CHAPTER 1

INTRODUCTION

Type 2 diabetes is one of the most common chronic diseases characterized by hyperglycemia as a result of impaired insulin secretion by pancreatic β-cells and by cellular resistance to insulin (American Diabetes Association, 2004). Diabetes mellitus is recognized by the World Health Organization (WHO) as a tremendously increasing worldwide epidemic with more than 285 million people worldwide afflicted in 2010 and it is estimated that the number of people with diabetes will increase to 439 million by 2030 (Shaw, et al., 2010). The WHO predicts that diabetes mellitus will become one of the leading causes of death within the next century (World Health Organization, 2011). International Diabetes Federation estimated that the number of people died from diabetes and its complications were 3.8 million of total world mortality in 2007, and making it the fifth leading cause of death in the world (Dieren, et al., 2010).

Type 2 diabetes accounts for 90 ~ 95% of all cases of diabetes and is largely associated with severe obesity and physical inactivity, which have been shown to lead to insulin resistance. The diets with higher in fats and sugar are significant factors to lead to obese and it is estimated that 80% of people who have diabetic are overweight (Yurgin, et al., 2008). The increase in this phenomenon around the world has been largely associated with people’s lifestyle, which refers to the combined detrimental effects of decreased exercise and bad diet. In terms of the total number of people afflicted globally, India, China and USA are the top three countries among the highest prevalence of diabetes for 2010. In India, 50.8 million people had diabetes in 2010 and this number is predicted to increase to 87 million by 2030. In China, 43.2 million had
diabetes in 2010 increasing to 62.6 million by 2030. In the USA, 26.8 million people had diabetes in 2010 and it is estimated to rise to 36 million by 2030 (Shaw, et al., 2010).

There is no effective cure for diabetes, but the progression of disease may be controlled considerably through proper diet and regular exercise. The current treatment of diabetes is aimed at maintaining strict control of glycaemia and while some patients may be achieved control of progression by healthy diet and regular physical activity, but in most cases, effective glycaemic control is required one or a combination of oral hypoglycemic agents. However, currently available oral antihyperglycemic agents, even when used intensively, they are often unable to control the hyperglycaemia and the disease progressively worsens with time. Therefore, there is a need for development of new anti-diabetic drugs.

Medicinal plants have been used traditionally throughout the world as remedies for the treatment of diabetes, especially in India and China where herbal medicine are extensively practiced. Sophora species are widely distributed in Taklimakan region, northwest of China. It can also be found in Oceania, the Pacific islands and some parts of Europe (Qiu, et al., 2004). In Chinese traditional medicine, the root of Sophora flavescens is used to treat hepatitis B virus (HBV) infection, tumor (Abbott, et al., 1966) and cancer (Fei, et al., 2009). In Uyghur traditional medicine, the seeds of this plant are used for diabetes. In previous studies by (Kim, et al., 2006) found that chemical compounds extracted from the roots of Sophora flavescens has α-glucosidase inhibitory activity. It has been shown that glycosidase inhibitors are deeply involved in several important biological processes in carbohydrate metabolism and biosynthesis of glycoprotein (Oh, et al., 2010). An extensive study conducted by (Sato, et al., 2007) revealed that flavonoids extracted from the roots of Sophora flavescens shows Na+-glucose co-transporter (SGLT) inhibitory activity. The biological activities of SGLT
inhibitors are known as decrease glucose reabsorption in kidney, and this could result in lower blood glucose level by increase of urinary glucose excretion (Ohsumi, et al., 2003). α-glucosidase inhibitors are believed to inhibit the enzymes that responsible for conversion of disaccharides to monosaccharides, and delay the digestion and intestinal absorption of carbohydrates, because only monosaccharides can be absorbed through the gut and thus result in reducing blood glucose level (Cheng and Josse, 2004). The recent studies (Li, et al., 2011) has revealed that sophocarpine isolated from Sophora alopecuroides preserves myocardial function in rats by inactivation of nuclear factor-kappaB (NF-kB). NF-kB activation is known to be associated with insulin resistance in type 2 diabetes and other non-diabetic disorders such as cancer and cardiovascular diseases (Barma, et al., 2009). However, to the best of our knowledge, it is noted that there is no one has examined the effects of Sophora alopecuroides seed on inhibition of glycogen phosphorylase and in vivo antidiabetic activities. In these properties, this medicinal plant was selected for study.

1.1 Research objective

1. To extract and determine the chemical compounds from Sophora alopecuroides seed using TLC and Q-TOF Mass Spectrometer.

2. To evaluate the antioxidant activity of crude extract from Sophora alopecuroides seed.

3. To determine antihyperglycemic activity of crude extract using glycogen phosphorylase enzyme assay.

4. To determine in vivo antihyperglycemic activity of Sophora alopecuroides seed extracts in streptozotocin - nicotinamide induced diabetic rats.

5. To determine the effect of Sophora alopecuroides seed extracts on serum lipid profiles in streptozotocin - nicotinamide induced diabetic rats.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Diabetes mellitus is one of the most common chronic diseases which lead to grow public health problem that result in reduced life expectancy in all around the world. The numbers affected are continuously increased as changing lifestyles associated with reduced physical activity and increased obesity. The disease is characterized by complex disorders of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion by pancreatic β-cell, insulin action or both (Sesti, 2006). Type 2 diabetes is a chronic metabolic disorder that results from insulin resistance and relative insulin deficiency in patients with diabetes. Even with current therapies, disease is progressively worsens with time, and some of its specific complications include retinopathy, nephropathy, neuropathy and cardiovascular disease (Meshkani and Adeli, 2009).

Insulin resistance is described an impairment of biological response to insulin, which causes hyperinsulinaemia and leads to pancreatic β-cell failure. In the early stage of insulin resistance, glucose homeostasis can be maintained through the excess insulin secretion by β-cells. Overt diabetes only develops when β-cells can no longer compensate for insulin resistance. It is reported that newly diagnosed patients with type 2 diabetes mellitus have approximately 50% β-cell function due to reduction in β-cell mass (Lupi and Del Prato, 2008). In the studies (Lin, et al., 2003; Luley, et al., 2011), inadequate glycaemic control in patient with treated diet alone or with anti-diabetic agent sulfonylurea treatment more than six years, was significantly associated with a
reduction in insulin levels due to progressive β-cell dysfunction. Therefore, improving β-cell function is critical in altering the progressive nature of the disease.

The United Nations (UN) has recognized the diabetes as a worldwide epidemic and a threat to entire world (United Nations, 2007). Apart from the human pain and suffering, diabetes imposes an increasing economic burden on the individuals with diabetes, national healthcare system and economy worldwide. International Diabetes Federation indicated that the global health expenditure on diabetes has been expected to account for USD 376 billion in the world in 2010, and estimated to increase USD 490 billion by 2030. An estimated average of USD 1330 per person with diabetes is expected to be spent on diabetes-related complications in 2010 globally. In the USA alone, total cost in 2010 has been estimated at USD198 billion which account for 52.7% of the global expenditure, and it is estimated to rise to USD 264.3 billion in 2030. In India, which has the largest population with diabetes in the world has been estimated to spent USD 2.8 billion which is account for less than 1% of the world total in 2010. The total cost in 2010 in china has been estimated at USD 5 billion and is estimated to rise to USD 14 billion by 2030. Healthcare costs for nations are vary by region and it range from 5-13% of their annual healthcare budgets (Zhang, et al., 2010). Because of the significant increase in the number of people with the diabetes, cost-effective therapies will be required to treat people, particularly those from developing parts of the world that cannot afford expensive medication. The solution to this problem is still remains unclear, and it will need a novel and concerted global effort that combines modern medicine with alternative medicine used throughout many parts of the world.

2.2 Diabetes

There are several pathogenic processes involved in the development of diabetes. According to the International Diabetes Federation (IDF), there are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes.
2.2.1 Type 1 diabetes

Type 1 diabetes is one of a group of metabolic disease that largely recognized as an absolute deficiency of insulin secretion. It is caused by an autoimmune reaction whereby the \( \beta \)-cells are destroyed by the body’s own antibodies. It is accounted for 5-10% of all cases of diabetes and no or very little insulin are produced people with type 1 diabetes. Since insulin can no longer be produced, the only effective treatment is to daily insulin injection.

2.2.2 Type 2 diabetes

Type 2 diabetes is sometimes called non-insulin dependent diabetes and it is estimated that 90-95% of diabetes are belong to this type. The disease is usually caused by a combination of resistance to insulin action, particularly in adipose tissue, muscle, liver and \( \beta \)-cell dysfunction. The disease can remain undetected in early stage of development. It is assumed that type 2 diabetes is mostly associated with people’s lifestyle which itself can cause insulin resistance and lead to progressive elevation in plasma glucose level, and thus result in continuously worsen the disease (Luley, et al., 2011).

2.2.3 Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as ‘carbohydrate intolerance during pregnancy (Carolan, et al., 2010) and studies demonstrated that gestational diabetes mellitus were associated with defect in \( \beta \)-cell function and obesity in pre-pregnancy (Hak, et al., 2003). It is estimated that 50% of women with GDM have been in the risk of development type 2 diabetes within five to ten years after delivery (United Nations, 2007).

The type 2 diabetes mellitus is widespread in all populations around the world and the prevalence is in a progressively increase. Prevention and treatment are still a
challenge in all types of diabetes. The World Health Organization (WHO) has chosen
the use of fasting plasma glucose (FPG) and two hour oral glucose tolerance test for the
diagnosis of the diabetes. Normally, fasting plasma glucose (FPG) concentrations are
strictly maintained within 5 mmole/l to 6.6 mmole/l but in type 2 diabetes the person is
unable to maintain glucose levels within this range. The disease is characterized by
fasting plasma glucose of ≥ 7 mmole/l or by a two hour oral glucose tolerance test
(OGTT) of ≥ 11.1 mmole/l. The onset of diabetes is preceded by an early diabetic state
with fasting plasma glucose between 6.1 and 6.9 mmole/l and is referred to as impaired
fasting glucose (IFG). Alternatively, it is recognized by a two hour OGTT between 7.8
and 11.1 mmole/l and is referred to as impaired glucose tolerance (IGT). International
Diabetes Federation (IDF) has recommended reducing the threshold for IFG from 5.6 to
6.9 mmole/l. The people with IFG or IGT have been referred to as having pre-diabetes,
and indicating that they have relatively high risk for development of diabetes in his
future life (World Health Organization, 2006).

Aside from FPG and 2 hour OGTT, the American Diabetes Association has
recently recommended the use of the glycosylated haemoglobin A1C (HbA1c) test to
diagnose diabetes. The report from Japan Diabetes Society showed that glucose in the
body may bind to haemoglobin to give glycosylated haemoglobin (HbA1c). HbA1c
concentration is directly proportional to blood glucose levels because it reflects blood
glucose concentrations over the previous 1-2 month. When comparison of FPG levels
with HbA1c concentration, the FPG levels of 7.0 mmole/l correspond to HbA1c of 6.5% and
results are not affected by the food intake prior to blood sampling (Shibata, et al.,
2005). In the criteria for the diagnosis of diabetes, present study indicates that if people
with A1C of 5.7-6.4%, is considered to be pre-diabetes and lowering their A1C to
below or around 7% has been shown to reduce micro-vascular and neuropathic
complications in both type 1 and type 2 diabetes. According to the most recent study
results, when compared to the accuracy of a FBG of \( \geq 7.0 \text{ mmol/L} \) to that of an HbA1c of \( \geq 6.5\% \) in the detection of hyperglycaemia, the result of FPG have been observed similar to A1c. The specificity and sensitivity of the test have been improved by combination measurement of FPG and HbA1c in the predicting of diabetes. Therefore, HbA1c test may be the most important indicator in diagnosis of diabetes and would be the ideal standards for assessing glycaemic control (Valdés, et al., 2011). However, the WHO still does not consider the HbA1c result alone a suitable diagnostic test for diabetes for following reasons. First, this test is not readily standardized and also not readily available many parts of the world. Secondly, HbA1c results are influenced by the patient’s clinical situation that completely unrelated to diabetes such as anaemia, pregnancy and uraemia (American Diabetes Association, 2010; Beard, et al., 2010). Thirdly, HbA1c sensitivity is vary for different age group (Tay, et al., 2011). To compare conventional tests (FPG and OGTT) with HbA1c test, conventional tests are the most applicable means of accessing glucose levels. Therefore, it remains most prime position as a standard for diagnosis of diabetes (World Health Organization, 2006).

2.3 Glucose metabolism

After meal, carbohydrates are degraded by enzymes and which result in formation of free glucose. Glucose is transferred to cell surfaces where it was utilized by cells as energy or stored as a glycogen in liver or in skeletal muscle. Glucose is the major energy source for cells and its concentrations are controlled by a number of hormones, the most importantly insulin and glucagon. Insulin is secreted by pancreatic \( \beta \)-cells when blood glucose concentration rises, and reduces glucose levels by two general mechanisms; (1) inhibition of hepatic glucose production (glycogenolysis and gluconeogenesis) and (2) increasing glucose uptake into muscle and fat tissue. Glucagon is a hormone secreted by pancreatic \( \alpha \)-cells and hepatic glucose output is
stimulated by this hormone. Glucagon is released into blood stream in response to low concentrations of glucose and is responsible for increase in glycaemia. It acts at the liver and opposes the effects of insulin by increasing glycogenolysis and gluconeogenesis and also by inhibiting glycogenesis and glycolysis through multiple mechanisms (Jiang and Zhang, 2003). Other hormones also participate in maintain normal glucose levels. These hormones include amylin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tolerant polypeptide (GIP). Amylin is actually secreted with insulin from pancreatic β-cells and functions in decreasing gastric emptying, which limits glucose excursions following a meal (Heptulla, et al., 2005). GLP-1 and GIP are incretins, gut derived hormones, which is secreted by intestinal L cells. They have a multitude of effects, both of which are to promote the synthesis and secretion of insulin from pancreatic β-cells, stimulates glucose uptake in muscle, and reduce glucagon-stimulated hepatic glucose production (Irwin, et al., 2006). Any change in the effect of these hormones leads to the progression of diabetes and its complications.

2.4 Glycogen metabolism

Glycogen is a polymer of glucose stored in the liver and skeletal muscle. It is degraded to yield free glucose when energy is needed for cells and is synthesized again when excess glucose is present in blood stream. Its degradation and release of glucose into the blood leads to elevate glucose level that is available between meals and is good source of energy for sudden need and starvation. The glucose is the only fuel for the brain tissue. Therefore, the role of glycogen metabolism in glucose homeostasis is extremely important (Pfeiffer-Guglielmi, et al., 2007).

Glycogen degradation and synthesis are regulated by two key enzymes, glycogen phosphorylase and glycogen synthase, and these enzyme activities are
controlled by several hormones such as glucagon, insulin and adrenalin. Glycogen degradation consists of three steps: (1) the formation of glucose 1-phosphate from glycogen. Glycogen phosphorylase is activated by phosphorylation, and which catalyzes glycogen to produce glucose 1-phosphate (G1P), (2) the remodeling of the remaining glycogen for further degradation, and (3) conversion of G1P to G 6-Phosphate (G6P) for further metabolism. G1P can be converted to G6P in the presence of enzyme, phosphoglucomutase. G6P can be used (1) as energy sources for anaerobic or aerobic metabolism in muscle and brain, or further broken down to produce pyruvate and lactate in glycolysis, (2) are catalyzed by glucose 6-phosphatase and converted to glucose in the liver by gluconeogenesis, and released into the blood for the use of other tissue, and (3) oxidized in the pentose phosphate pathway. Glycogen synthesis is started with G1P by glycogen synthase catalyze to produce activated intermediate, UDP-glucose. Glycogen synthase is activated by insulin to start synthesis glycogen when blood glucose concentration is too high and it is inactivated by hormones such as glucagon in order to response insulin-induced hypoglycemia. Glycogen metabolism is regulated by reversible phosphorylation of enzymes that adjust glucose level for the needs of the entire organism (Lang, et al., 2002; Toole and Cohen, 2007).

2.5 Pathogenesis and complications

The pathogenesis of diabetes is complex. Usually, destruction of insulin producing pancreatic β-cell by its own immune system is assumed to be the main pathogenic factor in type 1 diabetes (Weiss, et al., 2008), but in type 2 diabetes, it typically begins with insulin resistance at target organs such as liver, muscle and adipose. Increased insulin demand for tissue and progressive impairment of β-cell function which result in insulin resistant are the pathophysiologic defects in progression of hyperglycemia in type 2 diabetes. In order to compensate for this, there is a necessity
of initial increase in insulin production. This hyperinsulinemic state is only temporary and over with time insulin secretion decrease due to progressive pancreatic β-cell deterioration. The combined effects of insulin resistance and pancreatic β-cell dysfunction results in a decreased capacity to limit hepatic glucose production as well as to decrease uptake and utilization of glucose in muscle and adipose tissue.

Insulin resistance is a complex disease that typical feature of the metabolic syndrome and is the result of a number of defects along the insulin signalling cascade (Venieratos, et al., 2010). Other most likely factors include defective incretin activity, elevated concentrations of free fatty acids (Zinman, 2011), inappropriate glucagon production from the α-cells (Mayhew, 2010), activation of NF-kB (Barma, et al., 2009), and tumour necrosis factor-α (TNF-α) and the hormone resistin (Winkler, et al., 2002).

Incretins are gut-derived peptides that responsible for higher insulin release when glucose was taken orally compared to an intravenous glucose load. Impaired release of incretin hormones, especially GLP-1 and impaired action of glucose-dependent insulinstropic polypeptide (GIP) have been observed in patient with type 2 diabetes, and this phenomenon is called incretin deficiency. There are two main incretin hormones, glycogen-like peptide -1 (GLP-1) and glucose-dependent insulinstropic polypeptide (GIP), both of which are released rapidly after meal in order to response elevated postmeal glucose levels. It is believed that GLP-1 control glucose homeostasis through stimulation of insulin secretion, and inhibition of glucagon and gastric emptying. Elevated FFAs produce insulin resistance by inhibiting glucose uptake and its oxidation in skeletal muscle. FFA's also increase hepatic gluconeogenesis and activate NF-kB. It is believed that activated NF-kB cause insulin resistance in skeletal muscle. Both TNF-α and resistin are produced by adipose tissue in greater amounts in obese diabetic individuals. TNF-α impairs insulin action while resistin is known to antagonize the effects of insulin (Winkler, et al., 2002; Barma, et al., 2009).
Increased hepatic glucose production and reduced glucose uptake in type 2 diabetes are attributed to both hepatic insulin resistance and increased glucagon levels (Shiba, et al., 1998). Pancreatic β-cells can compensate for resistance by secreting more insulin. This hyperinsulinemic state is only temporary, as β-cells cannot maintain insulin levels required to maintain euglycemia. This is referred to as the "petering out" effect and occurs due to apoptosis of β-cells. High glucose and FFA's contribute to β-cell malfunction, in a condition called glucolipotoxicity. When insulin resistance can no longer be overcome transition to type 2 diabetes occurs.

Epidemiological study indicates that unregulated glucose control can lead to severe macro and micro vascular complications and it can result in long term damage to various organs and tissues. In fact, the correlation of these complications with glucose levels used to cut-offs for the diagnosis of diabetes has been mentioned above. Diabetes mainly affects the heart, blood vessels, eyes, kidney and nerves. Vascular complications are the fatal complications that lead to morbidity and mortality in patient with diabetes, and the complications in eyes and kidney are a leading cause of blindness and renal failure. Diabetes can cause gestational age in children during pregnancy. In general, micro-vascular complications refer to those affecting small blood vessels in the retina, kidney, and peripheral nerves, and can lead to retinopathy, nephropathy and neuropathy, respectively. Diabetic retinopathy occurs as a result of long-term damage to blood vessels in the retina and can lead to blindness or severe visual loss. Diabetes can also cause the development of cataract through the formation of sorbitol deposits on the lens of the eye. Sorbitol is a product of the polyol pathway formed by the action of aldose reductase, which becomes overexpressed in type 2 diabetes, and is believed to be intimately involved with organ damage. Diabetes is one of the leading causes of kidney failure and 10-20% of diabetics die from this disease. Diabetic nephropathy occurs as a result of increased in urine albumin excretion, and is a second cause of renal
replacement therapy. Diabetic neuropathy refers to a group of diabetes-related nerve disorders which was affected with 50% to 60% people in diabetes. It occurs as a result of damage to the nerves and results in tingling, pain, numbness and weakness in the extremities, which left untreated, can lead to infection, ulceration and possibly amputation. Macro-vascular complications refer to diseases affecting large blood vessels in the heart, brain and peripheral circulation leading to cardiovascular diseases such as atherosclerosis, heart attack and stroke, which are responsible for 50% of deaths of diabetics (World Health Organization, 2011).

It is highly hypothesized that there are some mechanisms by which hyperglycaemia induce glucose-mediated vascular complications. There are: (1) increased polyol pathway; (2) activation of protein kinase C (PKC); (3) increased advanced glycation end-products formation; (4) increased hexosamine pathway (Munusamy and MacMillan-Crow, 2009); (5) increased glucose flux through the aldose reductase pathway; (6) increased production of reactive oxygen species (ROS) (Nishikawa, et al., 2007) and (7) increased in fatty acid flux (Herlein, et al., 2010). A common effect of each mechanism is that they increase the production of superoxide by the mitochondrial electron-transport chain. Superoxide is a reactive oxygen species that leads to oxidative stress and subsequently cause the tissue damage that is observed during diabetes. This suggests that antioxidants, as free-radical scavengers, may be used therapeutically to prevent the development of diabetes associated complications (Munusamy and MacMillan-Crow, 2009).

2.6 Current oral anti-hyperglycemic agents

The current ultimate goal to treat diabetes are aimed at maintaining fasting blood glucose levels between 4.5 and 6.6 mmole, and HbA1c levels at or below 7%. The
control of HbA1c levels at or below 7% has been shown to decrease the risk of developing micro-vascular complications (Ten Brinke, et al., 2008). When proper diet and exercise fail to control hyperglycemia, the use of anti-diabetic agents becomes necessary. A variety of oral hypoglycemic agents are currently available and these can be generally classified as (1) insulin secretagogues, (2) biguanides, (3) insulin sensitizers, (4) α-glucosidase inhibitors, (5) dipeptidyl peptidase-IV (DPP-IV) inhibitors, (6) incretin mimetics, (7) glucose transporter inhibitors or (8) glycogen phosphorylase inhibitors.

2.6.1 The insulin secretagogues

The insulin secretagogues include the sulfonylureas and meglitinides and both stimulate insulin release from the pancreas by a common mechanism. Sulfonylureas and meglitinides stimulate insulin secretion by binding to the sulfonylurea receptor (SUR) of ATP sensitive potassium channel on pancreatic β-cell plasma membrane (Cyrino, et al., 2003). Meglitinides bind to the sulfonylurea receptor, but also bind to an additional site on the pancreatic β-cell to induce insulin secretion by blocking ATP-depended potassium channels. Because they secrete insulin independent of glucose concentration, hypoglycemia is a serious side effect of sulfonylureas and meglitinides (Del Prato and Pulizzi, 2006). Another side effect is their tendency to cause weight gain. This is undesirable especially considering that 80% of diabetics are already overweight. Despite these problems, sulfonylureas are considered a frontline treatment regimen. Meglitinides have similar side effects but they are less pronounced. Some patients do not respond to sulfonylureas while others who have responded may fail to do so after several years. After 10 years of monotherapy with a sulfonylurea, they generally become ineffective and most patients require a second agent to maintain glucose control (Davis, 2008).
All of the sulfonylureas have the same mechanism of action but they differ in pharmacological potency and pharmacokinetics which result in considerable clinical differences among the same classes, therefore, they are further classified as first generation (tolbutamide and chlorpropamide) and second generation (glibenclamide, gliclazide, glipizide, and glimepiride) drugs based on their structural features, pharmacological potency, time of onset, and duration of efficiency (Cyrino, *et al.*, 2003). Repaglinide is one of the meglitinide classes which belong to insulin secretagogues. It is believed to have a rapid onset and a short duration of action in liver, and is differ with sulfonylureas in structure (Van Gaal, *et al.*, 2001).

2.6.2 Biguanides

Biguanides include metformin and phenformin. Their mechanism of action is generally believed that they inhibit hepatic glucose production by activation of AMP-activated protein kinase (AMPK). Both of them may effect on glucose metabolism by increasing glucagon-like peptide-1 (GLP-1) biosynthesis and secretion, thereby decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization in liver. Recently, metformin has been shown to increases AMPK activity in skeletal muscle which leads to increase GLUT-4 protein content (glucose transporter) in the plasma membrane, and resulting in insulin-independent glucose uptake (Svendsen, *et al.*, 2009; Grisouard, *et al.*, 2010).

Biguanides have been shown improves glucose tolerance, lowering both basal and postprandial plasma glucose in patients with type 2 diabetes. Among the biguanides, metformin is frontline treatment option that may be used alone or in combination with other antihyperglycemic agents. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia (Liepinsh, *et al.*, 2011). Aside this, a beneficial side effect is
that it is associated with weight loss, and this makes it preferable to sulfonylureas to treat severely obese diabetics.

2.6.3 Insulin sensitizers

Insulin sensitizers include pharmacological ligands for the peroxisome proliferation activated receptor-gamma (PPAR-γ) such as thiazolidinediones (TZD). Thiazolidinedione drugs enhance insulin sensitivity in adipose tissue, skeletal muscle, liver and in fat by stimulating the nuclear PPAR-γ receptor which control proteins required for glucose and lipid metabolism and activate the glucose transporter gene (GLUT-4) in muscle and adipose tissue. Thiazolidinediones reduce hyperglycemia by improving β-cell function, reduction insulin resistant and released free fatty acid, increasing cellular glucose consumption, glucose uptake, and insulin sensitivity in muscle and adipose tissue. They do not affect insulin levels. It was shown that thiazolidinediones increase pre-adipose differentiation and as a result cause weight gain as a side effect (Smith, 2003; Meriden, 2004). To counteract this, combination with insulin or metformin as an anti-diabetic agent is being considered. Some example of TDZs class of drugs is Pioglitazone and Rosiglitazone.

2.6.4 α-Glucosidase inhibitors

α-Glucosidase inhibitors inhibit the enzymes that responsible for conversion of disaccharides to monosaccharides in intestine. They reduce blood glucose by preventing digestion and absorption of complex carbohydrates (such as starch) because only monosaccharides can be readily absorbed through gut. After a few hours of having meal, blood concentration is high. During this time, insulin is secreted from the pancreatic β-cells and glucose is immediately transferred to the cells for as an energy sources. In type 2 (non-insulin dependent) diabetes mellitus (NIDD), insulin secretion can be normal but
cells are not sensitive to the insulin, as a result, glucose cannot be absorbed properly. One of the strategies for reducing elevated glucose levels in NIDD is to delay rate of digestion of ingested carbohydrates, thereby lowering postprandial blood glucose levels. Previous studies shows that this can be achieved by administering drugs which inhibit the activity of enzymes, such as α-amylase and α-glucosidase that responsible for hydrolyse polysaccharide to glucose and other monosaccharides in the small intestine. Currently available α-glucosidase inhibitors include acarbose, voglibose and miglitol. These drugs are capable of decrease postprandial glucose levels without risk of hypoglycemia and should be taken with food for optimal effect. It has been observed that they have only modest antihyperglycemic activity by themselves. Therefore, these drugs are usually recommended to use in combination therapy. Side effects which are known include flatulence, diarrhea and abdominal pain (Borges de Melo, et al., 2006).

2.6.5 Dipeptidyl peptidase-IV (DPP-IV) inhibitors

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease that exists as both a membrane bound and plasma soluble form. It is expressed in all tissues and some cells with highest levels found in kidney, intestine, liver, pancreas, spleen, synovia, mammary gland and immune cell (e.g. T cell and B cell) leukocytes (Nielsen, 2005). It is a proline specific amino peptidase that responsible for the degradation of a number of biologically active peptides including glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP). GLP-1 and GIP are the hormones that secreted in the intestine after meal and are responsible for insulin release due to increased glucose levels and are rapidly degraded by DPP-IV. To enhance GLP-1 activity, inhibition of this enzyme is useful as a novel therapeutic approach in the treatment of diabetes through an enhancement of the incretin effects (Verspohl, 2009).
DPP-IV inhibitors decrease blood glucose levels by inhibiting the activities of enzyme DPP-IV, thus increase GLP-1’s ability to release insulin in response to elevated concentrations of blood glucose. Several DPP-IV inhibitors which have been reported (Thornberry and Gallwitz, 2009) include Sitagliptin, vildagliptin, saxagliptin and alogliptin. Vildagliptin and sitagliptin have been approved for clinical use in both the United States and Europe, saxagliptin has been approved only in United States, and alogliptin has been approved in Japan. Clinical trials have been proved that these drugs can be used safely with other oral antidiabetic agents such as metformin, sulfonylureas, and thiazolidinediones in patients unable to control blood glucose at desired levels. In previous studies showed that either monotherapy or combinations with other oral agent such as metformin, inhibition of DPP-IV with vildagliptin and sitagliptin were shown to reduce blood glucose and glycosylated haemoglobin (HbA1c) without significant changes on body weight in type 2 diabetes (Kendall, et al., 2009; Campbell, 2011). Studies conducted by (Duttaroy, et al., 2011) demonstrated that pancreatic β-cell mass was increased in animal model treated with vildagliptin, thus result in a rise in insulin release. Administration of sitagliptin has been observed to decrease in glucagon secretion following meals, decrease the rate of carbohydrate digestion, slow down the rate of gastric emptying, reduce food intake, and preserve β-cell function in type 2 diabetes (Zerilli and Pyon, 2007; Gustavson, et al., 2011).

DPP-IV inhibitors have a number of distinct advantages over current hypoglycemic agents. Since they function by enhancing the effects of GLP-1, they are capable of stimulating insulin secretion without causing hypoglycemia. The insulinotropic effect of GLP-1 requires that glucose concentration be at or above normal fasting concentration. Therefore, as glucose concentration falls to the normal range, the insulinotropic effects of GLP-1 diminish. In addition, they have the potential to control weight change and to restore β-cell mass. Hypoglycemic agents that are capable of
restoring β-cell mass are highly desirable. Because of this, the search for novel DPP-IV inhibitors is an active area of research (Gustavson, et al., 2011).

2.6.6 Incretin mimetics

Incretins are hormones that are secreted from intestinal cells into the blood in response to nutrient ingestion. The role of intestine in the regulation of insulin release was based on the observation that the amount of insulin secreted following an oral glucose dose higher than that of an equivalent dose administered intravenously. In diabetic and non-diabetic individuals, plasma insulin levels following an oral glucose dose were threefold higher than that after the same dose administered intravenously (Holst, et al., 2008). This phenomena was termed the "incretin effect" and was subsequently found to be primarily the result of two incretin hormones, GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide) (Verspohl, 2009). The effects of these hormones on glucose homeostasis were shown to activate GLP-1 receptor, which was located throughout the body, as a result, insulin secretion was increased and glucagon release was supressed in both patients with type 1 and type 2 diabetes (Mudaliar and Henry, 2009).

Incretin mimetics are functional analogous of the human incretin Glucagon-Like Peptide-1 (GLP-1) that capable of inhibit degradation by the enzyme dipeptidyl peptidase-IV (DPP-IV). Incretin mimetics reduce blood glucose by stimulating insulin secretion from pancreatic β-cell in a glucose-depended manner, suppress glucagon release, slow gastric emptying, reduce apatite, and enhance pancreatic β-cell function (Barber, et al., 2010). Currently available incretin mimetics include exenatide and liraglutide. The exenatide treatment in patients with type 2 diabetes showed that HbA1c has been reduced about 2% along with improved in glycemic control, and the body weight has been decreased up to 6 kg within three months without cause hypoglycemia
Incretin mimetics are generally used combination with other oral hypoglycemic agent such as metformin and sulfonylureas. These drugs cause weight loss. This side effect is beneficial in obese patients and those patients with other cardiovascular risk factors. Beside this, most common side effect currently reported is nausea and diarrhea (Mayhew, 2010).

2.6.7 Glucose transporter inhibitors

Glucose transporter, which was termed sodium-glucose co-transporter (SGLT), is a membrane protein that has isomers such as SGLT1, SGLT2, SGLT3, and SGLT4. It was well known that SGLT1 absorbs dietary glucose from gut, and SGLT2 responsible for maintain glucose homeostasis by reabsorption of glucose in the kidney. Therefore, it was expected that SGLT2 inhibitors could reduce elevated glucose levels by increasing glucose excretion in urine, thus, they have potential therapeutic value for the treatment of type 1 and type 2 diabetes (Derdau, et al., 2010).

Development of glucose transporters as an antidiabetic agent is relatively novel in pharmaceutical industries. However, there are currently no hypoglycemic agents available for use in this area, but they are attracting more attention of scientists to look for potent compounds that useful for treatment of diabetes. In previous studies, sodium-glucose cotransporter inhibition activity of flavonoids isolated from Sophora species have been reported (Sato, et al., 2007). Most recently, SGLT2 inhibitors synthesised by Sanofi-Aventis have been shown to reduce intestinal glucose absorption, and enhance renal glucose excretion in animal models (Derdau, et al., 2010). This suggests that inhibition of glucose transporter is useful for diabetes.
2.7 Glycogen phosphorylase inhibitors and diabetes

Glycogen phosphorylase (GP) is an enzyme that catalyses the reversible phosphorolytic cleavage of glycogen to produce glucose 1-phosphate which is the first step of glycogen degradation. GP exists in two forms which is phosphorylase-a and phosphorylase-b that can be found in the muscle and in the liver. In the muscle where glucose is produced for as energy source and hepatic glucose production is take place in the liver. Pharmacological activities of this enzyme are regulated by phosphorylation. The phosphorylase-b is unphosphorylated form of GP and is essentially inactive. It is transformed to the more active form (phosphorylase-a) by phosphorylation, and transformation process is controlled by several mechanisms. The most active form (phosphorylase-a) is responsible for hepatic glucose production in the liver and it is an important contributor in diabetic hyperglycemia. Glycogen phosphorylase a form is dephosphorylated by the action of protein phosphatases, whereby glycogen phosphorylase is returned to its less active b form (Hampson, et al., 2006; Bertus, et al., 2008).

Glucose is produced by glycogen degradation in the liver, and glycogen degradation is regulated by glycogen phosphorylase. Glycogen phosphorylase (GP) is an enzyme that catalyses glycogen to produce glucose-1 phosphate (G1P) which is a first step for glycogen metabolism. G1P is then further metabolized to glucose, and secreted into the blood stream for the use of other tissues, especially the central nervous system that relies on glucose as its major energy sources. It is well known that glucose released from glycogen degradation is the main contributor to elevated hepatic glucose output in patient with diabetes, and glycogen degradation is directly associated with regulation of blood glucose levels in the liver. Therefore, inhibition of Glycogen phosphorylase has been thought to reduce hepatic glucose production, and thus decrease blood glucose levels, which is considered potential antidiabetic agent. Some example of
currently available GP inhibitors include Corosolic acid (Yamada, et al., 2008) and Ingliforib (Bennett, et al., 2010). Ingliforib (CP368296) is a potent GPa inhibitor which is in the stage of phase II clinical trials. Corosolic acid (commercially known Glucosol™) is the first drug in the class of glycogen phosphorylase inhibitor that has been released to the market in Japan and United States for reducing blood glucose levels and weight-loss.

These oral hypoglycemic agents are may be used alone or combination with insulin or combination with themselves. Combination therapy is an option when one drug is no longer particularly effective. After 2 to 5 years follow-up studies, monotherapy with either a sulfonylurea or metformin, approximately 50% of patients have HbA\textsubscript{1c} above 7%, and after more than 5 years treatment this number increases to approximately 75% (Ceriello, et al., 2005; Brown, et al., 2010). In this case, a second agent of a different class is usually added to the regimen to restore glycemic control through an additive or synergistic effect. The most common combination is metformin with a sulfonylurea. Other useful combinations include metformin and a TZD, metformin with a meglitinide, or an α-glucosidase inhibitor with either metformin or a sulfonylurea. In the case when two agents are no longer effective a third agent of another class might also be added (i.e. TZD to a combination of metformin and a sulfonylurea). Finally, when oral hypoglycemic therapy has failed to achieve therapeutic goals in type 2 diabetes, subcutaneous insulin injections are necessary to prevent hyperglycemia.

There are the reasons why glycogen phosphorylase inhibitor (GPI) is important in diabetes. (1) GPI has been shown to be more potent at reducing hepatic glucose output in the presence of high glucose concentrations (Martin, et al., 1998); (2) GPI has been shown to significantly attenuate hyperglycemia without producing hypoglycemia (Oikonomakos, et al., 2000); (3) GPI has cardioprotective effects (Cai, et al., 2005); (4)
GPi has been shown to inhibit tumour inducer and has anticancer properties (Schnier, et al., 2003); (5) GPi has antioxidants activities (Guan, et al., 2010). The discovery of the primary role of glycogen phosphorylase in glycogen metabolism led to the suggestion that inhibition of this enzyme may be useful in the treatment of diabetes.

Glycogen phosphorylase inhibitors have variety of distinct advantages compare to other antihyperglycemic agent. It has been reported that potency of compound was significantly increased at higher glucose concentration and it was reduced when glucose concentration falls to the normal range (Ercan-Fang and Nuttall, 1997; Ercan-Fang, et al., 2005). Thus, this property is important for better protection of patients from hypoglycemia. Corosolic acid is a potent glycogen phosphorylase inhibitor used for the treatment of type 2 diabetes. Increased glucose uptake by insulin receptor phosphorylation and decreased the level of oxidative stress have been observed in diabetic rats treated with Corosolic acid (Yamaguchi, et al., 2006; Shi, et al., 2008). Nuclear factor-kB is a family of transcription factors, believed to be involved in pathogenesis of several inflammatory diseases including tumour, cancer and insulin resistance. Inhibition of GP activity in patient with type 2 diabetes was shown to significantly inhibit NF-kB overactivation and reduces insulin resistance (Barma, et al., 2009). Combined, these studies seem to validate the notion that inhibition of glycogen phosphorylase is a viable way of indirectly enhancing effective management of diabetes and its complications.

2.8 Antioxidants in diabetes

Oxidative stress has been suggested to be critically involved in the pathogenesis and progression of diabetic tissue damage. It has been shown that hyperglycemia is correlated with an increased production of the free radical superoxide and reactive nitrogen species (RNS). Overproduction of superoxide in the organ systems is
associated with nitric oxide which is a potent oxidant that causes nitrosative stress in the organ systems. It is an important feature of diabetic complications that can be found in both patients with type 1 and type 2 diabetes (Cai, et al., 2005). Pancreatic β-Cells are exceptionally vulnerable to the toxic effects of free radicals because the pancreas is the organ that has the lowest levels of antioxidant enzymes and these levels are further diminished in diabetes. It has been reported that most potent oral antidiabetic agent metformin have ability to reduce cardiovascular complications by significantly reduction of reactive oxygen species (ROS) (Hou, et al., 2010), and another novel antidiabetic agent repaglinide has been shown to prevent inflammation by significantly diminishing protein oxidation in diabetic rabbits (Gumieniczek, et al., 2005). Inflammation is the earliest and most common diabetic complications that caused by oxidative stress. Metformin has its origin in the plant and it is a potent enhancer for pancreatic β-cell function. Antioxidant mediated preservation of pancreatic β-cell function have been considered to effect on diabetes by slowing the progression of the disease. Plants including Sophora species often contain considerable quantities of antioxidants such as alkaloids, α-tocopherols (vitamin E), carotenoids, ascorbic acid (vitamin C), and polyphenols such as flavonoids and tannins.

2.9 Traditional medicine

In many parts of the world plants are still the main source of treatment for disease and the WHO estimates that 80% of the population in the developing countries depend on traditional medicine for their primary health care needs (Mukherjee and Wahile, 2006). The use of plants in the treatment of diabetes has a long and rich history. Herbal medicines have been in use for thousands of years before modern medicine began, and continued to provide mankind with novel remedies.
Traditional medicine systems developed through experience and experimentation. Knowledge of this was most likely obtained by using a variety of plants to treat a particular disease and observe their effects. Plants that had a positive effect in treating the disease were recorded. In the case of diabetes, many plants have been used to help control blood sugar levels. In recent times, the hypoglycemic activity of some of these herbs has been demonstrated in various animal models of diabetes and in some instances the active principles have even been isolated (Shokeen, et al., 2008; Daisy, et al., 2009). But, the majority of these herbs still in use today have far escaped scientific scrutiny and neither their mode of action nor the active principles are known.

The modern prevalence of diabetes has clearly led to a need for new drugs. Plants used traditionally for treatment of diabetes, particularly those that have been proven to reduce blood sugar, can potentially lead to the isolation of novel molecules with significant hypoglycemic activity. Because of a number of factors, such an approach can have some advantages over the conventional approach to drug discovery. This approach is referred to as ethnopharmacology and utilizes the information learned from various systems of traditional medicine in the search for new drugs. Since humans have used these plants for generations, it can be expected that bioactive compounds isolated would have low toxicity, though this is not always the case. Also, there is a tremendous degree of chemical diversity in a plant extract. This includes alkaloids, glycosides, saponins, polysaccharides, flavonoids, steroids, carbohydrates, terpenoids, amino acids and tannins. Such diversity can lead to interesting molecules that may be useful drug entities themselves or more likely serve as lead molecules in a medicinal chemistry program.

Interestingly, Glucosol™, potent glycogen phosphorylase-a inhibitor has its origins in many plants such as Lagerstroemia speciosa L, which was used to treat
diabetes in the USA and Japan. Its use as an antidiabetic agent was as a direct result of the isolation of corosolic acid as an active antihyperglycemic agent (Judy, et al., 2003).

2.10 Studied plant - *Sophora alopecuroides*

*Sophora alopecuroides* belong to the Leguminosae family and is a shrub with bead-shaped fruits. Its fruit is 3 to 7 cm long and contains oval-shaped yellow seed. It is locally called buya and foxtail-like Sophora in English. Plant is mostly originated in Taklimakan region which is central part of Asia and can be found some parts of the Europe. Different parts of the plant are used for throughout the world traditionally for a number of diseases. In Korea, roots are used for treatment of hair loss and fiver (Roh, et al., 2002). In china, roots and leaves are used for the treatment of viral hepatitis, cancer and cardiac diseases, and seeds are used for tumour and diarrhea (Li, et al., 2011). In Japan, the seeds are used for weight loss (Park, et al., 2009). The seeds are known to be rich source of alkaloids (Xiu, et al., 2010) and flavonoids (Guo, et al., 2011). In Uyghur traditional medicine, the seeds are prescribed for diabetes.

It is generally the case that herbal drugs operate by a number of mechanisms to elicit their effects. Indeed, the root of *Sophora* has already been shown to inhibit α - glucosidase enzyme which is responsible for carbohydrate digestion (Kim, et al., 2006). It was known that α-glucosidase inhibitors reduce blood glucose by preventing digestion and absorption of complex carbohydrates in intestine. Most recently, it has been reported that sophocarpine, which is an alkaloids, isolated from Sophora alopecuroides preserves myocardial function in rats by inactivation of nuclear factor-kappaB (NF-kB) (Li, et al., 2011). NF-kB activation is known to be associated with insulin resistance in type 2 diabetes and other non-diabetic disorders such as cancer and cardiovascular diseases (Barma, et al., 2009). Other evidence is that methanol extract
from *Sophora* roots has been shown to inhibit sodium-glucose cotransporter (SGLT) activity. SGLT was known to maintain glucose homeostasis by absorption of glucose in the kidney as a result increase glucose excretion in urine (Sato, *et al*., 2007).

Aside from the α-glucosidase and SGLT inhibitory effects, combined administration of high-fat diet with powdered fruit of some *Sophora species* significantly decreased body weight in non-diabetic mice, and it also improved serum lipid profiles that often result in cardiovascular complications. In diabetes, increased lipolysis results in increased levels of triglyceride and cholesterol. *Sophora species* exhibited lowering triglyceride and cholesterol effects while at the same time increasing HDL cholesterol (Park, *et al*., 2009).

Oxidative stress has been known to be involved in the pathogenesis of diabetic cardiomyopathy which is a leading cause for mortality in diabetes. Evidence from recent studies showed that the pathological changes in the heart caused by oxidative stress result in increased myocardial cell death, and increased generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Cai, *et al*., 2005). It has been shown that alkaloids and flavonoids isolated from *Sophora species* decrease oxidative stress by increasing levels of the free radical scavenger (Tai, *et al*., 2011). Antioxidants interfere with activation of free radicals and resulting in protecting the human body from free radicals that may cause some chronic diseases such as diabetes. (Jang, *et al*., 2010). Most plants contain alkaloids, flavonoids, and polyphenols which are the major antioxidants that have substantial effects on the prevention of cellular damage caused by oxidative stress (Cotelle, 2001; Zhao, *et al*., 2006).

Obesity is one of the common pathogens in patients with type 2 diabetes. As mentioned earlier (Park, *et al*., 2009), *Sophora* fruit powder significantly reduced fat mass in non-diabetic obese mice. This evidence suggests that active compounds in this
species may be useful for controlling obese-related metabolic disease such as diabetes and its complications.

**STATEMENT OF THE PROBLEM AND AIM OF THE STUDY**

Type 2 diabetes has become a worldwide epidemic which is predicted to become even worse. Currently there is no cure and until recently the available pharmacological agents were only able to control the hyperglycemia for some period of time. Traditionally, medicinal plants have been used throughout the world to treat diabetes. The literature is replete with research attempting to show the beneficial properties of the medicinal plant in the control of hyperglycemia through inhibition of α-glucosidase (Kim, *et al.*, 2006), increase of glucose uptake, enhancement of insulin secretion, inhibition of DPP-IV (Thornberry and Gallwitz, 2009), and inhibition of glycogen phosphorylase (Yamada, *et al.*, 2008). However, to the best of our knowledge, no one has studies the effects of *Sophora alopecuroides* seed on inhibition of glycogen phosphorylase enzyme. It is the purpose of this study to determine extracts of *Sophora alopecuroides* seed for inhibition of glycogen phosphorylase-a enzyme to identify novel chemical compounds, which may serve as leading molecule. In addition, my goal is to gain a clear understanding as to the mode of action of antihyperglycemic herb *Sophora alopecuroides* seed traditionally used for diabetes.