on blood glucose

and plasma insulin levels in rodents (Taha and Raza, 1996), the effect of different doses of *Pterocarpus santalinus* L. bark extracts in normal and diabetic rats on blood glucose levels evaluation (Rao *et al.*, 2001), etc.

In Thailand, many medicine plants had been studied the anti-hyperglycemic activity such as *Coccinia indica* (Choradol *et al.*, 1972) *Solanum sanitwongsei* Craib. and *Solanum trilobatum* L. (Hongvareewatana, 1976) *Pandanus odorus* (Peungvicha *et al.*, 1985; Peungvicha *et al.*, 1990), *Mimosa pudica* L. (Daechativong *et al.*, 1988), *Pluchea indica* (Peungvicha *et al.*, 1999), etc.

Recently, HAMM is widely used as traditional medicine in the north-eastern part of Thailand, particularly along the border of Lao people's Democratic Republic. It is claimed as a Laos traditional medicine and used for balancing blood pressure, detoxifying agent, for the treatment of high blood cholesterol and antihyperglycemic, etc. HAMM has been recognized as effective remedy anti-hyperglycemic. Dechwisissakul *et al.* (2000) reported pharmacognostic investigation of HAMM indicated that its botanical origin is *Coscinium fenestratum* (Gaertn.) Colebr., family Menispermaceae (Figure 9).

Singh *et al.* (1990) studied hypotensive action of *Coscinium fenestratum*. They found a 50% ethanol extract of *C. fenestratum* stem material that possess hypotensive action in anaesthetised dogs, rats and guinea pigs. The effect was more pronounced in spinal-transcted animals. The oral LD_{50} was estimated to be 1200 mg/kg in mice.

In 2002, a methanol extract of *C. fenestratum* stem powder was examined the antioxidant effect using carbon tetrachloride-intoxicated rat liver as the experimental model. The decresased activities of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reduction in carbon tetrachloride-intoxicated rats, and its retrieval towards near normalcy in the methanol extract co-administered animals revealed the effectiveness of *C. fenestratum* in combating oxidative stress due to hepatic damage (Venukumar and Latha, 2002).

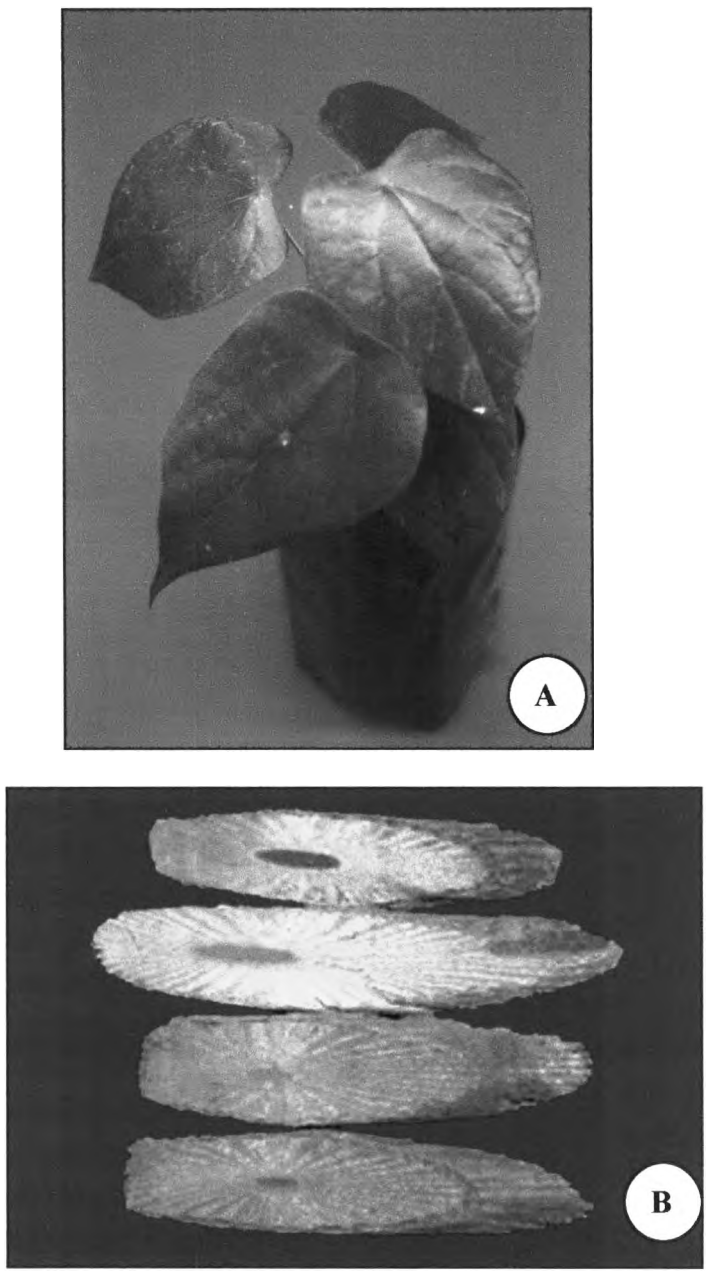


Figure 9. *Coscinium fenestratum* from Ubonratchathani province. A. *C. fenestratum* plants. B. Dried pieces stem of *C. fenestratum*

Ueda *et al.* (2002) tested for their antiproliferative activities against human HT-1080 fibrosarcoma cells using methanol extracts and methanol-water (1:1) extract of *C. fenestratum*. They showed selective activity against lung carcinoma and/or lung metastatic cell lines, human lung A549 adenocarcinoma, murine lewis lung carcinoma and murine B16-BL6 melanoma cells. Characteristic morphological change and

DNA fragmentation indicated the antiproliferative activity to be due to the induction of apoptosis.

However, hypoglycemic effect of *C. fenestratum* had not been reported. Therefore, it is interesting to examine anti-hyperglycemic of water extract from *C. fenestratum*.

Coscinium fenestratum Colebr. is a large liana with yellow wood and sap. Young twigs are hairy, there first leaves obviously peltate, hardly peltate base with concave margins. Leaves usually broadly ovate or ovate, rarely subpanduriform with basal, lateral lobes, 11-33 by 8-23 cm, base broadly rounded, truncate or shallowly cordate, rarely broadly obtuse, apex acuminate, upper surface glabrescent, usually drying smooth, midrib and other main nerves sunken; lower surface often whitish tomentellous, palmately 5-7 nerved at base and also usually two pairs of distal lateral nerves thinly coriaceous (Smitinand, 1991). The petiole 3-16 cm, inserted up to 0.8-2.7 cm from basal margin of lamina, with curved thickened base densely hairy, the inflorescences are axillary or cauliflorous with 6-12 florets (Keawpradub, 1992)

Male flowers sessile or with pedicels, up to 1 mm; tepals 9, imbricate in 3 whorls, externally sericeous; stamens 6, 1mm long, the outer 3 free with 1-locular introrse anthers, the inner 3 with connate filaments and with 2-locular extrorse anthers. Female flowers: tepals as in male, staminodes 6, claviform, 1 mm long; carpels 3, curved-ellipsoidal, 2mm long; densely pilose, style filiform recurved. Drupes subglobose tomentellous, brown to orange or yellowish, 2.8-3 cm diam.; pericarp drying woody, ca 1 mm thick; endocarp bony, 2.2-2.5 cm diam., wall 3 mm thick coverde with anastomosing fibrous ridges; condyle deeply intrusive, thickly clavate and containing 2 ducts, each linking the seed-cavity with a pore on the basal surface of the endocarp; seed subglobose, peltate, enveloping the condyle, endosperm surrounding the divaricate, folded and divide coteledons (Figure 10) (Forman, 1978). the plants producing these alkaloids, berberine and palmatine are mediating chemical defense against microorganisms, viruses and herbivores (Schmeller *et al.*, 1997; Park *et al.*, 1999)

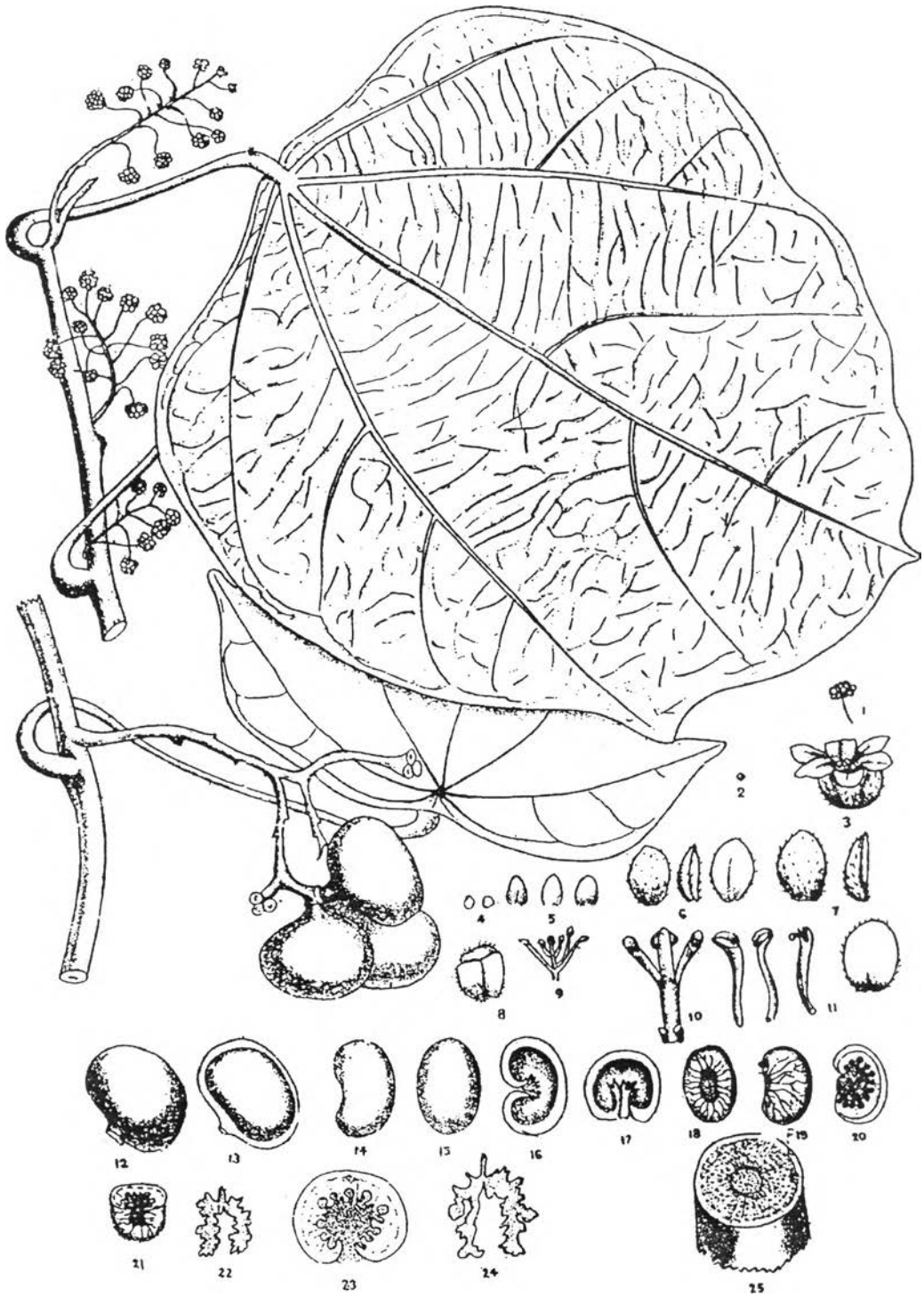
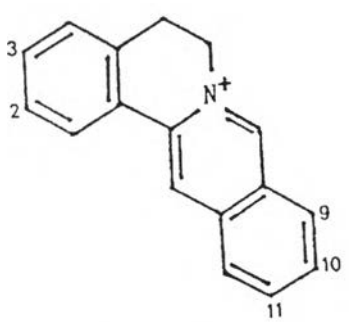


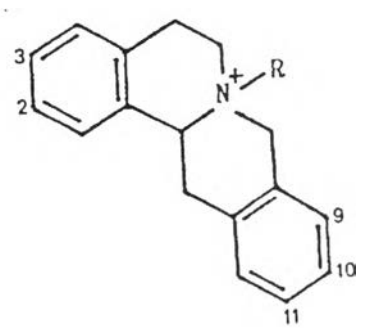
Figure 10. ***Coscinium fenestratum*** (Basu, 1980)

Siwon *et al.* (1980) reported the major alkaloids from the stem and root of *C. fenestratum* (Gaerth.) Colebr., family Menispermaceae. That contained major alkaloids as berberine and jatrorrhizine. Apperciable amounts of berberrubine and N, N-dimethyllindcarpine and small amounts if thalifendine and palmatine. Keawpradub (1982) reported the alkaloids from the stem *C. fenestratum* Colebr. That is four alkaloids, three of which being protoberberine alkaloids as berberine, jatrorrhizine and tetrahydropalmatine. The other on being aporphine alkaloids as crebanine. Furthermore, minor alkaloid from *C. fenestratum* as oxyberberine, tetrahydroberberine (canadine), sitosterol and stigmasterol (Malhotra *et al.*, 1989) (Figure 11).

Figure 11. Chemical structure of protoberberine alkaloids



Formula A



Formula B

Formular A	Formular B	2	3	9	10	11/R
Berberine		-O-CH_2- O-		OMe	OMe	-
Palmatine	Tetrahydropalmatine	OMe	OMe	OMe	OMe	-
Jatrorrhizine		OMe	OH	OMe	OMe	-
Thalifendine		-O-CH_2- O-		OMe	OH	-
Berberubine		-O-CH_2- O-		OH	OMe	

Yin *et al.* (2002) studied effect of berberine on glucose metabolism *in vitro*. The action of berberine was compared with metformin and troglitazone (TZD) by using HepG2 cell line, phenotypically similar to human hepatocytes. It was used for glucose consumption (GC) studies. They found the glucose-lowering effect of berberine decreased as the glucose concentration increased. The maximal potency was reached in the presence of 5.5 mmol/L glucose, and it was abolished when the

glucose concentration increased to 22.2 mmol/L. The effect was not dependent on insulin concentration, which was similar to that of metformin and was different from that of TZD, whose glucose-lowering effect is insulin dependent. These observstions suggest that berberine is able to exert a glucose-lowering effect in hepatocytes, which is insulin independent and similar to that of metformin, but has no effect on insulin secretion.

Tang and Eisenbrand (1992) studied chemical constituents of *Coptis chinenis*. The result is berberine, palmatine and jatrorrhizine which similar alkaloids from *C. fenestratum*. Berberine is the major active constituents as antimicrobial activities, antiinflammatory effect, antitumor activity and hypoglycemic activities. In the plants producing these alkaloids, berberine and palmatine are mediating chemical defense against microorganisms, viruses and herbivores (Schmeller *et al.*, 1997; Park *et al.*, 1999)

Furthermore, other alkaloids such as berberrubine and the ester derivatives of berberrubine had a strong anti-tumor activity (Hoshi *et al.*, 1976), minor alkaloid as sitosterol produced hypocholesterolemic activity (Wang *et al.*, 1999), hypoglycemic activity (Basnet *et al.*, 1993).