PRELIMINARY STUDY: EFFECT OF 95% ETHANOL EXTRACT OF *SENNA AURICULATA* FLOWER ON STREPTOZOTOCIN- INDUCED DIABETES RATS.

by

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CERTIFICATE

This is to certify that the dissertation entitled "PRELIMINARY STUDY: EFFECT OF 95% ETHANOL EXTRACT OF *SENNA AURICULATA* FLOWER ON STREPTOZOTOCIN- INDUCED DIABETES RATS " is the bonafide record of research work done by Ms Niswathul Haania Binti Zain Ali during the period from July 2009 to October 2009 under my supervision.

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LIST OF SYMBOL AND ABBREVIATION

α	= aplha
β	=beta
μ	= micro
°C	= degree Celsius
®	=Registered trade mark
>	= more than
<	= less than
=	= equals to
%	=percentage
ADP	=adenosine diphosphate
CAT	=catalase
CFEt	=aqueous extract of *Cassia auriculata* L. flower
CMC	=carboxyl methyl cellulose
CoA	=coenzyme A
DM	=diabetes mellitus
DNA	=deoxynuclotide acid
GCMS	=gas chromatography mass spectrometry
GI	=gastrointestinal
GLUT2	=glucose transporter
GPx	=glutathione peroxidase
g	=gram
GST	=glutathione S -transferase

GSH =reduced glutathione

H&E =Haematoxylin and Eosin

IP =intraperitoneal

kg =kilogram

L = liter

mL = mililitre

mg =milligram

mg/kg = milligram per kilogram

mmol/L =milimol per liter

NIDDM =non- insulin dependent diabetes mellitus

SA = *Senna auriculata*

SD =standard deviation

STZ = Streptozotocin

SOD =superoxide dismutase

WHO =World Health Organization

ABSTRAK

Diabetis mellitus ialah penyakit yang sering dikaitkan dengan gaya hidup yang mana penyakit ini terdapat di kalangan orang golongan kelas atasan kerana pengambilan makanan yang tidak seimbang dan oleh kerana itu diabetis mellitus digelar penyakit orang kaya, di mana penyakit ini memberi impak maksima ke atas kadar kematian global. Data-data yang diterbitkan pada jurnal menunjukkan bahawa ekstrak air dan ekstrak etanol *Senna auriculata* (SA) mempunyai kesan anti-hiperglysemik yang kuat. Ia dikatakan dapat mengurangkan kandungan glukosa dalam tikus aruhan diabetis terinduksi oleh streptozotosin (STZ). Objektif utama kajian ini adalah untuk menguji keberkesanan ekstrak etanol 95 % *Senna auriculata* ke atas tikus jantan aruhan diabetis melalui induksi STZ. Di dalam kajian ini, tikus jantan jenis Sprague Dawley berjisim badan 150-200 gram telah dipilih dan diagih secara rawak kepada 6 kumpulan, dengan 3 kumpulan kawalan dan 3 kumpulan ujian iaitu diberi 50, 100 dan 200 mg/kg *Senna auriculata.* Jisim badan, aras glukosa darah berpuasa dan perubahan fizikal diperhati sepanjang tempoh kajian. Pada akhir tempoh kajian, aras glukosa darah berpuasa diukur sebelum tikus dibunuh untuk mengambil pankreas untuk melakukan pemeriksaan histologi. Aras glukosa darah berpuasa didapati tidak berbeza secara signifikan ($p>0.05$) berbandingkan statistik keseluruhan dibuat untuk tempoh 25 hari selepas dirawat dengan ekstrak tersebut. Didapati ekstrak etanol 95 % bunga *Senna auriculata* tidak mempunyai kesan anti-hiperglisemik pada tikus jantan aruhan diabetis melalui induksi STZ.

ABSTRACT

Diabetes mellitus is a disease of lifestyle which was observed to be more common in the upper classes where they consume more unhealthy food and for this reason diabetes mellitus is called rich man's disease and it has a vast impact on global burden of mortality. Data published in journal showed that both of the aqueous and ethanol extract of *Senna auriculata* (SA), have strong anti-hyperglycemic effect in the short term to reduce blood glucose level in STZ- induced diabetic rats. The main objective of this study is to evaluate the effect of 95 % ethanol extract of *Senna auriculata* prepared via rotary evaporation method on streptozotocin (STZ) - induced diabetic male rats. In this study, Sprague Dawley male rats of body weighed 130-180 grams were selected and randomly assigned to 6 groups. The diabetic negative control rats were given orally carboxyl methyl cellulose (CMC) 2% as vehicle and positive control rats were given 15mg/kg metformin also orally. While the rats in treatment group were given 95 % ethanol extract of *Senna auriculata* via oral route at doses 50,100 and 200 mg/kg daily respectively for 25 days. The rats body weight, fasting blood glucose and physical changes were observed throughout the study period. At the end of the study period the blood glucose level were measured before the rats sacrificed after anesthetized. The rats' pancreas was taken for histological examination. The fasting blood glucose were not significantly different ($p>0.005$) in SA treated diabetic group compared to diabetic control group after treatment for 25 days with 95 % ethanol extract of SA. Based on the data produced it shows that 95 % ethanol extract of *Senna auriculata* flower have no anti-hyperglycemic effect on STZ- induced diabetic male rats.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Diabetes mellitus is a chronic disorder inherited or acquired and is characterized by inability of pancreatic cell to efficiently produce insulin. Based on the statistic been published by American Diabetes Association in 2004, the number of patient with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. For all age-groups diabetes prevalence worldwide is estimated to be 2.8% in year 2000 and will rise to 4.4% in the year 2030, while the total number of people suffering from diabetes is estimated as 171 million in 2000 and is expected to rise to 366 million in 2030. Men are more prone to diabetic than women but statistically there are more women with diabetic than men are. The urban population is projected to be doubled between years 2000 to 2030 in developing countries. Diabetes prevalence across the world appears to increase in the proportion of people aged more than 65 years old (Wild *et al.*, 2004).

Prevalence of diabetes in Malaysia is projected to increase with the number of population to have type II diabetes is estimated to be 10,671 in 1995 and will rise to 21,629 in 2025 (King *et al.*, 1998) which is a very large figure. Statistic record from the Ministry of Health Malaysia showed that the number of admissions to government hospitals in Peninsular Malaysia for diabetes cases had increased from 19,629 cases in 1991 to 30,661 cases in 2001, while mortality rates due to diabetes has also increased

from 254 deaths in 1991 to 380 deaths in 2001. These increases of 50 % show substantial evidence that diabetes mellitus is epidemic in Malaysia (Shafie *et al.*, 2004).

Many oral hypoglycemic agents are available but still there is no cure for diabetes as its complications are severe, furthermore side effects of the therapeutic agents increases mortality rate (Jarald *et al.*, 2008). Following are some of the side effects of currently available oral hypoglycemic agents where biguanides are found to cause lactic acidosis and gastrointestinal (GI) disturbances, sulphonylureas are associated with hepatotoxicity, hypoglycemia, weight gain and GI disturbances., meglitinides cause hypoglycemia at high doses and thiazolidinediones, the most widely used antidiabetic drugs, have been reported to cause hepatotoxicity, weight gain, fluid retention and congestive heart failure (Palsamy and Malathi, 2007). Due to this problem many patient prefer alternative medications by consuming herbal extract because of their effectiveness, less side effects and relatively low cost. The alternative medications are taken alone or together with oral hypoglycemic agents recommended by the physician. Some of the drugs available have been reported to be derived from plant, which has been a reservoir for the potential source of anti-diabetic agent property (Rema Razdan *et al.*, 2008).

Information from the ethno-botanical source stated that about 800 plants may possess anti-diabetic potential (Aguilara *et al.*, 1998). Several anti-diabetic potential posing traditional plants have been assessed by experimental models of diabetes. One of the plants that have anti-diabetic potential is the flower of the plant *Senna auriculata*

(pokok brendi) also known as *Cassia auriculata* L. Researches have been conducted to prove the anti-diabetic potential of *Senna auriculata* (SA). Research done by (Pari and Latha, 2002b) demonstrated suppression of elevated glucose and lipid levels in diabetic rats. There was another research done by Pari and Latha in year 2002. In their study via oral administration of the aqueous extract of *Cassia auriculata* L. flower (CFEt) for 30 days showed positive result, significantly reducing blood glucose level and an increase in plasma insulin. The extract also decreased free radical formation in tissues they have studied. They also have produced data on CFEt was more effective than glibenclamide due to the decrease in lipid peroxides and increase in reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S -transferase (GST) clearly showed the antioxidant properties of CFEt which was better than glibenclamide (Pari and Latha, 2002a).

In year 2003, Pari and Latha again have done research on the effect of *Cassia auriculata* flower extract (CFEt) on hepatic glycolytic and gluconeogenic enzymes. They have shown that aqueous extract possesses an antihyperglycemic effect and suggest that enhanced gluconoegenesis during diabetes was shifted towards normal and the extract also enhances glucose utilization through increased glycolysis (Pari and Latha, 2003). Investigation conducted by Pari and co-researcher (2007), where their research proved that the increase insulin binding activity in the rats when treated with *Cassia auriculata* flower extracts. The mechanism of *Cassia auriculata* flower extracts improved specific binding ability of insulin to erythrocyte membrane (Pari *et al.*, 2007).

Another study done by Hakkim and co-reasearcher (2007) showed antidiabetic potential of aqueous and ethanol extract of *Cassia auriculata* L. flowers. The study was assessed in alloxan-induced diabetic rats, oral administration of water-soluble fraction of the ethanol extract for 30 days exhibited a significant reduction in the blood glucose level and a marked increase in plasma insulin level compared to the aqueous extract-treated rats and diabetic control. In the laboratory study done by other researcher using alloxan-induced diabetic rats, the ethanol extract of *Cassia auriculata* showed significant antidiabetic activity by reducing serum glucose levels in diabetic rats (Hatapakki *et al.*, 2005).

This research project was carried out for about 25 days where the 95 % ethanol extract of SA flower were given through oral route and changes in fasting blood glucose level and physical changes were observed throughout the study period.

1.2 Purpose and Objective of Research

1.2.1 Purpose of the Research

The main purpose of this study is to provide evidence on the efficacy of 95 % ethanol extract of the SA flower in treating streptozotocin (STZ) - induced diabetes rats.

1.2.2 Objective of the Research

1. To identify the active ingredient of 95% ethanol extract of SA flower by screening using gas chromatography mass spectrometry (GCMS) analysis.
2. The main aim is to demonstrate the effects of 95% ethanol extract SA flowers on blood glucose level in experimental diabetes male Sprague Dawley rats.

1.3 Scope and Limit of the Research

This research includes the standard preparation the of 95% ethanol extract of SA flower. The preparation of 95% ethanol extract of SA flower is prepared via standard technique for the purpose of analysis through GCMS. STZ –induced diabetes rat model is used as a substitute for human subject. Physiological changes in the STZ -induced diabetes rat models were observed throughout the study period. Histopathology technique was applied in examining the microscopic structure of pancreatic tissue, stained by Haematoxylin and Eosin (H&E) staining method.

1.4 Research Hypothesis

Different doses of the 95% ethanol extract of SA flower; 50, 100 and 200mg/kg given orally to STZ –induced diabetes rats produce different effects.

1.5 Benefits of the Study

This research is done to provide evidence that SA flower can be used as complimentary medicine and will promote commercial value of plant to treat diabetes. The patient suffering from diabetes especially will benefit if this study is successful as this plant is useful to treat and control diabetes. Those populations at risk of developing diabetes can take this plant extract as a supplement to maintain blood glucose at normal level. The probability of the success of this study depends on the efficacy of the treatment which is determined by significance of this study. This study is expected to have significances as it could be used as alternative treatment for this dreadful disease as there is no total recovery by using synthetic therapeutic agent have been reported so far .

The hypoglycemic agents are used to control and treat the complication from this disease. By promoting the plant as a new product to treat diabetes, synthetic therapeutic agent and imported oral hypoglycemic agent can be reduced as it has many side effects and increases the mortality rate.

The experimental techniques applied are expected to be a guideline for researcher and scientist to study other plants to treat diabetes. Knowledge and information gained should be applied in modern medicine to produce more acceptable form of medication to treat this disease.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Senna auriculata*

2.1.1 Taxonomy of *Senna auriculata*

Senna auriculata (SA) from the Family-Leguminosae is a fast growing branched tall, evergreen shrub with reddish brown branches (Sabu and Subburaju, 2002). The scientific name of this plant is *Senna auriculata* and also known as *Cassia auriculata* Linn. Its common name in English is Tanner's Cassia, in Malay it is known as (Pokok Brendi), Avartaki (Sanskrit), Awal and Tarwar (Hindi), Tarod and Tarwad (Marathi), Avaraim and Avirai (Tamil), Avaram (Malayalam), Avarikke and Tangedi (Kannada), Tagedu (Telugu), Awala (Gujarati) and Peik-thingat (Myanmar).

Two names have been ascribed for this flower *Senna auriculata* and *Cassia auriculata* Linn. Both the names have been used in the researches done before. Since both names have been used interchangeably, this research uses the name *Senna auriculata.*

SA is branched shrub with smooth cinnamon brown dark and closely pubescent branchlets. Its leaves are alternate, stipulate, compound paripinnate with glands opposite to leaflets, numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval

oblong, obtuse, at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules are very large, reniform- rotund, produced at the base on side next petiole into a filiform point and persistent.

Its flowers are irregular, bisexual, golden yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, and erect and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The five sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also five are free, imbricate, and crisped along the margin, bright yellow veined with orange. The anthers 10 in number and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules. The fruit is a short legume, 7.5-11 cm long, 1.5 cm broad, oblong, obtuse, tipped with long style base, flat, thin, papery, undulate crimpled, pilose, pale brown. 12-20 seeds per fruit are carried each in its separate cavity.

From the geographical aspect, SA can be found at the roadside as this plant is used for landscaping roadways and home gardens. SA can tolerate drought and dry conditions, but not much cold. This plant is available in Asian country and worldwide known with different names depending on the location and languages used. There are hundreds of species of *Cassia* which occurs with more than 1000 names. 350 species have been ascribed in the genus with 80% of it occurring on the American continent, while 20% of the species remained as the member of the county tropical Africa,

Madagascar and Australia, a few species recedes in the southeastern Asia and some on the Pacific Islands (Irwin and Barneby, 1985, Randell and Barlow, 1998), while no native species are found in Europe though Europeans have used several species in traditional medication (Colladon, 1816). Some important species are *Cassia fistula, Cassia grandis, Cassia hirsutica, Cassia sieberiena, Cassia alata , Cassia tora, Cassia occidentalis, Cassia auriculata* and *Cassia Nigricans* (Ayo et al., 2007).

2.1.2 Chemical composition of *Senna auriculata*

Extensive studies carried out on chemical composition of SA showed that several laboratories involved in it. One of the studies is done by the Department of Pharmacy, University of Regensburg. These scientific studies done on several species of plant *Senna* have reported this plant to contain alkaloids, sitosterols, anthraquinone, glycosides, tannins and flavonoids.

There was a study done by Mazumder *et al.*, (2008), discovering the phytochemical and pharmacological activity in the diverse species of *Cassia* in that genus which is distributed in various part of world. The research aspect includes all part of plants in determination of the hypoglycemic, antioxidant and free radical properties. Their study discovered substances like anthraquinones, aloe, emodin and sisterols.

A laboratory study to isolate the bioactive constituents from the activity guided fraction was done by Hatapakki and co-researcher (2005). The active ethanol extract was subjected to qualitative chemical analysis to identify the active phytoconstituents. The

sterol, β sitosterol, was isolated from the ethanol extract by column chromatography. The ethanol extract showed the presence of sterols, triterpenoids, flavonoids and tannins.

In the study on antidiabetic potential of aqueous and ethanol extract of *Cassia auriculata* L. flowers was assessed in alloxan-induced diabetic rats where the phytochemical screening and antioxidant activity were made in these extracts. Antidiabetic agents (flavonoids and phenolic acids) and free radical scavenging activity in water-soluble fraction of the ethanol extract was higher compared to that of aqueous extract (Hakkim *et al.*, 2007).

Research done on laxative activity of *Cassia auriculata* L. flowers revealed another substance, anthracene derivatives which is responsible for laxative activity (Ganapaty and Vidyadhar, 2005).

2.1.3 Uses of *Senna auriculata* as Medicinal plant

A numbers of modern drugs have been isolated from natural sources and many of these isolations were based on the uses of the agents in traditional medicine. Advancement of the modern drug and therapeutic agents and the wide acceptance of them by public have great impact on tradition medication. Thereby traditional medicine has receded in occidental societies thus only over a very limited number of these medicinal plant has been scientifically studied and reviewed in detail. World Health Organization (WHO) should recognize this field of study and comprehensive investigation could be done.

Various species of Cassia are reported to have laxative, purgative, antidiabetic, anti-inflammatory, antimicrobial, antifungal, hepatoprotective, antipyretic, antineoplastic, antimalarial, antiasthmatic, antiviral and wound healing properties (Mazumder *et al.*, 2008). Further reported that in the Ayurvedic system of medicine these plants were also used for the treatment of fever and headache. In treating a wide range of ailments worldwide, plants in the *Cassia* species have been used, probably due to the synergistic action of its metabolites which the ingredient responsible for the beneficial effect of the plant.

The methanolic extract of *Cassia auriculata* L. flowers was assessed in rats for laxative activity suggested laxative property of the flower as indicated by increased fecal output in the rats tested (Ganapaty and Vidyadhar, 2005).

Other parts of plant also possesses medicinal property, where the bark is astringent, leaves and fruits suggested antihelmintic property, the seeds have been used to treat eye trouble and root employed in skin disease. It has been used for the treatment of ulcers, leprosy and liver disease (Kumar *et al.*, 2002). The dried flower bud powder is used as a substitute for tea in the case of diabetic patients and also supposed to improve the complexion in women (Siva and Krishnamurthy, 2005).

2.1.4 The Side Effects of *Senna auriculata*

Based on the current literature search done there was no claim of side effects of the SA were found.

2.2 Diabetes Mellitus

Diabetes mellitus is a chronic disorder of blood sugar metabolism mainly glucose, where it is not broken down to it metabolites in the blood stream. Glucose is necessary for cell nutrition and function. Type I diabetes also known as insulin dependent diabetes and juvenile diabetes is caused by body's inability to produce insulin due to autoimmune destruction of insulin producing beta cells of the pancreas. Destruction of the beta cells are permanent thus lacking of cells is unable to produce sufficient insulin to meet body's requirement, thus elevating the blood glucose level. In a healthy person the fasting blood glucose is 3.9-6.7 mmol/L whereas individual with type I diabetes may have elevated level about 11-12 mmol/L and this excess glucose reaches the renal threshold and cause glycosuria or glucose in the urine which cause frequent urination in patient.

Type I diabetes is lethal without exogenous insulin treatment, via injection to compensate the dysfunctional beta cell that could not produce sufficient insulin. Currently no preventive measures are available for type I diabetes and some developments are in progress and still investigated. Diet and exercise have always been helpful to control the disease. Laboratory test can be used to distinguish type I diabetes with type II diabetes via several assays, one of it is C-peptide assay that measure the endogenous insulin production while the external insulin has not indeed the C-peptide.

Type II diabetes account for 90 % of the diabetes cases (Rema Razdan *et al.*, 2008). The etiology of type II diabetes was found to be the development of insulin

resistance and the primary cause is the insulin secretion defect. Hyperglycemia results from both the loss of surviving pancreatic cell and its functional defect which causes reduced production of postprandial insulin and insulin sensitization declines (Taylor *et al.*, 1994, Polonsky *et al.*, 1998). Formerly diabetes mellitus is known as non- insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes with the characteristic features of high blood glucose due to the relative insulin deficiency and resistance. Obesity, diet, sedentary lifestyle, hereditary factor and environmental as well as external factor contribute to diabetes development.

Type II diabetes' prevalence have been dramatically rising and estimated to reach 370 million in year 2030, where this number reflect the total population world wide to be suffering from diabetes mellitus (Smyth and Heron, 2006). The available hypoglycemic and therapeutic agents used in treatment in the previous years have failed to reach the therapeutic goals. Therefore the urgency to develop new therapeutic strategies to treat this disease has become a priority in order to overcome the defect in the existing medication.

The insulin treatment and oral antidiabetic agent tends to have prominent side effect and unable to alter the complication from diabetes. Among the available oral hypoglycemic agent, one drug that has superior postprandial antihyperlycemic effect due to their potent insulinotropic action is sulfonylurea, used by diabetic patients and only a few primary non-responders are reported (Cavaghan *et al.*, 2000, Holman, 2006). One of

the undesirable side effects is hypoglycemia due to potent and persistent stimulation of insulin secretion even when the blood glucose is low (Stahl and Berger, 1999).

Gestational diabetes occurs due to glucose intolerant during early period of pregnancy. It is associated with hereditary factor to genetically transmit type I and type II diabetes. Even though, gestational diabetes is lost after child birth, the women are at high risk of developing diabetes during the menopausal age.

2.2.1 Pathophysiology of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic disorder characterized by hyperglycemia, a condition of high blood glucose concentration due to insulin deficiency which is often combined with insulin resistance. Fasting plasma glucose concentration > 7.0 mmol/L or postprandial (2 hours after meal) plasma glucose concentration > 11.0 mmol/L. Hyperglycemia results from uncontrolled glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis, thus when the renal threshold for glucose reabsoprtion is exceeded, glucose spills over into urine (glycosuria) and cause osmotic dieresis (polyuria). Polyuria causes dehydration which results in thirst and increased drinking (polydipsia). As a consequence of insulin deficiency, wasting of glucose occurs through increased breakdown and reduced synthesis of protein. Diabetic ketoacidosis develops in the absence of insulin due to accelerated glucose breakdown of fat to acetyl- CoA, which in the absence of aerobic carbohydrate metabolism is converted to acetoacetate and β-hydroxybutyrate, causing acidosis and acetone (a ketone) results.

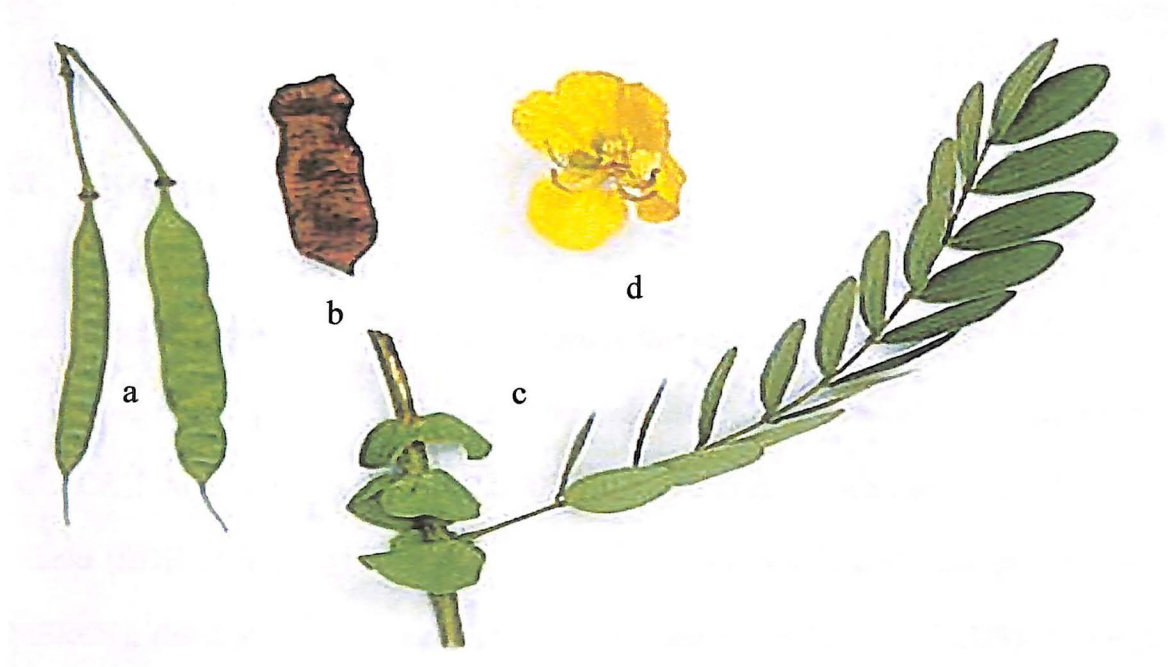
Various complications of diabetes develop due to metabolic derangement of diabetes. Mostly disease of blood vessels, either large (macrovascular disease) or small (microangiopathy) and vascular endothelium dysfunctions leading to development of vascular complications. Accelerated atheroma and its thrombotic complications results as macrovascular disease, while microvascular disease particularly affects retina, kidney and peripheral nerves. DM is the commonest cause of chronic renal failure, which itself is a huge and rapidly increasing problem, the cost of which to society as well as to individual patients are staggering. Contemporaneous hypertension promotes progressive renal damage and treatment of hyper tension slows the progression of diabetic nephropathy and reduces myocardial infarction. Angiotensin converting enzyme inhibitors is effective in preventing diabetic nephropathy than other antihypertensive drugs, perhaps by preventing the proliferative actions of angiotensin II and aldosterone. Neuropathy due to diabetes is associated with accumulation of numerically active metabolites of glucose, produced by the action of aldose reductase but aldose reductase inhibitors have been disappointing as therapeutic drugs (Rang *et al.*, 1999).

2.2.2 Rat Animal Model of Diabetes

Currently there is no animal model to duplicate the human diabetic but syndromes that resemble human diabetes can occur in some animal species. There are a few models to induce diabetes in the rats, which includes treating animal with drugs or viruses, total pancreatomy of the rat (excising their pancreases) and manipulating their diet and each model has advantages and disadvantages. Spontaneous animal model of both type I and type II diabetes also exist (Yang and Santamaria, 2006, Rees and

Alcolado, 2005, Chen and Wang, 2005). The most commonly used is chemically induced animal model with alloxan or streptozocin (STZ).

In this study STZ- induction were used, due to its availability and lesser side effects on the study animal. STZ is an antibiotic that is produced by *Streptomyces achromogenes*. STZ induction produces diabetes that resembles type I and type II diabetes (Matteucci and Giampietro, 2008). STZ action is by destruction of the beta cell of Islets of Langerhans and declines the insulin production. One of the disadvantages is that the STZ- induced diabetes model does not resemble the diabetic conditions in human but it is believed to illustrate various types of etiological and pathogenic mechanisms which might resemble humans (Porta *et al.*, 2007). The mechanism of STZ action is on the β cells of islet cell of Langerhans where the route of STZ entrance is via a glucose transporter (GLUT2) and alkylation of DNA which results in damage. The damage induces activation of poly ADP- ribosylation, which is a process important for diabetogenicity of STZ than to cause DNA damage itself. STZ action on the β cells results in necrosis.



Remark

a. Fruit of the flower (legume)

b. Dried fruit of the flower

c. Stalk and leaf

d. *Senna auriculata* flower

Picture 2.1: *Senna auriculata* plant parts differentiated.

CHAPTER 3

MATERIAL AND METHOD

3.1 Material

3.1.1 Chemicals

The following chemical has been used in this research project:

95% ethanol pro analysis (MERCK), normal saline, formaldehyde min. 37-41 % (MERCK), streptozotocin (STZ) (SD130-1G) from Sigma Aldrich Co.USA, Eosin sprit soluble (BDH stains), xylene pro analysis (MERCK), absolute alcohol pro analysis (MERCK), distilled water, tissue embedding medium (PARAPLAST PLUS), carboxyl methyl cellulose (medium viscosity), DPX (BDH stains), acid alcohol, hexane (MERCK), methanol pro analysis (MERCK), ammonium solution 25 % (MERCK) and microscopy Harris Haematoxylin (BDH stains).

3.1.2 Streptozotocin (STZ)

Streptozotocin was obtained from Sigma Chemical Co. (USA) from the local supplier. It is of 98 % purity based on HPLC analysis and was packed in 1 gram vial. The powder was yellow in color.

3.1.3 *Senna auriculata* (SA)

SA flower were collected from Penang. The plant was indentified and authenticated by Prof.Syed Mohsin Sahil Jamalullail.

3.1.4 Equipments

The equipment used in this research projects are measuring cylinder, beaker, pipette, gavage, glass filter funnel, aluminium foil, spatula, round bottom flask, conical flask, specimen jar, universal bottle, Schott bottle, glass slide, glass cover slip, microcentrifuge tubes, homogenizer, dissecting set, parafilm, filter paper, syringe, needle 22G & 26G, mould, tissue cassette, tissue embedding ring, disposable gloves, petri plates, Easy Mate® glucometer, Easy Mate® I glucose strips, low profile microtome blade, Tissue Processor (Model: Leica), light microscope (Olympus: BX 41), Image Analysis System (Model: BX41), incubator (Binder BLY 15), water bath (Memmert), rotary evaporator (Heidolph- Rotavac), electrical grinder (Panasonic), fridge (National NR-B53FE), electronic weighing scale (METTLER TOLEDO), digital camera (Sony) vortex mixer (EVM 6000,ERLA), Microtome (Model Microm: HM 325), Hotplate (Model: Leica H11220), Tissue Floatation Bath (Model Thermo: 3120057) and Tissue Embedding Center (Model: Leica H11220).

3.1.5 Sprague Dawley Rats

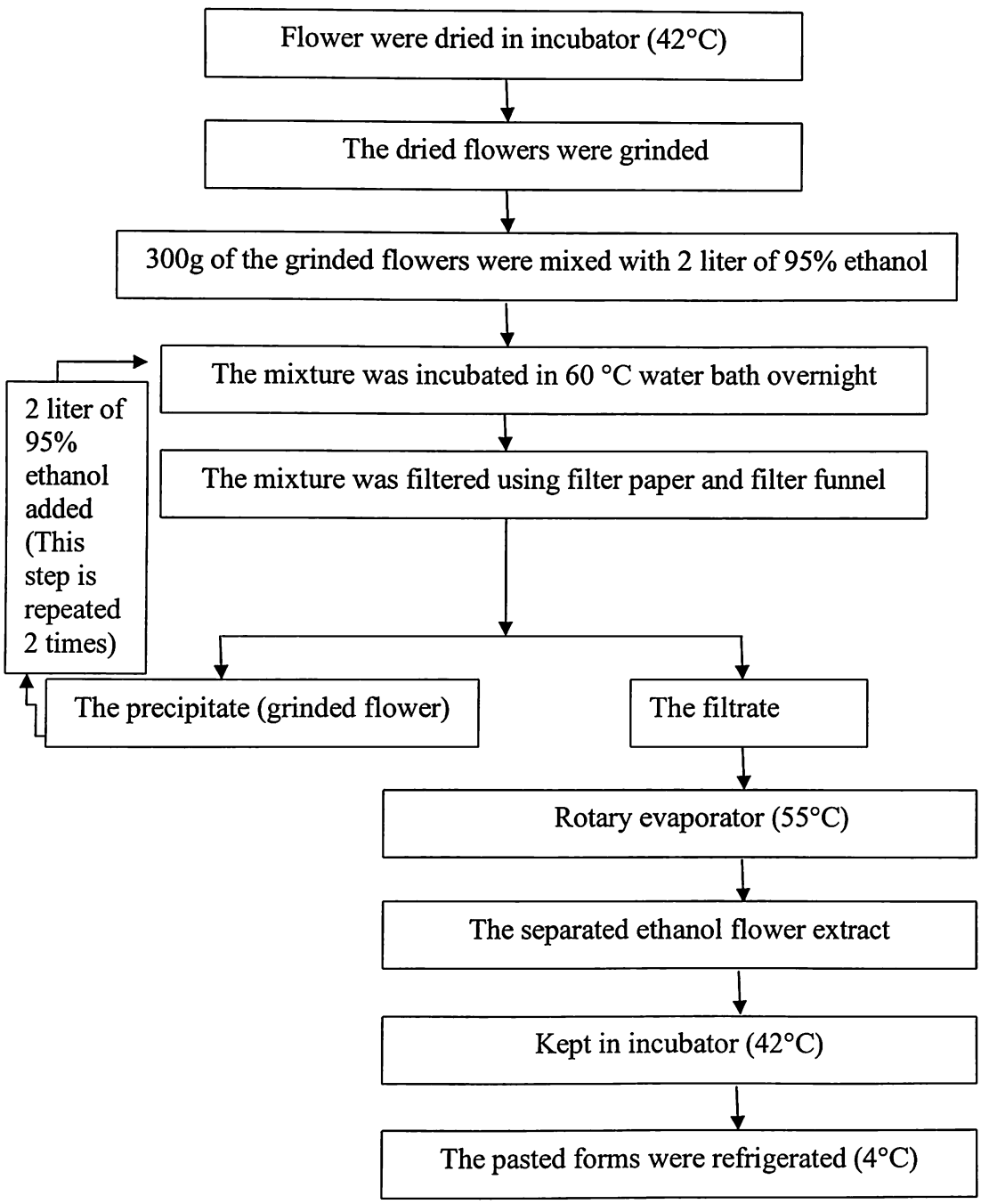
Male Sprague-Dawley rats aged 46 to 50 days old, weighed 130-180 g was obtained from Laboratory Animal Research Unit, Health Campus, USM. The animals were housed in well ventilated spacious plastic cages with 12-hour light/12-hour dark cycle and provided with adequate supply of food pellets and water *ad libitum*. A period of one week is allowed for acclimatization. Procedures involving animals and their care were conducted in conformity with the guidelines of the institute animal ethics committee, which also approved the study (please refer to appendix 7). The animals

were randomly divided into six experimental groups; group 1 were diabetic non-treated, group 2 were treated with 50 mg/kg of 95 % ethanol extract of SA flower, group 3 were treated with 100 mg/kg 95 % ethanol extract of SA flower, group 4 were treated with 200 mg/kg 95 % ethanol extract of SA flower, group 5 were treated with 15 mg/kg of Metformin and group 6 were normal rats and non treated. Each group had three animals. All the above treatments were made for 25 days. Blood glucose determinations were performed in overnight fasted rats at every three days of the treatments.

3.2 Method

3.2.1 95 % Ethanol Extract of *Senna auriculata* Flower

95 % ethanol extract of SA flower were prepared in the laboratory in PPSK. 300 grams of grinded dry flowers of SA were mixed with 2000 mL of 95 % ethanol and incubated in water bath at 60°C for over night. Next day the mixture were filtered, the filtrate (approximately 1500 mL) were kept at room temperature while the flower (precipitate) were again were mixed with 1000 mL of 95 % ethanol and incubated in water bath at 60°C for over night. . Next day the mixtures were filtered, the filtrates (approximately 1000 mL) were kept at room temperature while the flowers (precipitate) were dried using incubator. A total of 2500 mL of filtrate were evaporated by using rotary evaporator at 60°C. The separated pure flower extract were collected in a bottle and kept in incubator at 42 °C until the pure flower extract were turned into paste form.(please refer to flow chart 3.1)



Flow chart 3.1: Preparation of 95% ethanol extract of *Senna auriculata* flower.

3.2.2 Experimental Design

In this study, Sprague-Dawley male rats (n=18), aged 46 to 50 days old, with average body mass in range 130-180 g were chosen. These rat were randomly divide into two groups, that is normal control and test group where normal control group consist of 3 rats and test group consist of 15 rats, in different cages. A week before giving the extracts, all the rat were observed, only normal and healthy rat was used. All the rat were fasted for overnight (16-18 hours) and the fasting glucose level were measured as base line reading, the rats in the test group were then given 0.2 ml fresh solution of STZ (50 mg/kg) via intraperitoneal (IP) route. The rats in the control group were given 0.2 mL normal saline via intraperitoneal route in same amount as STZ.

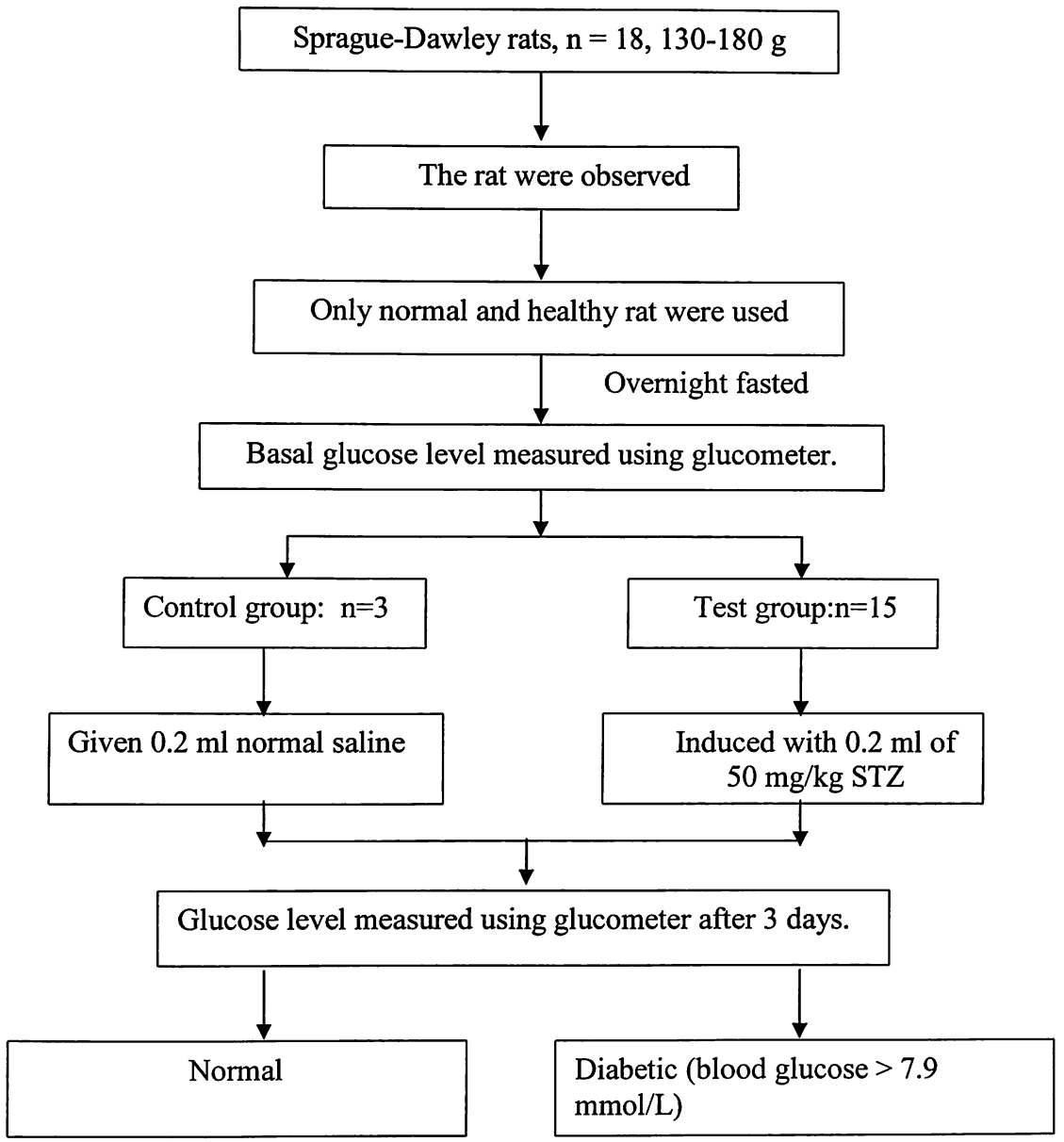
Three groups act as control group, where one of the groups is non-induced (normal control), another group was treated with metformin (15 mg/kg) (positive control) and the other group is given carboxyl methyl cellulose (CMC) 2 % of equal volume (negative control). While the three other groups were chosen as test group and were given the 95 % ethanol extract of SA in doses 50,100, and 200 mg/kg. The study was conducted for 25 days. At the end of study period, the rats were sacrificed and pancreas were taken for histopathological examination and fixed in 10 % formalin.

Body weight and fasting blood glucose were measured before and after induction and at every 3 days interval for 25 days. Body weight was measured using electronic weighing scale. Physical changes throughout the study period were recorded and at the end of study period before the rats were sacrificed, photo of the rats were taken using

digital camera. The rats were sacrificed after anesthetized using carbon dioxide inhalation for 2-3 minutes. Pancreas were excised for histopathological examination and fixed in 10 % formalin for 12 days to preserve the tissue structure before tissue processing is done. Histopathology examination was done under light microscope to observe the difference in the islet cells between the control groups and treatment groups. (please refer to flow chart 3.2 and 3.3).

3.3 Screening of active ingredient of 95 % ethanol extract of *Senna auriculata* by using GCMS

The extracts were analyzed with gas chromatography mass spectrometer (GCMS) for screening of active compound in the extracts. The active compounds were then compared to the compounds in the database to know whether, the active compound that has antihyperglycemic property and any new interesting compounds which are medically important were present in the crude extract.



Flow chart 3.2: STZ induction in rat.