PRELIMINARY STUDY ON THE EFFECTS OF WINTER MELON (*Benincasa hispida)* ON GLYCEMIC PROFILES: *IV VIVO* TEST ON STREPTOZOTOCIN SPRAGUE-DAWLEY RATS

By

FLORANCE GEDUIN

SCHOOL OF HEALTH SCIENCES

UNIVERSITI SAINS MALAYSIA

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Preliminary Study on the Effects of Winter Melon (*Benincasa hispida*) on Glycemic Profiles: *In vivo* Test on Streptozotocin-Induced Diabetic Sprague-Dawley Rats

Florance Geduin

Bachelor of Health Sciences (Biomedicine)

School of Health Sciences
Universiti Sains Malaysia
Health Campus
16150 Kubang Kerian, Kelantan

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CERTIFICATE

This is to certify that the dissertation entitle "Preliminary Study on the Effects of Winter Melon (*Benincasa hispida*) on Glycemic Profiles: *In vivo* Test on Streptozotocin-Induced Diabetic Sprague-Dawley Rats" is the bona fide record of research work done by Ms Florance Geduin during the period from September 2013 to March 2014 under my supervision.

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CHAPTER FOUR: RESULTS

LIST OF ABBREVIATIONS

ADA = American Diabetes Association

ADP = adenosine diphosphate

AMP = adenosine monophosphate

BH = *Benincasa hispida*

BHE = *Benincasa hispida* extract

DM = diabetes mellitus

DNA = deoxyribonucleic acid

ED = effective dose

EDTA = ethylenediaminetetraacetic acid

FBG = fasting blood glucose

GLUT2 = glucose transporter

HbA1c = glycosylated hemoglobin

IDDM = insulin dependent diabetes mellitus

IDF = International Diabetes Federation

NHMS = National Health and Morbidity Survey

NIDDM = non-insulin dependent diabetes mellitus

OGTT = oral glucose tolerance test

SD = Sprague-Dawley

STZ = streptozotocin

T2DM = type 2 diabetes mellitus

WHO = World Health Organization

LIST OF SYMBOLS

°C	= degree Celcius
%	= percentage
<	= less than
>	= more than
g	= gram
g/kg	= gram per kilogram
h	= hour
kg	= kilogram
mg/kg	= milligram per kilogram
ml	= milliliter
mmol/l	= millimole per litre
rpm	= revolutions per minute
s	= second
α	= alpha
β	= beta
μ	= gamma

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ABSTRAK

Benincasa hispida (BH) atau dikenali sebagai Kundur adalah sejenis sayuran yang digunakan secara meluas di negara-negara tropika dan telah digunakan untuk mengawal penyakit kencing manis secara tradisional dalam kalangan masyarakat Asia. Bahagian yang berlainan telah terbukti mempunyai kesan hypoglsemik dan anti-diabetik. Walaubagaimanapun, kurang kajian yang telah dilakukan untuk mengetahui kandungan hipoglikemik dan anti-diabetik yang dimiliki oleh isi Kundur. Kajian tentang kesan hipoglikemik ekstrak ini telah dijalankan ke atas tikus normal manakala kesan anti-diabetik BH dijalankan ke atas tikus makmal yang telah disuntik dengan Streptozotocin dengan membandingkan kesan dos ekstrak cair yang berbeza ke atas tikus diabetik semasa ujian toleransi glukos dan memberikan dos ekstrak cair yang berkesan (250 mg/kg setiap hari) selama 28 hari kepada tikus diabetik. Perubahan dalam paras glukos dalam darah semasa berpuasa, berat badan, gula dalam air kencing dan tahap HbA1c telah dipantau. Keputusan menunjukkan tahap gula dalam darah menurun sebanyak 14.2% untuk ujian tahap gula semasa berpuasa tetapi kenaikan sebanyak 8.5% semasa OGTT ke atas tikus normal. Dos 250 mg/kg dikenalpasti sebagai dos yang berkesan kerana telah menghasilkan penurunan sebanyak 10.5% dalam paras glukos semasa ujian toleransi glukos ke atas tikus diabetik. Selepas 4 minggu rawatan, paras glukos dalam darah menurun sebanyak 29% dan berat badan bertambah sebanyak 36.7% berbanding dengan tikus diabetik tanpa rawatan. Tahap HbA1c menurun sebanyak 7.3 % tetapi paras gula dalam air kencing tidak menunjukkan perubahan positif selepas empat minggu. Ekstrak BH tidak memberikan perubahan yang signifikan ke atas profil glisemik daripada tikus diabetik STZ - teraruh. Walau bagaimanapun, ia terbukti membantu memperbaiki profil glisemik jika paras glukosa darah sebelum rawatan adalah di bawah 20 mmol / l.

Kata kunci: *Benincasa hispida;* Ekstrak cair; Kencing manis; Kesan anti-diabetik; Kesan hipoglikemik; Streptozotocin

ABSTRACT

Benincasa hispida (BH) or known as Winter Melon is a widely used vegetable in tropical countries and has been used to control diabetes traditionally among the citizen, particularly among the Asian people. Different parts of the BH were shown to have hypoglycemic and anti-diabetic effects. However, less study was done to study the hypoglycemic and anti-diabetic properties of the flesh of BH. The hypoglycemic effects of the extract were studied on normal rats. Results showed that blood glucose level reduced by 14.2% during fasting blood glucose test. During oral glucose tolerance test (OGTT) in diabetic rats, blood glucose level reduced by 10.5%. The anti-diabetic effect were studied in Streptozotocin (STZ)-induced diabetic animals by comparing the effect of different aqueous extract doses on blood glucose levels in rats during OGTT and using the effective dose of extract (250 mg/kg daily) for 28 days in diabetic rats. The change in fasting blood glucose, body weight, urine sugar and HbA1c level were monitored. The dose of 250 mg/kg was found to be an effective dose as it produced a fall of 17.8% in blood glucose level during OGTT in diabetic rat. After four weeks of treatment, the blood glucose level reduced by 29% and the body weight increased by 36.7%, compared to diabetic rats without treatment. The HbA1c level produced a fall of 7.3% but urine sugar showed no improvement after four weeks. The *Benincasa hispida* extract has not significantly improved the glycemic profile of the STZ-induced diabetic rats. However, it is shown to help improving the glycemic profile if the baseline blood glucose level is below 20 mmol/l.

Keywords: *Benincasa hispida*; Aqueous extract; Diabetes mellitus; Anti-diabetic effect; Hypoglycemic effect; Streptozotocin

CHAPTER ONE

INTRODUCTION

1.1 Background study

The prevalence and incidence of diabetes mellitus is increasing worldwide. Recent estimates indicate there were 382 million people in the world living with diabetes in the year 2013 and this will rise to 592 million by 2035 (IDF, 2013). Diabetes mellitus is a heterogeneous metabolic disorder characterized by persistent hyperglycemic with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Bastaki, 2005).

The major burden of diabetes mellitus is now taking place in developing rather than in developed countries. Eighty percent of cases of diabetes mellitus worldwide live in less developed countries and areas. Asia has emerged as the 'diabetes epicentre' in the world, as a result of rapid economic development, urbanization and nutrition transition over a relatively short period of time. Among the 10 countries with the largest numbers of people predicted to have diabetes mellitus in 2030, five are in Asia (China, India, Pakistan, Indonesia and Bangladesh) (Chen *et al.*, 2012). Despite the predominantly urban impact of the epidemic, type 2 diabetes mellitus (T2DM) is fast becoming a major health concern in rural communities in low- and middle-income countries. Indigenous communities are among those especially vulnerable to diabetes (IDF, 2013). According to World Health Organization (1999), diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia

of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2008). It is caused by a complex interaction of genetic, immunological and environment factors as well as life-style choices (Bailes, 2002).

Type 2 diabetes mellitus (T2DM) or known as non-insulin dependent diabetes mellitus (NIDDM) was relatively rare in developing countries some decades ago. However, higher rates observed in Asian Indian and Chinese populations in Mauritius, as well as in Asian immigrants in Western countries strongly predicted the potential epidemic of T2DM that has now emerged in mainland China and India (Chen *et al.*, 2012). Compared with developed countries, the proportion of young to middle-aged individuals with T2DM is higher in developing countries. Furthermore, T2DM is not necessarily less prevalent in rural than in urban areas of developing countries, as is generally believed. The rural–urban difference in prevalence is predicted to narrow owing to urbanization, rural to urban migration and its associated lifestyle changes.

The incidence and prevalence of people with diabetes in Malaysia is increasing together with the rapid phase of industrialization and urbanization in recent decades. Statistics available from Ministry of Health for the last two decades showed that energy intake, fats and sugars increases besides the 'westernization' of global eating habits contributing to the increase of diabetes cases (Wan Nazaimoon *et al.*, 2013). At the population level, a high prevalence of obesity results from a complex interaction between changes in the population's lifestyle, involving a higher energy and fat consumption and an increasingly sedentary existence (Ismail *et al.*, 2002). According to National Health and Morbidity Surveys NHMS (2011), 15.2% or about 2.6 millions of Malaysian population aged 18 years and above have diabetes, compared to 11.6% in 2006 (Letchuman *et al.*, 2010).

The development of commercial medicine to control diabetes including metformin posed several side effects such as hypoglycemia, liver problems and lactic acidosis (Palsamy and Malathi, 2007). The decreasing efficacy of synthetic drugs and the increasing contraindications of their usage leading to the exploration on medicinal plants and herbs (Petrovska, 2012) which is believed to be safer and cheaper. *Benincasa hispida* is a vegetable that commonly consumed by Asian people and are believed to possess hypoglycemic and anti-diabetic properties. This preliminary study is carried out to elucidate the anti-diabetic effects of *Benincasa hispida* extracted on streptozotocin (STZ)-induced diabetic rats.

1.2 Research objective

1.2.1 General objective

The general objective of this study is to provide evidence on hypoglycemic and anti-diabetic effect of aqueous *Benincasa hispida* extract (BHE) on streptozotocin (STZ)-induced diabetic Sprague-Dawley (SD) rat *in vivo*.

1.2.2 Specific objectives

1. To determine the hypoglycemic effect of BHE on normal SD rat
2. To establish the effective dose (ED_{50}) for the BHE
3. To evaluate the effects of the BHE on the glycemic profile of the STZ-induced diabetic SD rat

1.3 Research hypothesis

The BHE will significantly improve the glycemic profile of STZ-induced diabetic SD rats.

1.4 Significance of study

Despite the available information regarding biological capabilities of BH, little scientific finding has documented the potential medicinal effect of BH on glycemic profiles. Thus, the primary concern of this research was to determine the possible benefits of this extract on diabetes. This study would also help establish a rat-diabetic model for natural product study as well as *in vivo* hypoglycemic effects of oral administration of BH extracts. Furthermore, this study also served as a preliminary basis to further investigate the active compounds in BH affecting the glycemic profile.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Benincasa hispida* (BH)

BH is a widely used vegetable in India and other tropical countries and belongs to the family Cucurbitacea (Chopra *et al.*, 1956). Most of the plants belonging to this family are frost sensitive and drought-tolerant (Whitaker and Bohn, 1950). It is also known as Kundur (Malay), ash gourd or winter melon (English), *Bhuru Kolu* or *Safed Kolu* (Gujarati), *Petha* (Hindi), *Kushmanda* (Sanskrit), *Dōngguā* (Chinese) and *Beligo* (Indonesian). The watermelon-shaped fruits range from oblong to round and may reach 4 feet in length and 2 feet in diameter. Young fruit is fleshy, succulent and hairy while the mature fruit has thickly deposited hairs with easily removable waxy bloom (Figure 2.1a) (Grover *et al.*, 2001) and the seeds are oval, flat and light brown (Figure 2.1a). It has thick, furrowed stems with coarse hairs and tendrils. The flowers are golden yellow in color with 2 ½ to 3 ½ inches wide. The flowers are monoecious and are pollinated by bees. Female flowers are borne on ¾ to 1 ½ inch long hairy stalks, while stalks bearing male flowers are 2 to 6 inches long (Stephens, 2012).

This plant require a long periods of warm, dry weather (Whitaker and Davis, 1962) in a rich well-drained soil and plenty of moisture (Bown, 1995) for their optimal growth. It can tolerate a pH of soil between ranges of 5.8 to 6.8 and requires stable temperature in order to grow well.

Figure 2.1a: The BH with waxy bloom on the outer rind.

Figure 2.1b: The seeds (oval, light brown) and flesh (white, spongy area) of BH

(Zaini *et al.*, 2011)

The BH has been used as a food and medicine for thousands of years in the Orient. According to an old Korean medical encyclopedia, the "Donguibogam", the BH is efficacious against diabetes, dropsy, diseases related to liver, leucorrhea, and good for the detoxification of minerals, the removal of fever, and to strengthen the function of bladder and small and large intestines (Choi *et al.*, 2001). In Ayurvedic medicine, the seed is used in the treatment of coughs, fevers, excessive thirst and expel tapeworms (Chevallier, 2001). Besides, it has been used in many empirical applications in India for various ailments such as GIT problems like dyspepsia and burning sensation, heart disease, vermifuge, diabetes and urinary disease (Jayasree *et al.*, 2011).

Study by Mohana and Mohan (2013) has shown an aqueous extract of BH stem significantly decreased the elevated blood glucose levels in alloxan-induced hyperglycemia in rabbit model. The hypoglycemic action of the extract may be due to potentiating the insulin effect of plasma by stimulating insulin release from the remnant pancreatic β-cells or its release from the bound form. It might also involve an extra-pancreatic action in these

alloxan-diabetic rabbits, which might include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis. Besides, the antihyperglycemic activity of aqueous extract may be due to the presence of phyto constituents present in the plant such as flavonoids, carbohydrates, tannins, phenols, glycosides and alkaloids. Flavonoids has insulinomimetic properties and stimulates lipogenesis and glucose transport in the adipocytes hence lowering blood sugar (Glauce *et al.*, 2004).

2.2 Diabetes mellitus

The signs and symptoms of diabetes have been observed and recorded since the beginnings of civilization. Ancient physicians has long described the disease, with the first reference to the condition being found in the Ebers Papyrus, an Egyptian medical treatise dated to *circa* 1500 B.C (Grauer, 2011). The term "diabetes" was first introduced by Araetus of Cappodocia (81-133AD). Later, the word mellitus (honey sweet) was added by Thomas Willis in 1675 after rediscovering the sweetness of urine and blood of patients. It was only in 1776 that Matthew Dobson firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness (Kirchhoff *et al.*, 2008).

Diabetes mellitus (DM) is not a single disease entity but it is a group of metabolic disorders characterized by defective regulation of carbohydrate, fat and protein (Ozougwu, 2011). In most cases, DM is characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia resulting from an absolute deficiency of serum insulin due to autoimmune antibody induced destruction of insulin secreting β-cells of pancreatic islets of Langerhans (type 1 DM or IDDM) (Sarkar *et al.*, 2013) or resulting from resistance to insulin release from β-cells as well as desensitization of peripheral tissue to insulin and down regulation of insulin receptors (type 2 DM or NIDDM) (Tripathi, 2004). Based on WHO (1999), fasting blood glucose concentration of more than 7.0 mmol/l and postprandial blood glucose level of more than 7.8 mmol/l (IDF, 2007) is defined as DM.

Diabetes is associated with a number of complications. The resulting complications can be grouped into vascular (stroke and myocardial infarction) and premature microvascular disease (retinopathy, nephropathy and neuropathy) which likely to have impaired vascular responsiveness, thus reduced distribution of blood flow to the tissue. Cardiovascular disorders in diabetes manifested as myocardial infarction and stroke. The formation of 'plaque' in the blood vessels or known as atherosclerosis may lead to vascular complication.

Diabetic retinopathy occur due to lesions within retina which can lead to blindness among adults aged 20 – 74 years (Frank, 2004) while diabetic nephropathy is characterized by the development of proteinuria with a subsequent decline in glomerular filtration rate (Forbes and Cooper, 2013). Patient with diabetes are at high risk of developing neuropathy, where nerve is damaged due to prolonged exposure to high blood glucose.

There are 4 classes of drugs that being used for glycemic regulation; sulphonyureas, biguanides (metformin), α-glucosidase inhibitors and endogenous insulin. Metformin is a widely prescribed antihyperglycemic agent for type 2 diabetes mellitus. There are many proposed mechanism of metformin action. Study by Scheen (1997) found that the glucose-lowering effect of metformin in patients with NIDDM is mainly by decreasing the hepatic glucose output and enhance peripheral glucose uptake. While Zhou *et al.* (2001) proposed that the metabolic effects of this drug may be due to its ability to phosphorylate and activate AMP-activated protein kinase. This action wills thereby reducing activity of acetyl-CoA carboxylase and lowering expression of a lipogenic transcription factor as well inhibiting hepatic gluconeogenesis. Previous study by Cheng *et al.* (2006) on the other hand, suggest that metformin exerts its anti-hyperglycemic effect primarily through enhancement of β-endorphin secretion from adrenal glands to stimulate opioid μ-receptors located on peripheral tissues, leading to the amelioration of GLUT-4 gene expression and an attenuation of raised hepatic PEPCK gene expression in rats with insulin-deficient diabetes.

2.3 Streptozotocin

Streptozotocin (STZ) (2-deoxy-2-(3-methyl-3-nitrosouriedo)-D-glucopyranose) is a naturally occurring compound, produced by the soil microorganism *Streptomycetes achromogenes*, that exhibits broad spectrum of antibacterial properties (Eileen, 1997). It is a mixture of α- and β-stereoisomers that appear as pale yellow or off-white crystalline powder (Eleazu *et al.*, 2013).

Several hypotheses have been proposed for the cytotoxic actions of STZ in pancreatic β-cells including the possibility that it may alter the metabolism of pyridine nucleotides, induce the formation of toxic free radicals and activate the enzye poly(ADP-ribose) synthetase. The generation of nitric oxide (NO) in the diabetogenic activity of STZ was implicated by O'Neill *et al.* (1993). The first report on diabetogenic effect of STZ on rats and dogs was done by Rakieten *et al.* (1963) who attributed it to disruption of the pancreatic islets. Later, more study confirmed this diabetogenic action in the rat but studies suggested that it is resulted from degranulation of pancreatic β-cells or other interference with production or release of insulin, rather than from actual destruction of β-cells (Evans *et al.*, 1965; Arison *et al.*, 1967). STZ is a toxic glucose and N-acetyl glucosamine analogue which will is taken up by pancreatic β-cells via the GLUT 2 transporter where it causes β-cell death by fragmentation due to the nitrosourea moiety (Ventura-Sobrevilla *et al.*, 2011).

Study from Akbarzadeh *et al.* (2007) showed that 60 mg/kg dose of STZ is the ideal dose to ensure the induction of type 1 DM in rats. Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of STZ treatment. Within one week to ten days, the amount of the relevant factors was almost stable, which indicates irreversible destruction of Langerhans islets cells. Studies done in mouse showed that at high doses, STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds. At lower doses, STZ

elicits an immune and inflammatory reaction, presumably related with the release of glutamic acid decarboxylase auto antigens. Under this condition, the destruction of β cells and induction of the hyperglycemic state is associated with inflammatory infiltrates including lymphocytes in the pancreatic islets. On the other hand, study by Arikawe *et al.* (2012) showed that 45 mg/kg is the ideal dose to induce type 2 DM in rats. The rats showed similar diabetic characteristics as Akbarzadeh's (2007) finding. However, we found that 40 mg/kg is enough to induce type 2 DM.

STZ also posed adverse effects on experimental animals including hepatotoxicity and nephrotoxicity. In addition to causing acidosis that can result from renal tubular damage in the kidney, STZ also cause type B lactic acidosis due to its nitrosourea toxic effects. Study by Eleazu *et al.* (2013) demonstrated that STZ can induce tumors in rat kidney, liver and pancreas when administered intravenously. In human, STZ can lead to acute complications such as irritation, nausea, headache, vomiting and chronic complications such as reproductive disorders, and general deterioration of health as well as blindness if in contact with the eyes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

Fresh fruits of BH (6 kg) were obtained from the local market, located in Kota Bahru, Kelantan during the months of October – December 2013. The fruits were washed thoroughly with clean water after collection.

3.1.1 Aqueous extract

The rind of the fruit was peeled off and the seeds were removed before cutting the flesh into a small dices. The fleshes were then weighed by using an analytical scale. Next, the fleshes were blended together with using Waring blender water with ratio of fleshes to water 1:1 for about 2 minutes or until the fleshes were crushed. The extract was then transferred into a 500 ml beaker and was boiled with hot plate stirrer at 42 ° C and at speed of 500 rpm and was left for 30 minutes once the temperature reached 80 ° C. After 30 minutes, the beaker was left at room temperature until it cool. Once the beaker cooled down, the extract was filtered using a sieve filter to obtain the juice. The juice was then centrifuged at 5000 rpm for 10 minutes at 24 ° C. The supernatant was transferred into a universal bottle, while the pellet was discarded. The universal bottle was stored in a freezer for a day and then placed in an air-oven. It was subsequently left to dry 55 ° C for 3 days. The freeze-dried extract was then stored at – 20 ° C until used for later experimentation. The process of aqueous extract preparation is summarized in Figure 3.1.1.

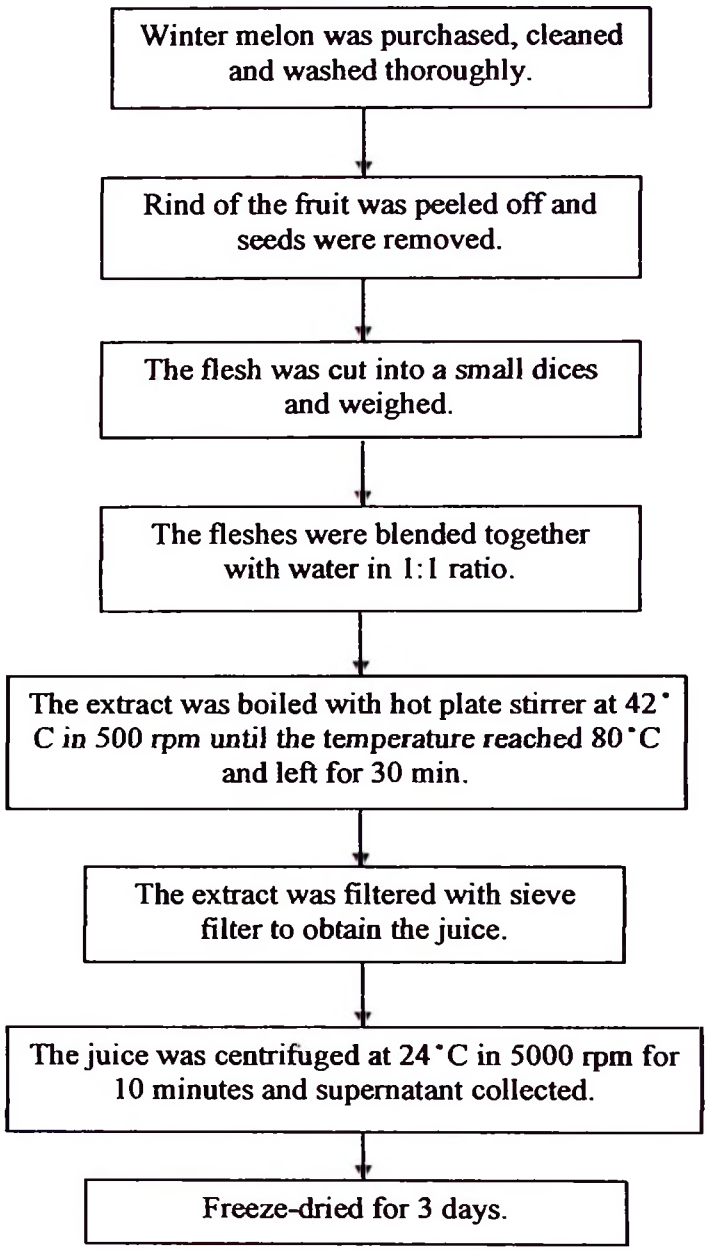

Figure 3.1.1: Preparation of aqueous extract of BH (winter melon).

3.2 Experimental animals

Eighty four SD rats were used in this study, of which 9 were female rats and the rest were male rats. Animal were obtained from Animal Research and Service Center (ARASC), Universiti Sains Malaysia, Kelantan and were housed in polypropylene cages at an ambient temperature of 25 – 30 °C and relative humidity of 45 – 55 % with a 12 hour of dark and light cycle. The animals were fed with pellet diet which provided by ARASC and water *ad-libitum*. The study was approved and conducted with conformity from Animal Ethics Committee USM (304/PPSK/61311083).

3.3 Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ at the dose of 40 mg kg^{-1} in 0.1 M citrate buffer (pH 4.5) to a group of overnight fasted rats. After three continuous days of STZ administration, blood glucose levels were measured and recorded.

3.4 Experimental design

The initial screening of extract for the hypoglycemic activity was done in normal healthy rats. The anti-diabetic effect were studied in STZ-induced diabetic rats by studying the effect of different doses of the aqueous extract on blood glucose levels of diabetic rats during OGTT and by giving the most effective dose of extract (250 mg/kg) daily once for 28 days to STZ-induced diabetic rats and observing the change in fasting blood glucose (FBG), body weight, urine sugar, and HbA1c level.

3.4.1 Assessment of hypoglycemic effect in normal healthy rats

Twenty one normal rats were fasted overnight and divided into seven groups of three rats each (Figure 3.4.1). Control group (Group I) was given vehicle (distilled water) while Group II, III, IV, V and VI received aqueous extract orally at doses 100, 250, 500, 750 and

1000 mg/kg; respectively. Group VII received standard drug (metformin) at dose 150 mg/kg. Blood glucose levels were checked before and after 2, 4 and 6 hour of oral administration of BHE. The blood samples were collected from tail vein.

3.4.2 Assessment of hypoglycemic activity by OGTT in normal healthy rats

Fifteen normal rats were fasted overnight and divided into five groups of three rats each (Figure 3.4.2). Group I served as a control group and was given distilled water only while Group II, III and IV were given aqueous extract orally at doses 100, 250 and 500 mg/kg, respectively. Group V received standard drug (metformin) at dose 150 mg/kg. The FBG was checked initially before orally administered distilled water, aqueous extract and metformin to the respective group. Blood glucose level was taken after 90 minutes of treatment. The rats were then orally administered with 2 g/kg of glucose and their blood glucose was measured hourly for 3 hours.

3.4.3 Assessment of hypoglycemic activity by OGTT in diabetic rats

Fasting blood glucose (FBG) was checked in overnight fasted rats and were divided into 6 group consist of 3 rats each (Figure 3.4.3). Group I served as a normal rats and Group II served as a control diabetic rats; both received distilled water only. Variable doses of 100, 250 and 500 mg/kg of aqueous extract and a dose of 150 mg/kg of standard drug metformin were given to Group III, IV, V and VI respectively. All rats in each group were given 2 g/kg of glucose after 90 minutes of the extract and drug administration. Blood samples were collected at 1, 2 and 3 hour after glucose administration.

3.4.4 Assessment of anti-diabetic activity of BHE in diabetic rats

Twelve rats were divided into four groups of three rats each (Figure 3.4.4). Group I served as control normal and Group II as diabetic control; both received vehicle (distilled water) only. Group III and IV were treated with single dose of 250 mg/kg of extract and 150

mg/kg of standard drug metformin respectively, once a day for up to 28 days. The levels of FBG, weight and urine sugar were checked every week.

3.4.5 Acute toxicity study

Eighteen normal rats fasted overnight were divided into three groups of six rats each (3 females and 3 males) (Figure 3.4.5). Group I was received distilled water only while Group II and III were given ten (2500 mg/kg) and fifteen (3750 mg/kg) times of effective dose of aqueous extract respectively. The FBG was taken before given aqueous extract of BH through oral administration. Rats were then observed for their gross behavioral, autonomic, neurologic and toxic effects up to 24 hours. Food consumption, urine and fecal output were also examined at 2, 4, 6, 12 and 24 hour after aqueous extract administration. At 24 hour interval, FBG was taken and recorded. The rats were euthanized with sodium pentobarbital (100 mg/kg) at the end of the study (24 hours).

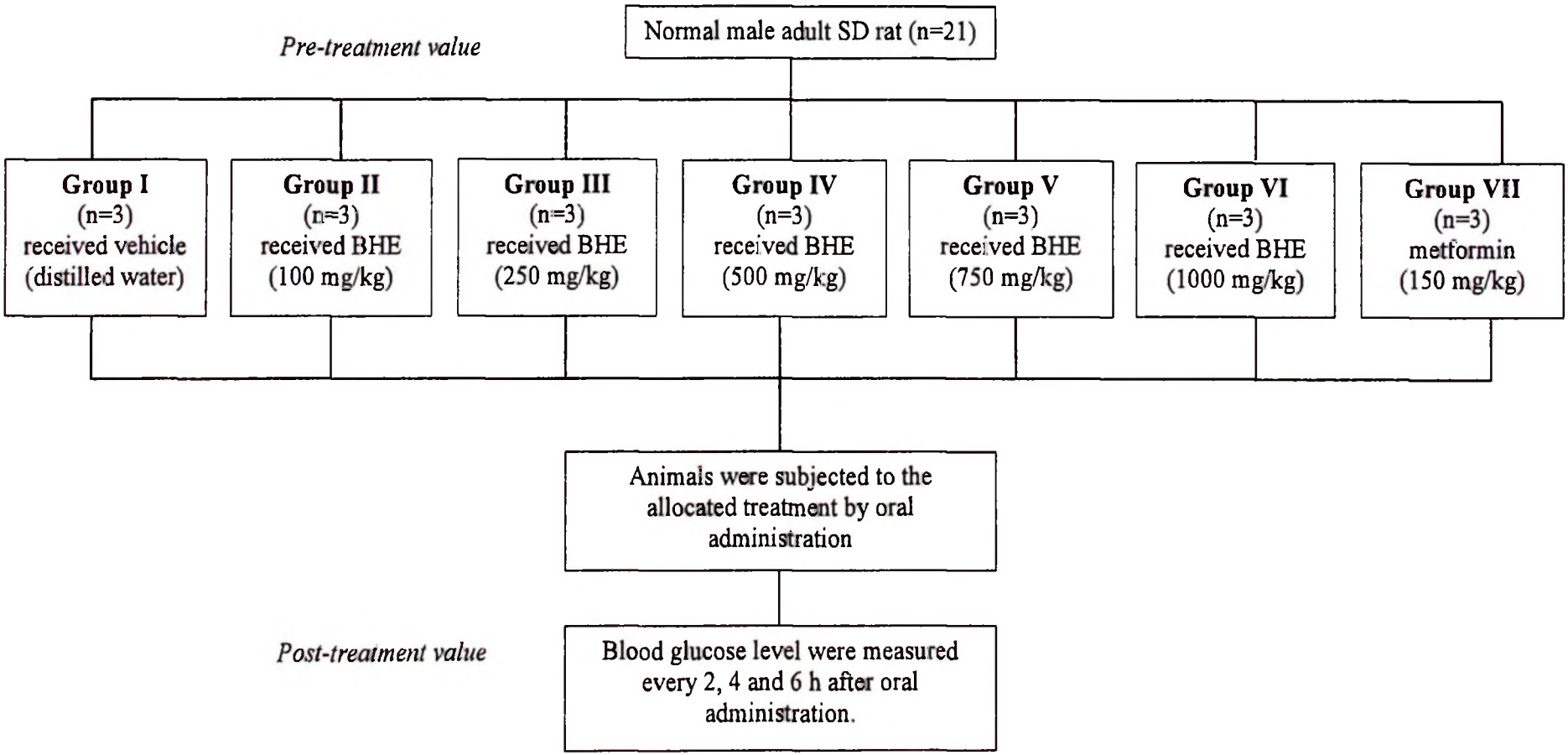

Figure 3.4.1: Evaluation of hypoglycemic effects in normal rats. Pre- and post-treatment value refers to the measurement of blood glucose level before and after 2, 4 and 6 h of treatment; respectively.

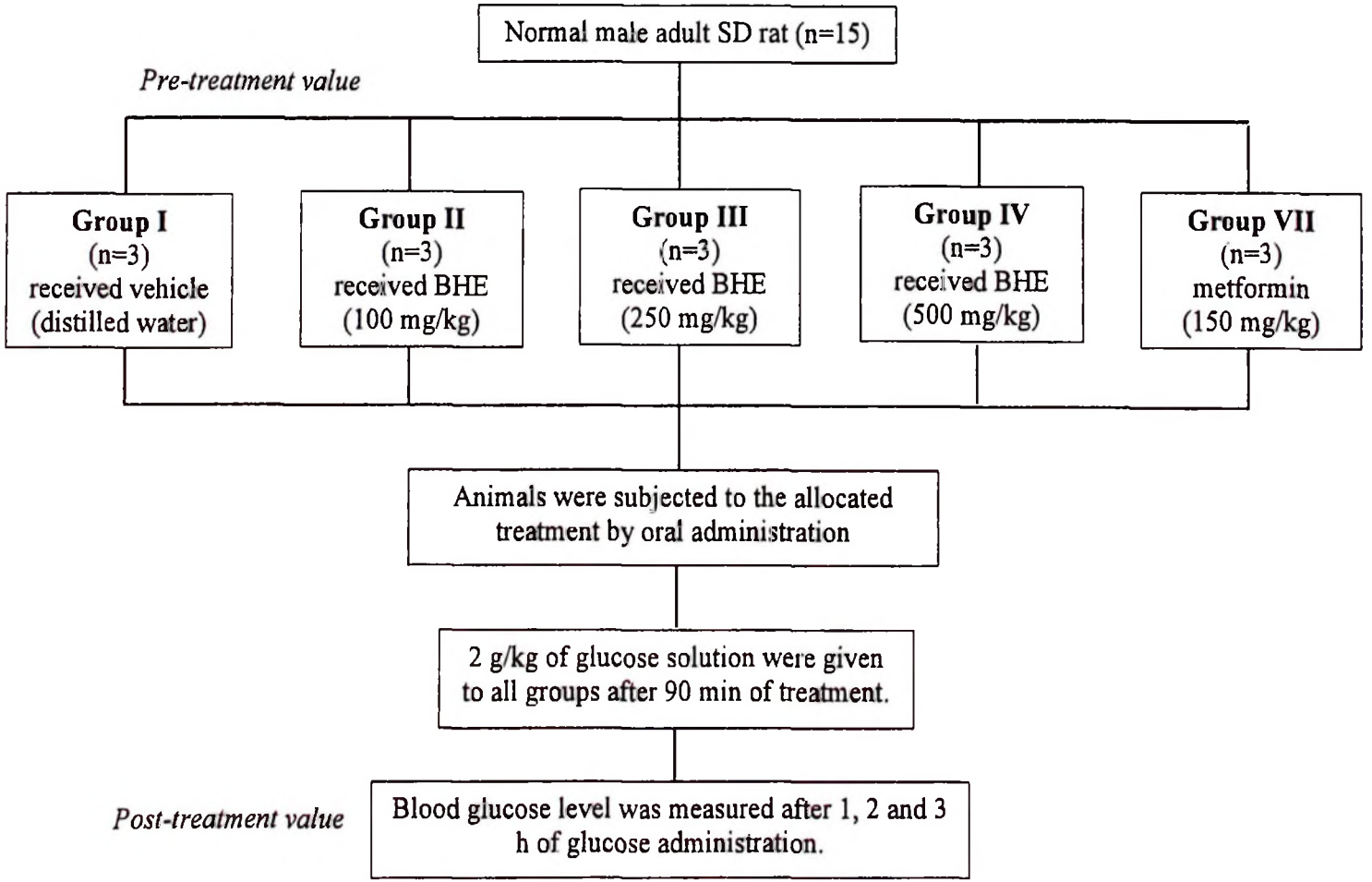

Figure 3.4.2: Evaluation of hypoglycemic effects during OGTT in normal rats. Pre- and post-treatment value refers to the measurement of blood glucose level before and after 1, 2 and 3 h of glucose administration, respectively.

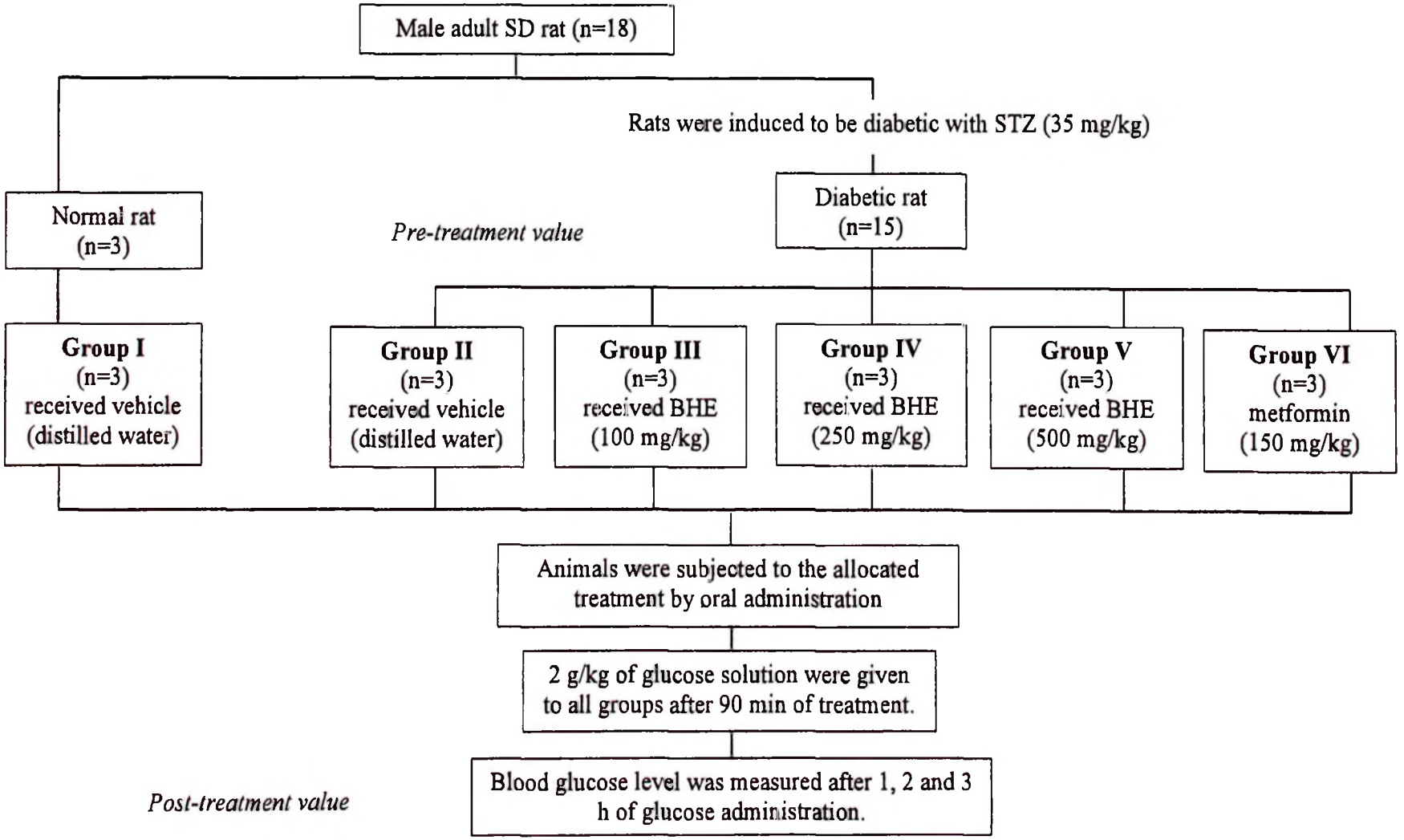

Figure 3.4.3: Evaluation of extracts effects during OGTT in diabetic rats. Pre- and post-treatment value refers to the measurement of blood glucose level before and after 1, 2 and 3 h of glucose administration, respectively.

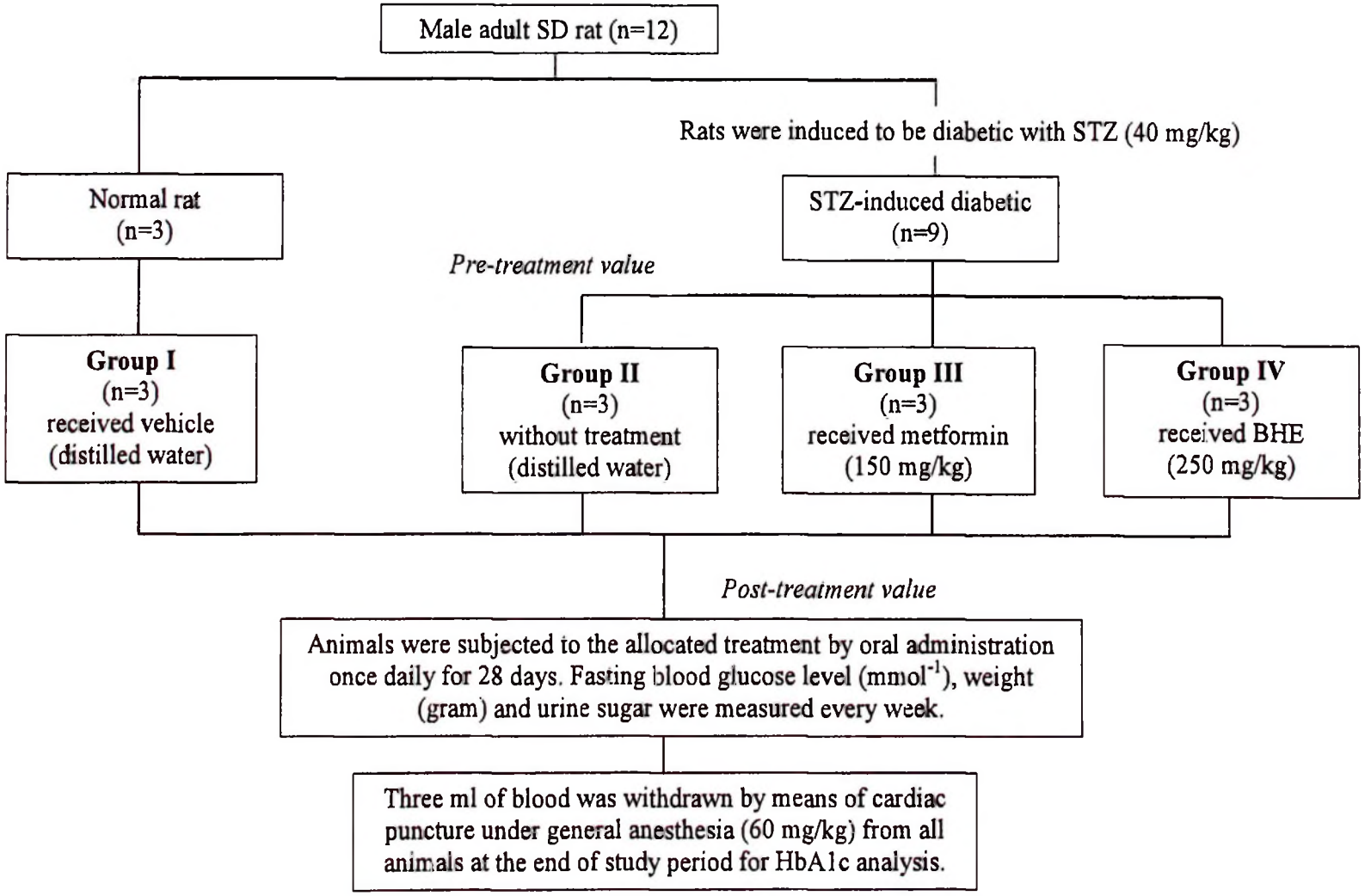

Figure 3.4.4: Effects of BHE on rat's glycemic profile. Pre- and post-treatment value refers to the measurement FBG, weight and urine sugar before treatment started.

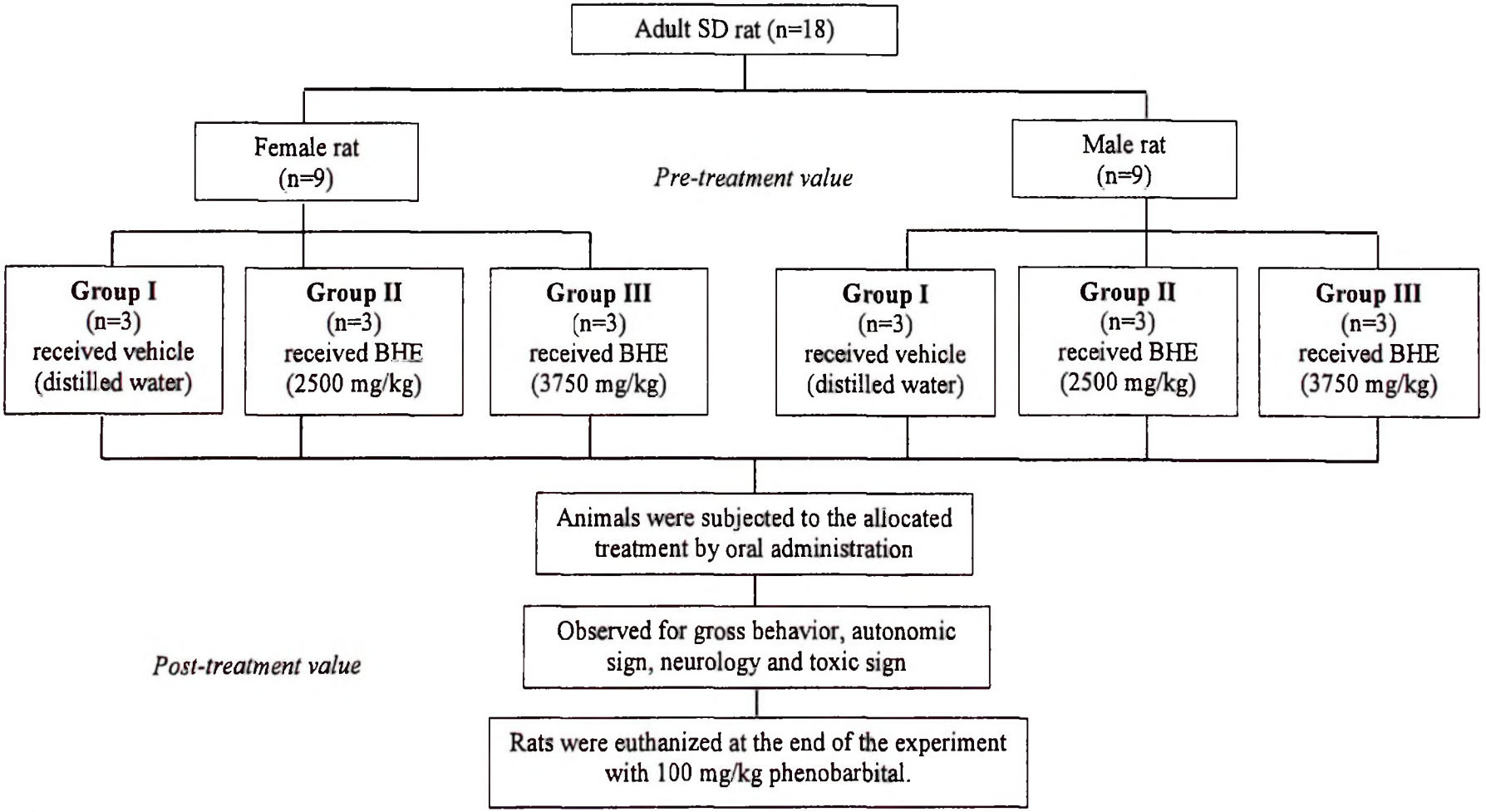

Figure 3.4.5: Acute toxicity study. Pre- and post-treatment value refers to the measurement of blood glucose level before and after treatment, respectively.

3.5 Oral administration

In this procedure, a bulb tipped gavage needle was used and attached to a syringe and used to deliver the extract into the stomach. Prior to performing the oral administration procedure, the distance from the oral cavity to the end of the xyphoid process (caudal of the sternum) was measured with the needle on the outside of the restrained animal. The correct needle is equal to the distance from the mouth to just beyond the last rib. The animal were firmly restrained to immobilize the head but not such that the animal vocalizes or shows other signs of distress (Figure 3.5). The position of the rat was maintained in an upright position and the gavage needle was passed along the side of the mouth. Following the roof of the mouth, the needle was advanced into the oesophagus and toward the stomach. After the needle is passed to the correct length, the appropriate volumes of extracts were administered slowly. After all of the extract delivered, the needle was removed in the opposite direction from the insertion and the rat was returned to its cage.

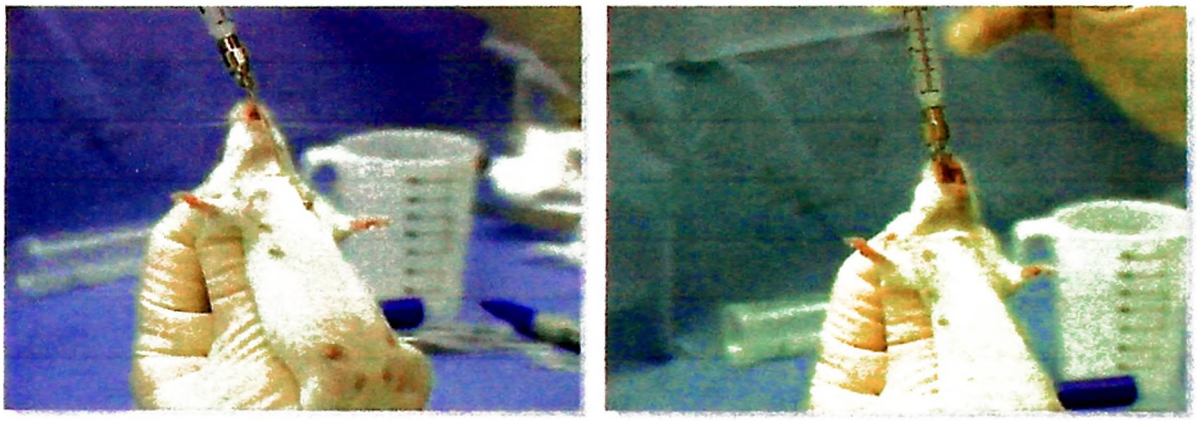

Figure 3.5: The right length of needle that is allowed to pass through oral of the rat (right) and the needle is passed to the correct length (left).

(LSSU, n.d)