Study on Extra-pancreatic Action of *Asteracantha longifolia* in Long-Evans Rats

A research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy.

Submitted by

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2013-1-70-064

Submitted to

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July, 2017

Declaration by the Research Candidate

I, Summaya Islam Tania, ID: 2013-1-70-064, hereby declare that the dissertation entitled **'Study on Extra-pancreatic Action of *Asteracantha longifolia* in Long-Evans Rats'** submitted by me, carried out under the supervision and guidance of **Dr. JMA Hannan** , Professor, Department of Pharmacy, East West University in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy. This thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

……..

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Certificate by the Supervisor

This is to certify that the thesis entitled **'Study on Extra-pancreatic Action of *Asteracantha longifolia* in Long-Evans Rats'** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Summaya Islam Tania**, ID: 2013-1-70-064 during the period 2016-2017 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

……..

Dr. JMA Hannan
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Certificate by the Chairperson

This is to certify that the thesis entitled **'Study on Extra-pancreatic Action of *Asteracantha longifolia* in Long-Evans Rats'** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Summaya Islam Tania**, ID: 2013-1-70-064 in 2016-2017.

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Dr. Chowdhury Faiz Hossain
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Dedication

To

My Beloved Parents

&

Research Supervisor

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Abstract

Ethanolic extract of *Asteracantha longifolia* have been used to investigate anti-diabetic activity in Long Evans rats. The plant extract was subjected to anti-diabetic study through assessing Disaccharidase activity and six segment method which was performed to assess the amount of sucrose remaining in the GIT at six different positions. In Six Segment method, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 100mg/kg extract at 30 minutes, 1hour, and 2hour. In assessing the effect of the plant materials on intestinal disaccharidase activity, the amount of unabsorbed sucrose in Pancreatic Enzymes are evaluated in control rats vs rats fed with 100mg/kg extract .The extract caused a significant ($p<0.05$), dose dependent inhibition of glucose absorption and showed hypoglycemic effects in Long-Evans rats weighing about 100-200 gm. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples collected after the experiment. In conclusion, these observations provide evidence and possible mechanisms of action for the anti-diabetic properties of plant *Asteracantha longifolia* claimed in Ayurveda medicine.

Keywords: Anti-Diabetic, *Asteracantha longifolia*, Hypoglycemic, Glucose, Sucrose.

CHAPTER 1

INTRODUCTION

1. Introduction

Diabetes, often referred to by doctors as **diabetes mellitus**, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria and they will become increasingly thirsty and hungry. Diabetes mellitus is a life-long disease affecting more than 150 million people all over the world and WHO has predicted the number will be doubled by the year 2025. Type 1 diabetes accounts for 5-10% of the diabetic population. Type 2 diabetes accounts for 90 - 95% of the people with diabetes and is more prevalent in adults (WHO 2002).

1.1 Diabetes Mellitus

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes.

All types of diabetes mellitus have something in common. Normally, body breaks down the sugars and carbohydrates eaten into a special sugar called glucose. Glucose fuels the cells in body. But the cells need insulin, a hormone, in bloodstream in order to take in the glucose and use it for energy. With diabetes mellitus, either body doesn't make enough insulin, it can't use the insulin it does produce, or a combination of both.

Since the cells can't take in the glucose, it builds up in blood. High levels of blood glucose can damage the tiny blood vessels in your kidneys, heart, eyes, or nervous system. That's why diabetes specially if left untreated can eventually cause heart disease, stroke, kidney disease, blindness, and nerve damage to nerves in the feet.

Insulin is a hormone that is produced by the pancreas. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop.

A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because the body does not produce enough insulin,

produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of glucose, the cells are not getting it for their essential energy and growth requirements.

(American Diabetes Association 1995)

1.1.1 Types of diabetes

The most common types of diabetes are type 1, type 2 and gestational diabetes.

Type 1 diabetes

Type 1 diabetes is also called insulin-dependent diabetes. It used to be called juvenile-onset diabetes, because it often begins in childhood.

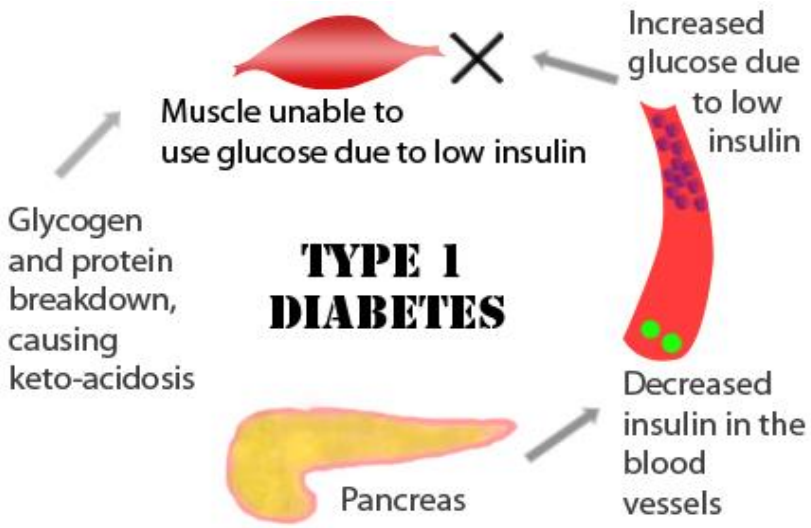

Figure 1.1: Type 1 Diabetes

(Alberti, 1998)

If one has type 1 diabetes, the body does not make insulin. Our immune system attacks and destroys the cells in the pancreas that make insulin. People with type 1 diabetes need to take insulin every day to stay alive.

Type 2 diabetes

The most common form of diabetes is type 2 diabetes, accounting for 95% of diabetes cases in adults. Some 26 million American adults have been diagnosed with the disease.

If one has type 2 diabetes, then body does not make or use insulin well. One can develop type 2 diabetes at any age, even during childhood. However, this type of diabetes occurs most often in middle-aged and older people. Type 2 is the most common type of diabetes.

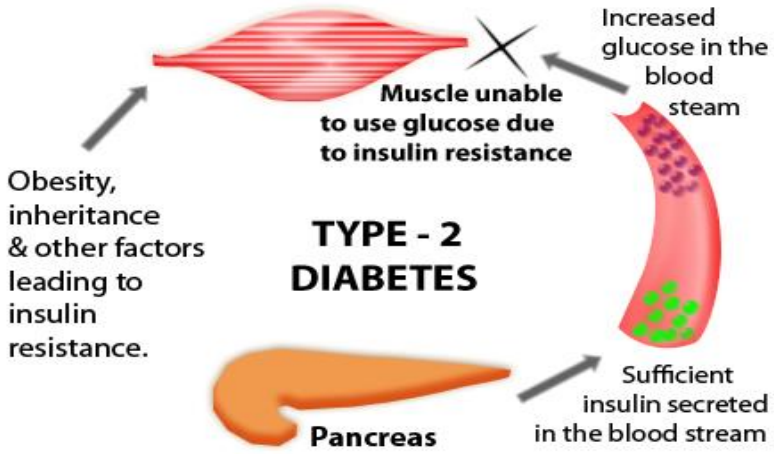

Figure 1.2: Type 2 Diabetes

(Alberti, 1998)

Gestational diabetes

Gestational diabetes develops in some women when they are pregnant. Most of the time, this type of diabetes goes away after the baby is born. However, if one has gestational diabetes, she has a greater chance of developing type 2 diabetes later in life. Sometimes diabetes diagnosed during pregnancy is actually type 2 diabetes.

Other types of diabetes

Less common types include monogenic diabetes, which is an inherited form of diabetes, and cystic fibrosis-related diabetes.(Genuth S et al)

Figure 1.3: Classification of Diabetes

1.1.2 Difference between type 1 and type 2 diabetes

Type 1 diabetes	Type 2 diabetes
Symptoms usually start in childhood or young adulthood.	Usually the disease is discovered in adulthood, but an increasing number of children are being diagnosed with the disease.
Hypoglycemia is common	There are no episodes of low blood sugar level, unless the person is taking insulin or certain diabetes medicines.
It can't be prevented	It can be prevented or delayed with a healthy lifestyle, including maintaining a healthy weight and exercising regularly

Table 1.1: Difference between type 1 and type 2 diabetes

(Alberti, 1998)

1.1.3 Epidemiology

Diabetes mellitus is a pandemic disease and is one of the main threats to human health. In the recent estimate of International Diabetes Foundation (IDF), it was mentioned that worldwide there were 366 million people with diabetes in 2011 and 371 million people with diabetes in 2012, with China (92.3 million), India (63 million) and the United States (24.1 million) leading the way and 4.8 million people died due to diabetes and also 4 out 5 people with diabetes live in low and middle income countries.

The greatest number of people with diabetes is between 40 to 59 years of age and diabetes caused more than 471 billion dollars to spend on healthcare globally. The prevalence of diabetes in the world is 8.3%, in Saudi Arabia, 23.4%, in Pakistan, 7.89% and in Australia the prevalence of diabetes has reached to 9.55%. (Whiting, 2011)

The Figure presents the global projection for the diabetes epidemic from 2011-2030.

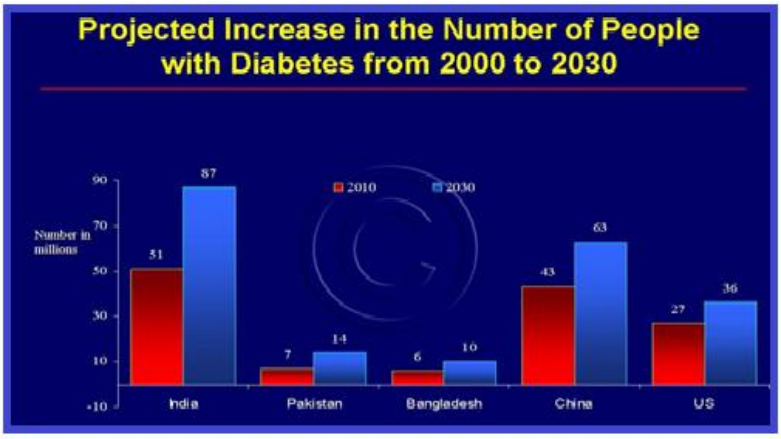

Figure 1.4: Prevalence of Diabetes

(WHO Expert Consultation, 2004)

As of 2014, 29.1 million people in the United States, or 9.3 percent of the population, had diabetes. More than 1 in 4 of them didn't know they had the disease. Diabetes affects 1 in 4 people over the age of 65. About 95 percent of cases in adults are type 2 diabetes. (Nita Gandhi Forouhi, Nicholas J. Wareham, 2014)

1.1.4 Health problems due to diabetes

Over time, high blood glucose leads to problems such as

- heart disease
- stroke
- kidney disease
- eye problem
- nerve damage
- Skin conditions
- Hearing impairment
- Alzheimer's disease.

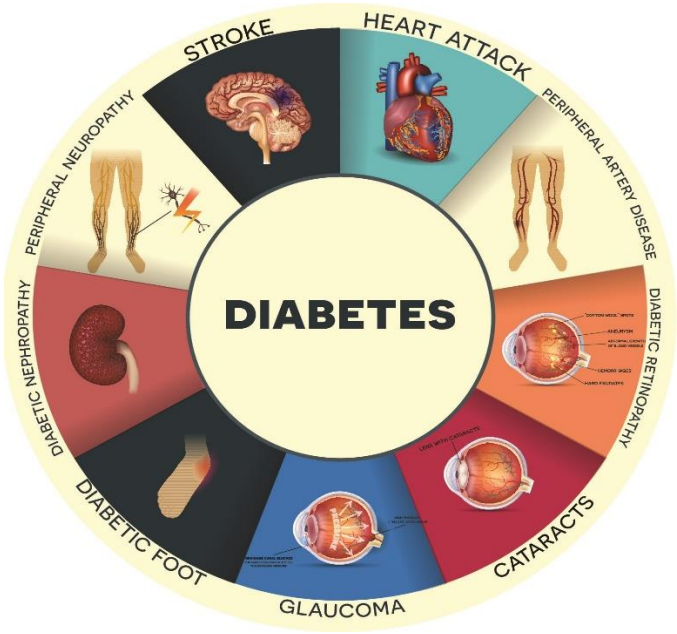

Figure 1.5: Health problems of Diabetes

(World Health Organization, 1999)

1.1.5 Facts on Diabetes

- Diabetes is a long-term condition that causes high blood sugar levels.
- In 2013 it was estimated that over 382 million people throughout the world had diabetes (Williams's textbook of endocrinology).
- Type 1 Diabetes - the body does not produce insulin. Approximately 10% of all diabetes cases are type 1.
- Type 2 Diabetes - the body does not produce enough insulin for proper function. Approximately 90% of all cases of diabetes worldwide are of this type.
- Gestational Diabetes - this type affects females during pregnancy.
- The most common diabetes symptoms include frequent urination, intense thirst and hunger, weight gain, unusual weight loss, fatigue, cuts and bruises that do not heal, male sexual dysfunction, numbness and tingling in hands and feet.
- If you have Type 1 and follow a healthy eating plan, do adequate exercise, and take insulin, you can lead a normal life.
- Type 2 patients need to eat healthily, be physically active, and test their blood glucose. They may also need to take oral medication, and/or insulin to control blood glucose levels.
- As the risk of cardiovascular disease is much higher for a diabetic, it is crucial that blood pressure and cholesterol levels are monitored regularly.
- As smoking might have a serious effect on cardiovascular health, diabetics should stop smoking.
- Hypoglycemia - low blood glucose - can have a bad effect on the patient. Hyperglycemia - when blood glucose is too high - can also have a bad effect on the patient.

(American Diabetes Association, 1995)

1.1.6 Symptoms of Diabetes

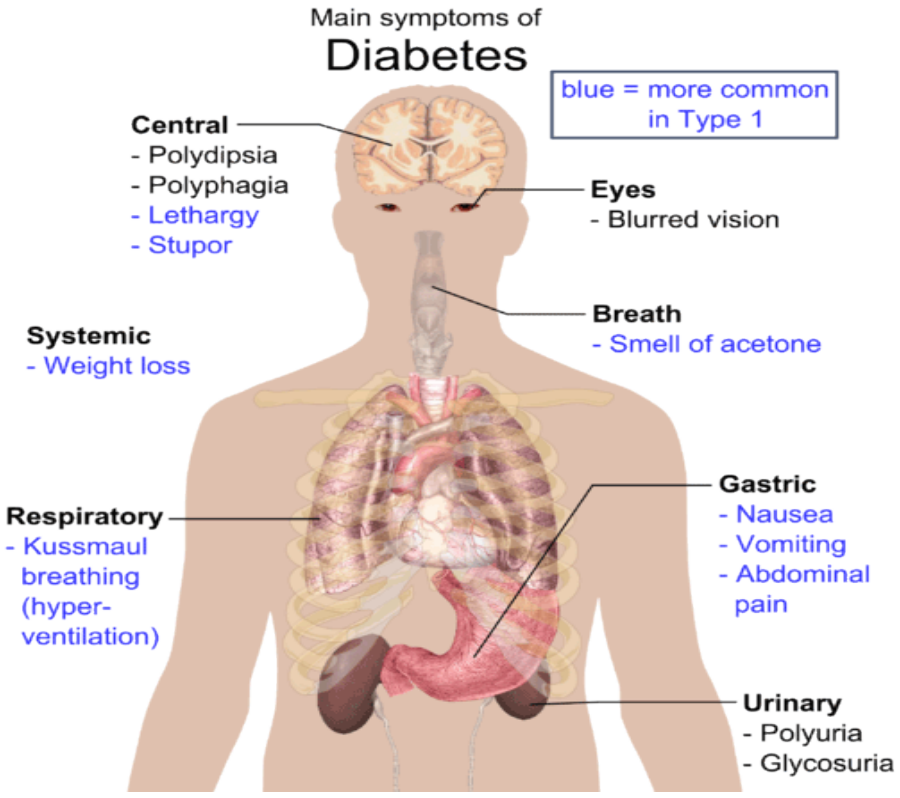

Figure 1.6: Symptoms of diabetes

(Jackson et al, 1991)

1.1.7 Complications linked to uncontrolled diabetes

- **Eye complications** - glaucoma, cataracts, diabetic retinopathy, and some others.
- **Foot complications** - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
- **Skin complications** - people with diabetes are more susceptible to skin infections and skin disorders
- **Heart problems** - such as ischemic heart disease, when the blood supply to the heart muscle is diminished
- **Hypertension** - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke
- **Mental health** - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders
- **Hearing loss** - diabetes patients have a higher risk of developing hearing problems
- **Gum disease** - there is a much higher prevalence of gum disease among diabetes patients
- **Gastroparesis** - the muscles of the stomach stop working properly
- **Ketoacidosis** - a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.
- **Neuropathy** - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
- **HHNS (Hyperosmolar Hyperglycemic Non-ketotic Syndrome)** - blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition.
- **Nephropathy** - uncontrolled blood pressure can lead to kidney disease
- **PAD (peripheral arterial disease)** - symptoms may include pain in the leg, tingling and sometimes problems walking properly
- **Stroke** - if blood pressure, cholesterol levels, and blood glucose levels are not controlled, the risk of stroke increases significantly
- **Erectile dysfunction** - male impotence.

- **Infections** - people with badly controlled diabetes are much more susceptible to infections

(American Diabetes Association, 1997)

1.1.8 Diagnosis of Diabetes

Doctors can determine whether a patient has a normal metabolism, prediabetes or diabetes in one of three different ways - there are three possible tests:

1. **Insulin Glucose Challenge Test –** This should be done with a 2-hour glucose challenge, 75 grams measuring fasting, 1- and 2-hour blood sugar AND insulin. The blood sugar should be less than 80 fasting and never rise above 110 or 120 after one to two hours. Insulin should be less than 5 fasting and should never rise above 30 after one to two hours.
2. **Hemoglobin A1C Test –** This is an important measure of glycated hemoglobin, which can be an early indicator of sugar problems. It measures sugars and proteins combining into glycated proteins called AGEs (advanced glycation end products), like the crust on bread, or the crispy top on creme brule. These create inflammation and oxidative stress throughout the body, and promote heart disease and dementia and accelerating aging. The hemoglobin A1C should ideally be less than 5.5. Anything over 6 is considered diabetes.

Figure 1.7: Hemoglobin A1C Test

3. **Lipid Profiles –** An HDL or good cholesterol level under 60 and triglycerides over 100 may be due to insulin resistance. An HDL under 40 and a triglyceride level over 150 usually means diabetes.

4. **NMR Lipid Profile –** This test is slightly different from the one above as it identifies the size of your cholesterol particles. With insulin resistance or Type 2 diabetes, one may develop small LDL and HDL cholesterol particles. They are much more dangerous than larger particles and lead to increased risk of atherosclerosis or heart disease.
5. **High Sensitivity C-Reactive Protein Test –** This is a measure of inflammation, one of the classic conditions that is both the cause and result of insulin resistance and diabetes. It should be less than 1, and is often associated with diabetes. In fact, anyone with a high C-reactive protein has a 1,700 percent increased risk of getting diabetes.
6. **Homocysteine Test –** Homocysteine levels are often abnormal in people with diabetes. The test is a measure of folic acid deficiency. It should be between 6 and 8.
7. **Fibrinogen Test –** This measures your risk of clotting, which can cause heart attacks and strokes. It is also a sign of inflammation and is associated with insulin resistance and diabetes. It should be less than 300.
8. **Check Ferritin Levels –** These are often elevated in people with diabetes. It is a nonspecific marker of inflammation associated with the disease. It also can mean an overload of iron in the body. It should be less than 150.
9. **Uric Acid Test –** Your level should be less than 6. Higher levels indicate problems with insulin resistance. This can lead to gout, which is related to insulin resistance and Type 2 diabetes.
10. **Liver Function Tests –** Elevated liver function can result from insulin resistance. This is the major cause of fatty liver and elevated liver function in this country. This is entirely due to sugar and carbohydrates in our diet that cause fatty liver, liver damage, and even cirrhosis.

(Genuth S et al)

1.1.9 Insulin

Insulin is a small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains held together by disulfide bonds.

The amino acid sequence is highly conserved among vertebrates, and insulin from one mammal almost certainly is biologically active in another. Even today, many diabetic patients are treated with insulin extracted from pig pancreas.

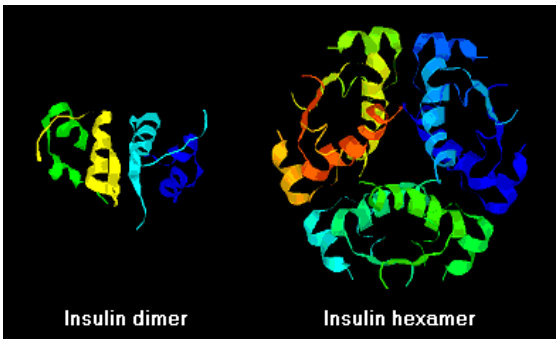

Figure 1.8: Insulin

1.1.9.1 Structure of Insulin

Insulin is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids

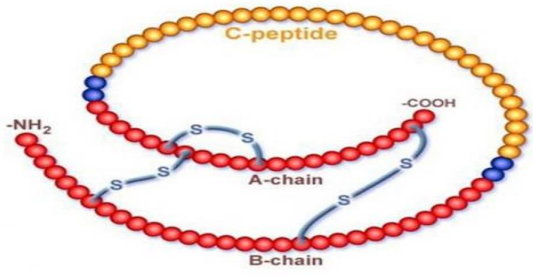

Figure 1.9: Structure of Insulin

1.1.9.2 Biosynthesis of Insulin

Insulin is synthesized in significant quantities only in beta cells in the pancreas. The insulin mRNA is translated as a single chain precursor called preproinsulin, and removal of its signal peptide during insertion into the endoplasmic reticulum generates proinsulin.

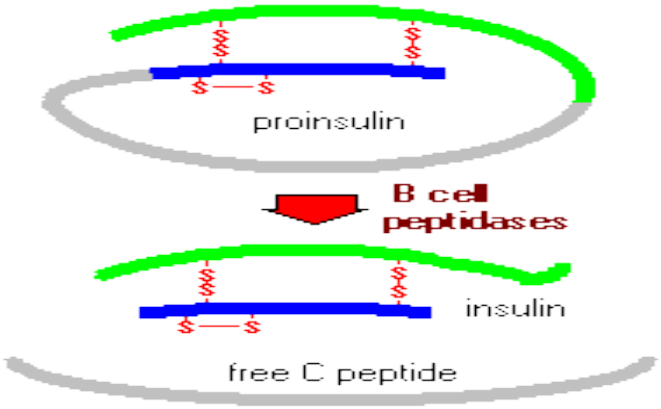

Figure 1.10: Synthesis of Insulin

(Katsoyannis, 1966)

Proinsulin consists of three domains: an amino-terminal B chain, a carboxy-terminal A chain and a connecting peptide in the middle known as the C peptide. Within the endoplasmic reticulum, proinsulin is exposed to several specific endopeptidases which excise the C peptide, thereby generating the mature form of insulin. Insulin and free C peptide are packaged in the Golgi into secretory granules which accumulate in the cytoplasm.

When the beta cell is appropriately stimulated, insulin is secreted from the cell by exocytosis and diffuses into islet capillary blood. C peptide is also secreted into blood, but has no known biological activity. (Patrik Rorsman, 2005)

1.1.9.3 The insulin gene

The insulin gene is evolutionary remarkably conserved across species, and diverged from its sister molecule insulin-like growth factor-1 (IGF-1) early in the course of chordate evolution. The human gene lies on the short arm of chromosome 11. The regulation of insulin gene expression is of course influenced by glucose but other factors such as Glucagon-Like Peptide-1 (GLP-1) and Growth Hormone play a part. The product of the insulin gene is the elongated, single-chain preproinsulin (98 amino acids) which is processed in the rough endoplasmatic reticulum to proinsulin, by removal of the so-called signal peptide (12 amino-acids). In the endoplasmatic reticulum, the single chain proinsulin folds back onto itself, aligning the future A- and B-chain and creating the disulfide bonds in this process. The A-chain and B-chain are still connected by the Connecting Peptide (C-peptide). In the Golgi-complex, the proinsulin is stored in so-called beta-granules. These contain the proteolytic enzymes that will cleave and remove the C-peptide from proinsulin, resulting in equimolar amounts of insulin and C-peptide in the mature beta-granule.

1.1.9.4 Insulin secretion

The mature beta-granules form a large storage pool for insulin, well in excess of the daily requirement. Insulin is released into the circulation by fusion of the granules with the beta-cell membrane and exocytosis. A series of events triggers insulin secretion. Physiologically, glucose enters the beta-cell through an insulin independent process (probably involving the glucose transporter 1, GLUT-1). There it is phosphorylated by the enzyme glucokinase and metabolized through glycolysis and entry into the mitochondrial TCA cycle. This results in the generation of ATP which is transferred back to the cytosol and increases the ATP/ADP ratio. This increased ATP/ADP ratio leads to closure of the ATP-dependent potassium channel (K_{ATP} channel) which leads to depolarization of the beta-cell membrane. The depolarization of the cell membrane activates voltage-sensitive Ca^{2+} channels, leading to an influx of Ca^{2+} into the cell. This forms the final trigger for insulin exocytosis. The granule membrane is recycled to the Golgi apparatus following release of insulin. (Hales, C.N. and Barker, D.J., 1992)

1.1.9.5 Mechanism of Insulin secretion

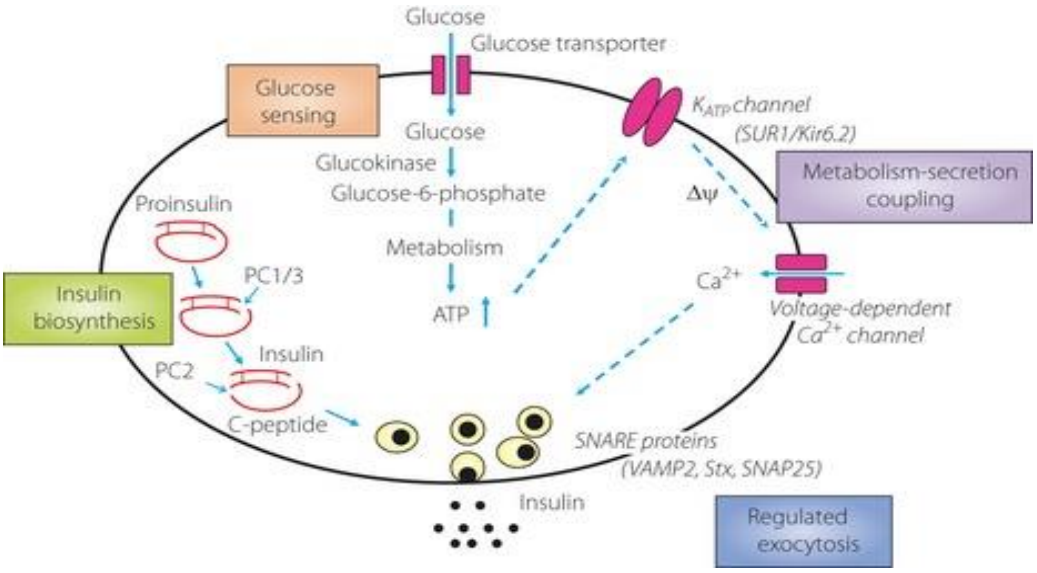

Figure 1.11: Mechanism of Insulin Secretion

(Kohtaro Minami, 2013)

1.1.9.6 Insulin degradation

Insulin has a short half-life in the circulation following release, estimated at 4-6 minutes, allowing minute-to-minute regulation of metabolism. Circulating insulin is cleared by the liver as it passes through the portal circulation, which means that portal levels of insulin are higher than those in the systemic circulation. The kidney is largely responsible for insulin clearance in the systemic circulation, and delayed insulin clearance may cause problems with control in those with kidney disease. Some degradation occurs within the insulin granule, and insulin is degraded in other tissues after binding to the insulin receptor. In this receptor-mediated degradation, the insulin-insulin receptor complexes come together on the plasma membrane of the target cell, forming groups that are sequestered in so-called coated-pits. These invaginate to fuse with intracellular lysosomes, in which the insulin is enzymatically degraded. **(**Duckworth, et al 1998)

1.1.9.7 Control of Insulin Secretion

Insulin is secreted in primarily in response to elevated blood concentrations of glucose. This makes sense because insulin is "in charge" of facilitating glucose entry into cells. Some neural stimuli (e.g. sight and taste of food) and increased blood concentrations of other fuel molecules, including amino acids and fatty acids, also promote insulin secretion.

- Glucose is transported into the beta cell by facilitated diffusion through a glucose transporter; elevated concentrations of glucose in extracellular fluid lead to elevated concentrations of glucose within the beta cell.
- Elevated concentrations of glucose within the beta cell ultimately lead to membrane depolarization and an influx of extracellular calcium. The resulting increase in intracellular calcium is thought to be one of the primary triggers for exocytosis of insulin-containing secretory granules. The mechanisms by which elevated glucose levels within the beta cell cause depolarization is not clearly established, but seems to result from metabolism of glucose and other fuel molecules within the cell, perhaps sensed as an alteration of ATP:ADP ratio and transduced into alterations in membrane conductance.
- Increased levels of glucose within beta cells also appear to activate calcium-independent pathways that participate in insulin secretion.

Stimulation of insulin release is readily observed in whole animals or people. The normal fasting blood glucose concentration in humans and most mammals is 80 to 90 mg per 100 ml, associated with very low levels of insulin secretion.

The figure depicts the effects on insulin secretion when enough glucose is infused to maintain blood levels two to three times the fasting level for an hour. Almost immediately after the infusion begins, plasma insulin levels increase dramatically. This initial increase is due to secretion of preformed insulin, which is soon significantly depleted. The secondary rise in insulin reflects the considerable amount of newly synthesized insulin that is released immediately. Clearly, elevated glucose not only simulates insulin secretion, but also transcription of the insulin gene and translation of its mRNA. (Porte, 1973)

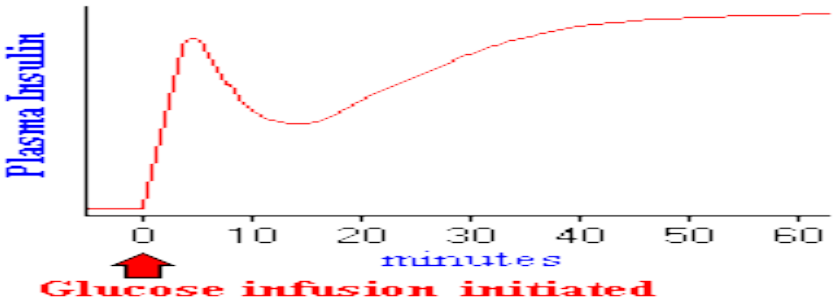

Figure 1.12: The effects on insulin secretion

1.1.10 Drugs used as Antidiabetic agents

- **Insulin secretagogues agent: Sulfonylureas (glibenclamide, gliclazide, glipizide, glimepiride)**

The sulphonylureas (SUs) were initially developed in the 1920s and have become indispensable in the management of type 2 DM.

The mechanism of action involves a direct secretory effect on the pancreatic islet beta-cells. Adenosine triphosphate (ATP)-sensitive potassium channels (K) of the beta-cells play an essential role in the release of insulin and consist of two components: a pore and a regulatory subunit (SUR-1).

The sulphonylureas act to enhance the sensitivity of the beta-cell to glucose and, when bound to the transmembrane sulphonylurea receptor (SUR-1), mediate the closing of the potassium-sensitive ATP channels on the cell membrane. Cellular efflux of potassium is reduced and membrane depolarisation takes place. Calcium influx is mediated by the opening of voltage-dependent Ca channels that promote the release of pre-formed insulin granules which lie just adjacent to the plasma membrane.

- **Rapid-acting prandial insulin releasers (repaglinide, nateglinide)**

The meglitinides stimulate rapid, short-lived insulin release. The mechanism of action of prandial insulin releasers indicate that they bind to the SUR-1 receptor in much the same way as the sulphonylureas.The short half-life of these drugs potentiates the effect of the first phase of insulin secretion, but the effect on the second phase is not sustained.

Repaglinide can be used in patients who do not achieve glycemic control on diet and lifestyle measures alone or who live irregular lifestyles where meals are missed or taken irregularly. These drugs should be taken immediately before meals. The lower risk of hypoglycemia, compared with a sulphonylurea, makes these drugs an attractive choice in elderly patients; they may cause minimal weight gain.

- **Insulin sensitizers: Biguanides (Metformin)**

The mainstay of action of this class of drug can be attributed to its hepatic effects. Hepatic sensitivity to insulin is increased, thereby reducing gluconeogenesis as well as glycogenolysis, which contributes to the post-prandial plasma glucose-lowering effects. Skeletal muscle and adipocytes undergo up-regulation of the insulin-sensitive GLUT-4 and GLUT-1 transporters to the cell membranes, thereby increasing glucose uptake.
Biguanides are generally considered the drugs of choice in obese type 2 diabetics. Metformin can be used in combination with any other class of oral antidiabetic drug or with insulin. Metformin is also used in the treatment of polycystic ovarian syndrome (PCOS) to improve insulin sensitivity and to lower circulating androgen levels. It also improves ovulation and menstrual cycle. The USA's Food and Drug Administration still considers this an unlicensed indication of this drug in the absence of diabetes. The American Association of Clinical Endocrinologists recommends that metformin be considered as the initial intervention in most women with PCOS, particularly those who are obese and overweight.

- **Thiazolidinediones (pioglitazone, rosiglitazone)**

With the introduction of this new class of drug in 1997, the world has watched the peroxisome proliferator activated receptor (PPAR)-γ agonists with anticipation. The net effect of these drugs results from stimulation of a nuclear PPAR-γ that regulates the transcription of genes culminating in an increase in insulin sensitivity.

Thiazolidinediones (TZDs) mediate their function through binding to the PPAR-γ receptor that is expressed predominantly in adipocytes. It is expressed to a lesser extent in muscle and liver tissue. Binding of the PPAR receptor in turn mediates binding to the retinoic-X receptor (RXR-receptor). This heterodimer then binds to a nuclear response element which then switches on gene transcription.

Many of the genes that are activated play a central role in carbohydrate and lipid metabolism. TZDs, like metformin, require the presence of insulin to mediate a blood glucose-lowering effect. Interestingly, the thiazolidinediones also suppress the expression of TNF-α by adipocytes.

- **Glucosidase inhibitors (Acarbose)**

Acarbose was the first glucosidase inhibitor and was introduced to the market in the early 1990s. This class of drug has the advantage of reducing postprandial hyperglycemia without associated weight gain. Its usage is at present hampered by unfortunate gastrointestinal side-effects despite a good safety record. The α-glucosidase inhibitors inhibit the activity of the glucosidase enzymes which are present in the brush border of enterocytes in the intestinal villi. Disaccharide and oligosaccharide cleavage is prevented with a net decrease in intestinal carbohydrate absorption.

Overall, the α-glucosidase inhibitors reduce postprandial insulin concentrations through the attenuated rise in postprandial glucose levels. Less than 2% of the drug is absorbed. It is broken down by intestinal amylases and certain intestinal bacteria. Some degradation products are taken up and subsequently eliminated in the urine.

- **Incretins (exendin-4, liraglutide, vildagliptin, sitagliptin)**

The small intestine secretes glucagon-like peptide-1 (GLP-1) as well as glucose-dependent insulinotropic polypeptide (GIP, previously called gastric inhibitory peptide) in response to food intake. These hormones stimulate insulin secretion, insulin gene expression and pancreatic beta-cell growth. Furthermore, they mediate the incretin effect which augments insulin secretion following oral administration of glucose. The GLP-1 molecule is subject to rapid degradation by the DPP-IV (dipeptidyl peptidase) enzyme.

a) Liraglutide

This drug is currently in phase III of clinical development. The results look extremely promising. In

June 2007, a 26-week study was conducted in which liraglutide was compared with insulin-glargine. This formed part of the greater liraglutide effect and action in diabetes (LEAD) programme. At the end of the 26 weeks, the liraglutide group showed that >50% of patients reached the HbAgoal of <7% as well as an average weight loss of 3.5 kg. This drug, given as a once-daily subcutaneous injection, has a plasma half-life of 12 hours.

b) Vildagliptin

This drug is taken in oral form as a once-daily dosage. Inhibition of dipeptidyl peptidase-IV (DDP-IV) stimulates the secretion of insulin in a glucose-dependent fashion, so minimising possible hypoglycaemic side-effects. Inhibition of DDP-IV is dose-dependent. Recent data suggest restorative effects on pancreatic islet cells, thereby fuelling the hope that the DDP-IV inhibitors could potentially slow or reverse the course of beta-cell failure.

c) Sitagliptin

This drug is also a DDP-IV inhibitor and can be used as monotherapy in type 2 diabetes or in combination with metformin, the SUs or the TZDs if the existing regimen no longer provides adequate glycaemic control. It has not yet been studied in combination with insulin. Sitagliptin is taken orally and has been shown to reduce HbA levels by 0.6 - 1%.

- **Amylin analogues (Pramlintide)**

Human amylin is a 37-amino acid glucoregulatory peptide that is co-secreted with insulin by the pancreatic beta-cells. Pramlintide, a synthetic analogue, exerts its effect by slowing down gastric emptying and increasing satiety. Post-prandially, it decreases glucose levels and reduces the re-introduction of glucose in the circulation.

Pramlintide is administered as a subcutaneous injection immediately before a meal.

1.1.11 Dosage of Anti-diabetic agents

Antidiabetic agent	Recommended dosage and/or administration
Insulin	400 IU per vial - 40 IU per day (mean value)
Gliclazide (Diamicron)	80 mg/tablet - 1 to 4 tablets per day
Glibenclamide (Daonil) or Glyburide (Micronase, Glynase, Diabeta)	5 mg/tablet - 1 to 3 tablets per day (Glibenclamide); 1.25 to 6 mg/tablet - 1 to 2 tablets per day (Glyburide)
Glipizide (Glucotrol, Glibenese)	5 mg/tablet - 1 to 4 tablets per day
Glimepiride (Amaryl, Amarel)	1 to 4 mg/tablet - 6 mg per day maximum
Chlorpropamide (Diabinese)	250 mg/tablet - 125 to 1000 mg per day per day
Tolbutamide	500 mg/tablet - 1 to 4 tablets per day
Repaglinide (Prandin)	0.5 to 16 mg per day

Table 1.2: Drugs used as antidiabetic agents

(Drugs.com, 2017)

1.2 Medicinal Plants as Anti-diabetic

Among the most common chronic diseases in the world, Diabetes mellitus (DM) is an extremely studied and widely manifested multi-factorial disease which deliberately requires multi-modal therapeutic strategies. It has an age-old history of being recognized and even symptomized in various cultures of the world majorly as glycosuria (sweet urine). Hence, the treatment strategies for DM have been in the process of development and documentation since a long time in traditional medicine systems. Back then the nature of drug used to be mostly unorganized and crude. The major difference now in the modern era is that the treatment strategies basically concentrate on identifying, isolating, modifying or searching alternatives of the lead compounds and exact active principles which attribute to the desired therapeutic nature of the plant. The aim of this paper is to acknowledge the various treatment methods available for Diabetes mellitus and to review the Traditional Indian herbs and plants which are most efficiently, safely and widely accepted medicament for DM and source of future lead compounds and family-wise segregation of these plants. This review is in total compliance with the strong and effective traditional medicinal systems of India.

Diabetes mellitus is a global metabolic epidemic affecting essential biochemical activities in almost every age group. Diabetes mellitus is not a single disease but rather a group of metabolic disorders. Hyperglycemia in diabetes results from defect in insulin secretion and or insulin action. Conventionally insulin dependent diabetes mellitus is treated with exogenous insulin, and non-insulin dependent diabetes mellitus is treated with synthetic oral hypoglycemic agents like sulphonyl urea's and biguanides. Synthetic oral drugs produce adverse health effects. Different medicinal systems are using the active plant constituent which discovered as natural hypoglycemic medicine came from virtue of traditional knowledge. Herbal drugs are considered free from side effects than synthetic one.

A large diversity of animal models has been developed to better understand the pathogenesis of diabetes mellitus and new drugs introduced in market to treat this disease. This review also studied the animal model used in testing of drug. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. The active principal present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of

insulin resistance. The ethanobotonical information reports about 800 plants that may possess antidiabetic potential and more than 1200 species of plants have been screened for activity on the basis of ethanopharmacology.

In India indigenous remedies have been used for treatment of diabetes since the time of charaka and sushruta. The World Health Organization has recommended the evaluation of traditional plant treatments for the diabetes. Diabetes mellitus can be induced by pharmacological, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals.

(Saad, 2017)

1.2.1 Traditional herbal anti-diabetics

It is now internationally accepted and acknowledged that traditional medicines systems of India and other ancient origins report, advocate and justify the significance of floral biodiversity as an effective and reliable treatment strategy of hyperglycemia and related malfunctions.

Several disadvantages associated with insulin and synthetic drugs and their failure to divert the course of diabetic complications have opened up tremendous horizons for searching possibilities in complementary and alternative medicine (CAM) for diabetes as well as many other chronic diseases. Plants, herbs and their derivatives owing to their wide spectrum of active principles representing numerous chemical compounds hold promising potentials for their consistent usages in the treatment of Diabetes. According to WHO, 21,000 plants around the globe have been reported for medicinal uses. India is posted to have an enormous medicinal flora of some 25,000 species, out of these 150 species are commercially exploited for medicinal extractions or drug formulation. There are about 800 plants species reported having the probability of possessing antidiabetic potentials in the ethnobotanical surveys. The antidiabetic effects of the plants are attributed to the wide range of chemicals and secondary metabolites. Reports have essayed approximately 200 pure compounds from plant sources to show blood glucose lowering effect. These compounds range vividly in chemical nature like alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoid, triterpenoid, peptides and amino acids, lipids, phenolics, glycopeptides, and iridoids. (Patel, 2012)

1.2.2 Medicinal Plants with reported Antidiabetic Effect on experimental models

Plant(Family)	Part of Plant Used	Material	Result
Annona Sqamosa (Annonaceae)	Fruit peel	Alcohol, ether, ethyl acetate	Significant increase body weight and diminished blood glucose level
Calamus erectus (Arecaceae)	fruit	Methanolic extract	Reduction of blood glucose level
Momordica Charantia (Cucurbitaceae)	Plant	Alcoholic extract	lower the blood sugar level
dactylifera linn (Arecaceae)	dried dates	Aqueous extract	reduction in blood glucose level
Zizyphus nummularia (Rhamnaceae)	Leaves	aqueous and 12% ethanolic extract	reduction in blood glucose level
Swertia Chirata (Gentianaceae)	Whole plant	aqueous and 12% ethanolic extracts	Significant antidiabetic activity
Tamarandus indica Linn (Caesalpiniaceae)	Fruit pulp	ethanolic extracts	Antidiabetic effect
Parmelia Perlata. Ach (Permeliaceae)	Leaves	Aqueous extract	reduced the fasting blood glucose
Psidium guvajava (Myrtaceae)	Leaves	Ethanolic extract	reduction in blood glucose level

Table 1.3: Medicinal Plants with reported Antidiabetic Effect on experimental model (Kirtikar, 1998)

1.3 *Asteracantha longifolia*

Asteracantha longifolia is a source of the ayurvedic drug, 'Kokilaksha' and the Unani drug, Talmakhana. The seeds are acrid, bitter, aphrodisiac, tonic, sedative, used for diseases of the blood. The plant is known to possess antitumor, hypoglycemic, aphrodisiac, antibacterial, free radical scavenging and lipid peroxidation and hematopoietic activity. It contains lupeol, stigmasterol, butelin, fatty acids, and alkaloids. The present review article is focused on phytochemical, pharmacological and other important aspects of Talmakhana.

Figure 1.13: *Asteracantha longifolia*

It is a spiny, stout, annual herb, common in water logged places. Leaves subsessile, oblong-lanceloate or linear lanceolate, spines yellowish brown, 2-3 cm long, Flower yellowish brown, fruit two celled, linear oblong, compressed about 8 cm long, pointed, 4-8 seeded. Seed ovate, flat or compressed, 0.2-0.25 cm long and 0.1-0.15 cm wide, hairy but appearing smooth; when soaked in water immediately get coated with mucilage, light brown: taste slightly bitter and odor not distinct

(Chauhan et al, 2017)

1.3.1 Traditional uses

The urinary infections, edema and gout. It is classified in ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of diabetes, dysentery etc (Nadkarni, 1978). Whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain.

Figure 1.14: *Asteracantha longifolia*

1.3.2 Scientific Classification

Kingdom: Plantae

Division: Angiospermae

Class: Equisetopsida C. Agardh

Order: Personales

Family: Acanthaceae

Genus: *Asteracantha*

Species: *Asteracantha longifolia*

1.3.3 Chemical constituents

The plant contains lupeol, stigmasterol and hydrocarbons, the seed have sterols and the flowers have apigenin glucuronide.

Seeds of this plant are very good aphrodisiac. Ayurveda Acharya's recommend this herb in male infertility. The seeds effectively increase sperm count and sperm motility. Hence the conditions like low sperm count can be improved by use of this herb. The seeds of Kokilaksha are best herbal ayurvedic remedy for Erectile Dysfunction. The erectile dysfunction capsule Rejuzoa from Moolika Ayurveda contains this herb.

Researches have shown that this plant has hypoglycemic activity and improves blood sugar level in diabetic patients. Hence Rejuzoa Capsules which contain kokilaksha are recommended in Vajikarana Therapy for diabetic Patients. It has dual benefits i.e. it controls blood sugar level and also helps to rectify erectile dysfunction.

This plant has very good diuretic properties. It increases urine output. Due to this property Kokilaksha is used in conditions like urinary calculi and cystitis.

(Chauhan et al, 2017)

1.3.4 Phytochemical studies

The phytochemical investigation of the *A. longifolia* as carried out so far contains various compounds with varying structural patterns. *A. longifolia* seed oil pale yellow in color about 23% contain about 72% of linoleic, 10% of oleic, 12% of stearic, and 6% of palmitic and myristic acids. Mineral elements Mn, Mg, Zn, Ca, Fe, Ni, Cr, Na, K and Al were found in the *A. longifolia* determined by using Flame photometer, Atomic Absorption Spectrometer and Inductively Coupled Plasma. Plant also contains minerals Fe, Cu, Co. Root contain stimagsterol. Aerial parts of *A. longifolia* have been reported to contain lupeol, stigmasterol and butelin while the seeds of the plant are reported to contain mainly fatty acids. Petroleum ether extract of *A. longifolia* root found lupeol and lupenone. Misra et al (2001) isolated the two aliphatic esters (25-oxo-hentriacontanyl acetate, and methyl 8-n-hexyltetracosanoate, and betulin from the aerial parts of *A. longifolia*. The HPTLC

estimation of lupeol and sitosterol in various part like root, leaves, seeds and stems was reported in solvent system toluene:ethylacetate:methanol 15:3:1.5 (% v/v). The whole plant contains lupeol, stigmasterol, an isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases. From the seeds isolation of asterol I, II, III, and IV, asteracanthine and asteracanthicine have been reported. Flowers contain apigenin 7-*O*-glucuronide. Also, amino acids histidine, lysine and phenyl-alanine have been detected in the seeds. From the plant collected from Saharanpur, lupeol, betulin and stigmasterol isolated; betulin was found to be absent in aerial parts and stigmasterol in roots.

1.3.5 Pharmacognostic studies

Plants having spines, having perennial root stocks; bluish-purple 2-lipped flower; leaves - sessile, multi-chambered thick-walled sclerotic cell, among the epidermal cells of midrib, large flat bunch-shaped calcium carbonate crystals and needle-shaped calcium oxalate crystals in the epidermal cells of lamina and in the cortical cells of midrib respectively; stomata-caryophyllaceous; stomatal index on upper surface: 23.46±47); lower surface: 27.44±52); palisade ratio: 10.23±19) and vein islet number: 25.8±69).

1.3.6 Tissue culture

High Plant regeneration frequency in *A. longifolia* was achieved from leaf explant implanted on MS basal medium supplemented with NAA (0.5 mg/L) + BA (2.0 mg/L) through intervening callus phase. Protein and total soluble sugar contents were maximum during organogenesis and multiple shoot induction phase compared with non-organogenic callus and root induction phase. Esterase and catalase activities were maximum during organogenic differentiation, while activities were minimum at non-differentiated callus stages. Peroxidase activities were higher during rhizogenesis whereas acid phosphatase activities were high during organogenesis and declined during rhizogenesis (Panigrahi et al., 2007).

1.3.7 Medicinal activity

The medicinal activities are given below-

1.3.7.1 Anti-diabetic (hypoglycemic) effects

Muthulingam investigated the effect of leaf extract of *Asteracantha longifolia* on diabetic rats. It appears that *Asteracantha longifolia* increased insulin secretion which brought glucose back to normal levels. The antidiabetic effect of leaf extracts of *Asteracantha longifolia* may be due to increased release of insulin from the existing beta-cells of pancreas similar to that observed after glibenclamide (antidiabetic drug) administration.

1.3.7.2 Liver Damage Protection

Many animal studies are suggesting that seed as well as root of *Asteracantha longifolia* extract may possess liver damage protection effects.

1.3.7.3 Hematopoietic activity

Petroleum ether extract of root from A. longifolia increases WBC count significantly. Ethanolic extract (100 and 200 mg/kg) of the aerial parts of H. spinosa significantly increased the hemoglobin, hematocrit, RBC and total WBC, as compared with vehicle treated control rat. In anemic male albino rats, the extract significantly increased hemoglobin, hematocrit and RBC count. Petroleum ether and chloroform extract of leaves show hematopoietic activity as it significantly increases erythrocyte count, leukocyte count, and hemoglobin count

(Pawar et al, 2006)

1.3.7.4 Antioxidant Activity

Aqueous extract of leaves of A. longifolia shows potent antioxidant activity in various in vitro model. (Sathya, 2012)

1.3.7.5 Aphrodisiac Activity

The ethanolic extract of seeds shows androgenic as well as improvement of sexual behavior of rat in dose dependent manner, it also improve the histoarchitecture of testis and increase the concentration of sperm count in epididymis and also increase testosterone level

(Chauhan et al, 2017)

1.3.7.6 Miscellaneous activity

Petroleum ether extract of root potentiated the sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone, chlordiazepoxide and protected against strychine-induced convulsions. Preliminary study shows it possess diuretic activity. Aqueous extract of root and leaves cure patient suffering from dropsy .Ethanolic extract of whole plant showed diuretic effects in rats.

1.3.8 Non medicinal use

Talmakhana is used in the preparation of fuchka in Bangladesh. It is a popular snack in Bangladesh.

1.3.9 Mechanism of Action

Scientific findings on the action mechanisms of the plant compounds have proposed many means in which they act to provide the anti-hyperglycemic and anti-hyperlipidemic effects. Some of them relate to their effects on the activity of pancreatic ß cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. Other mechanisms involve improved glucose homeostasis including an increase of peripheral utilization of glucose, an increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycogenic index of carbohydrates, reduction of the effect of glutathione.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant Material

Plant sample of *Asteracantha longifolia* were used for the experiments. They were processed in the laboratory.

2.1.1 Collection of plant

The Plant sample *Asteracantha longifolia* was collected and washed with water several times.

2.1.2 Drying and grinding

The collected plant sample were washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried leaves was then grinded to a powdered form and stored in there refrigerator at +4°C for a few days.

2.1.3 Extraction (Ethanol extraction)

300 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 800 ml of 80% ethanol, sealed and kept for a period of 2 days with occasional shaking and stirring. It was then filtered first by cotton material and twice through whatman filter paper to obtain a finer filtrate. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Eyela n 1000, Tokyo Rikaki Kai Co. Ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65◦C temperature. The separated filtrate was found to be a precipitate of dark green color and the gum my concentrate was designated as the crude ethanol extract. It was then dried in the freeze drier and preserved at +4°C for two weeks.

2.1.4 Extraction Procedure

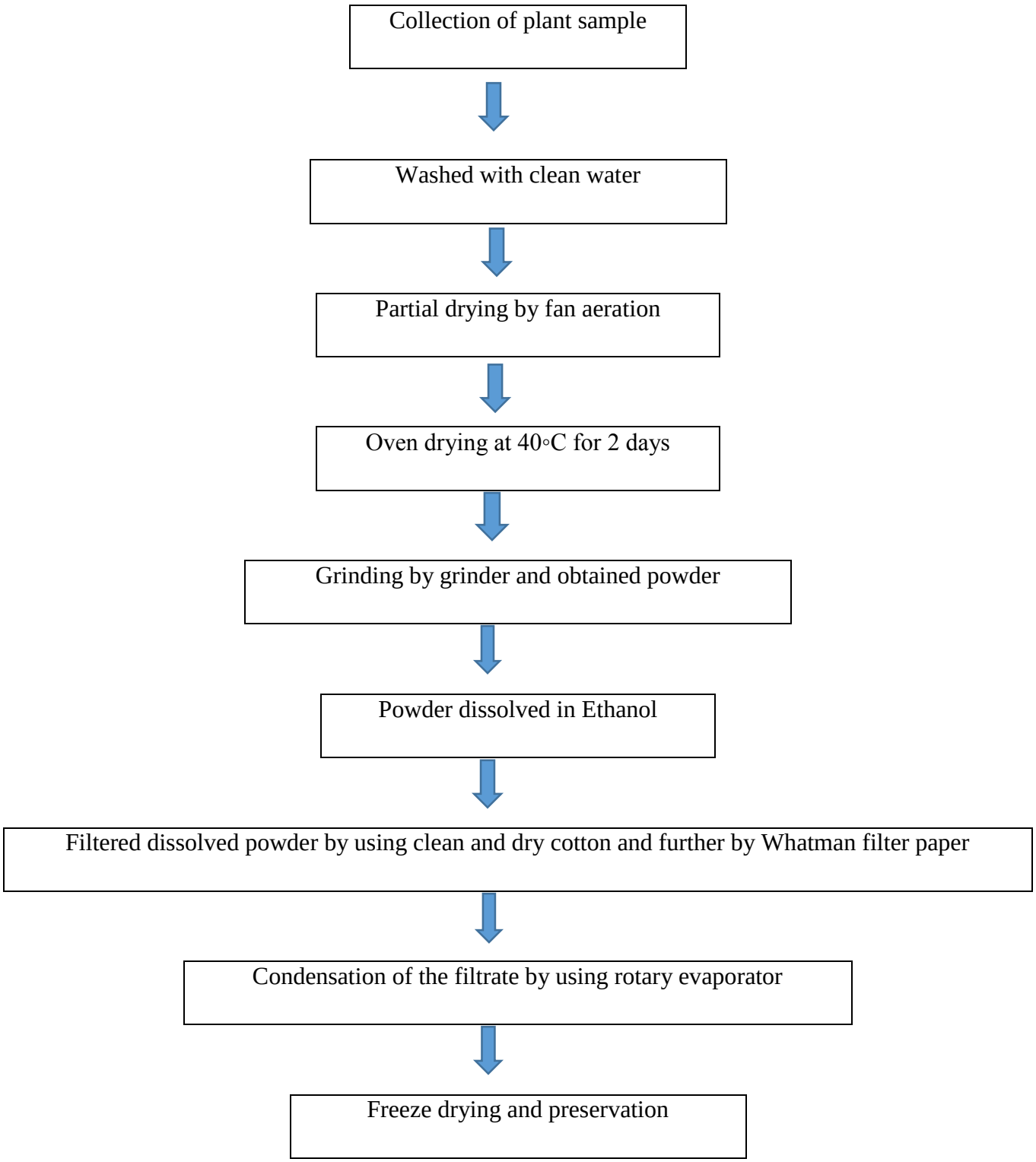

Figure 2.1: General Plant Extraction Procedure

2.2 Experimental animals

Long-Evans rats (male and female), weighing 80-200g of either sex are bred in ICDDR, B and grown in the animal house of the Department of Pharmacy, East West University. All the animals acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2°C, and 12 hours light dark cycle). The animals were fed with standard diet from ICDDR, B and had free access to filtered water (M.K. Sharif et al, 2011)

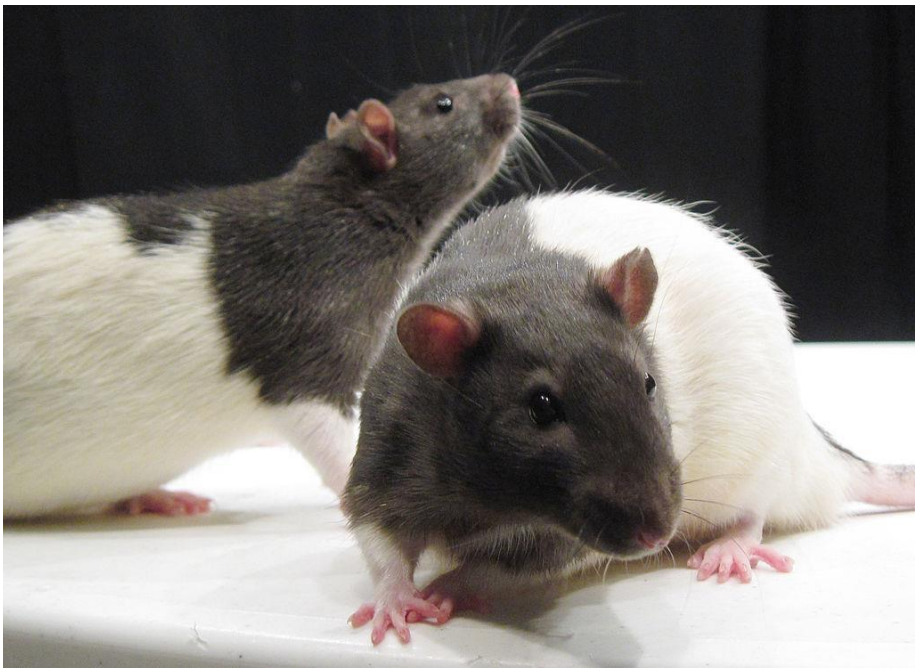

Figure 2.2: Long-Evans rats

2.2.2 Biomedical research

Rats have a prevalence within biomedical research second only to humans and they share 90% of the genome with humans. Almost all disease-linked human genes we currently know of have equivalent genes within the rat genome, making them a suitable research tool.

Figure 2.3: Long-Evans rat

Rats were the first mammalian species specifically domesticated to be used in the laboratory.

Records dating back to the 1850s show these animals were derived from those bred by rat fanciers who collected them for their unique coat colors and behavioral characteristics.

The success of the rat in research today has been linked to the Wistar Institute in America and their development of the Wistar albino strain. There are currently 117 albino strains of the laboratory rat, all of which can be traced genetically back to the one rat, likely to have arisen as a mutation from a hooded (piebald) rat strain. Since their development as a laboratory species, rats have been used to answer a wide range of basic science questions ranging from physiology, immunology, pharmacology, toxicology, nutrition, behavior and learning.

2.3 Screening for the possible inhibition of carbohydrate absorption by plant material

2.3.1 Chemicals and reagents

Normal saline, 2N H_2SO_4, 1N NaOH, Sucrose (2.5g/Kg body weight of rat in 5ml deionized water)

Drug: 100mg/Kg body weight of rat

Kits:

Glucose kit was used for the determination of Glucose.

2.3.2 Procedure

Rats were fasted for 20hours before experiment. Sucrose (2.5g/Kg/5ml, average 443 mg) with or without extract (effective dose of hypoglycemic effect). Each segment was washed out with ice-cold saline (10ml), acidified with H_2SO_4 (2ml) and centrifuged at 3000rpm for 10minutes. The supernatant thus obtained was boiled for 2hours to hydrolyze the Sucrose and then neutralized with NaOH (approximately 2.5ml). The blood glucose level and the amount of Glucose liberated from residual Sucrose in the gastrointestinal tract were measured by Glucose Oxidase (GOD-PAD) Method. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose.

2.3.3 Steps of the experiment

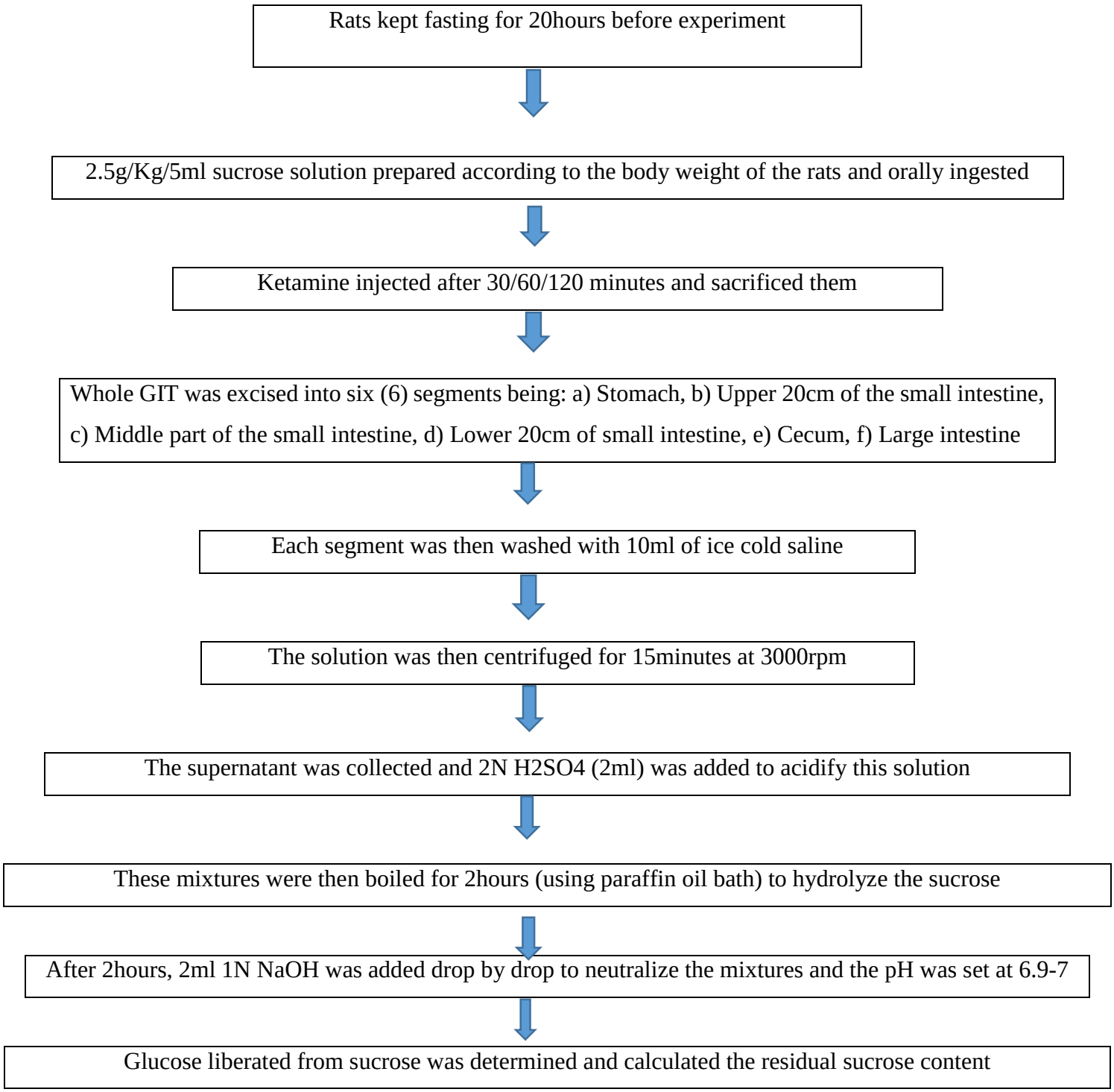

Figure 2.4: Flowchart of the experiment

2.4 Assessment of the effect of plant materials on intestinal disaccharidase activity

2.4.1 Assessment of conditions

All rats were fasted overnight (12hours) before being tested but still allowed free access to distilled water. Extract is administered orally to experiment group and water to control group.

2.4.2 Mucosa/Tissue Collection

After one hour ileocaecal junction. The lumen of the intestine is washed out with 50ml of ice cold saline. Intestine is then placed on ice-cold glass plates over ice and cut longitudinally. The mucosa is isolated bt scrapping of drug administration, rats are anesthetized with pentobarbital-Na/ether, the entire length of the small intestine (from pylorus to ileocaecal junction) is carefully removed from the pylorus to the with glass microscope slides and homogenized with 10ml of saline for 20seconds at medium speed in a Heidolph Diax 600 homogenizer.

2.4.3 Enzyme activities

Disaccharidase activity is assessed using the Dahlqvist method with modifications. Twenty (20) µl of mucosal homogenate were added in duplicate to 40 mM sucrose and incubated at 37◦C for 60minutes. The glucose converted from sucrose and total protein (using Lowry's methods) in the homogenate are measured. Disaccharidase activity will be calculated by glucose concentration converted from sucrose as µmol-mg glucose/protein/h.

2.4.5 Steps of the experiment

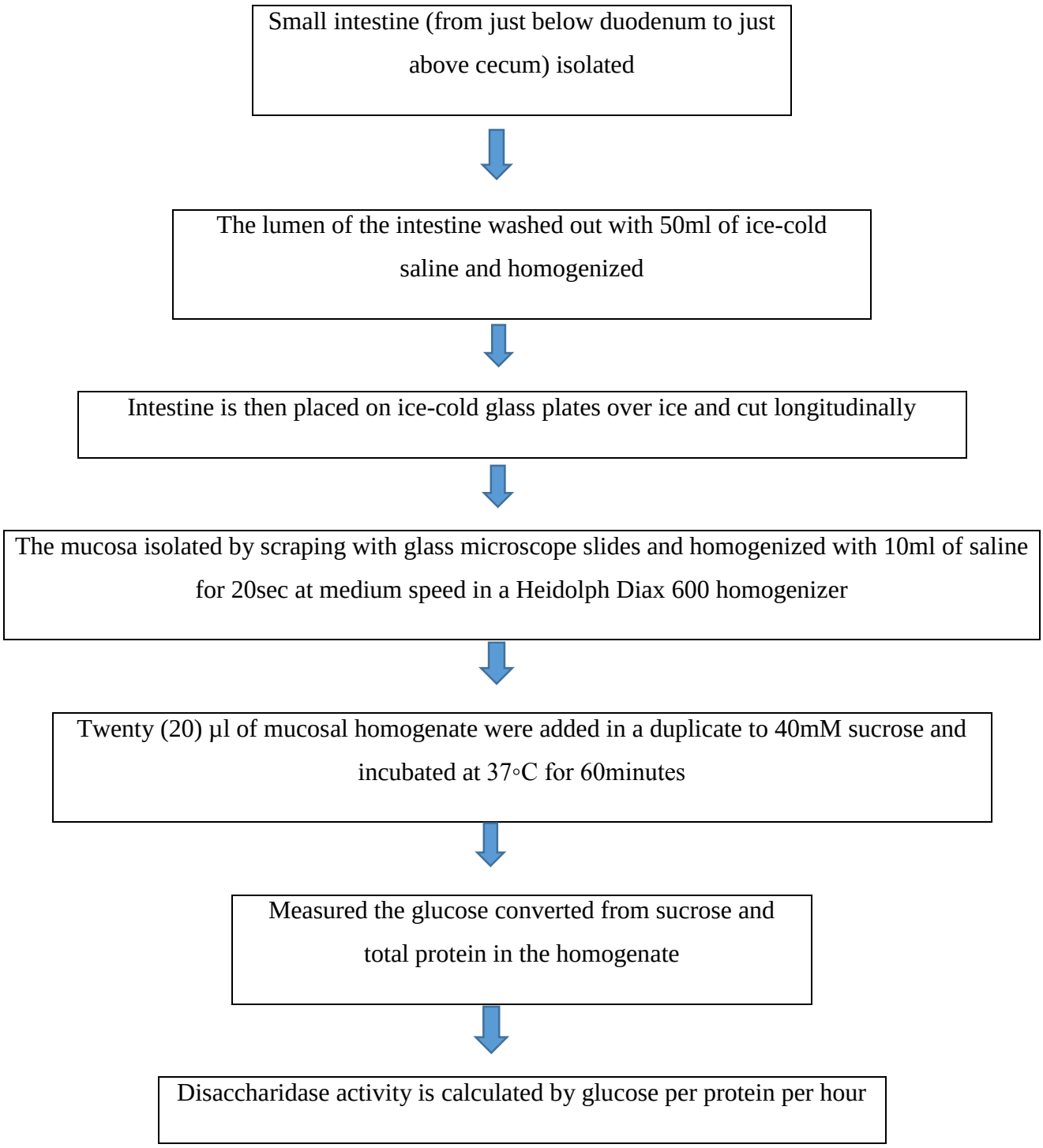

Figure 2.5: Flowchart of the experiment

CHAPTER 3

RESULT

3.1 Effect of *Asteracantha longifolia* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with ***A. longifolia*** (100mg/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1hour.

		30 min	60 min	120 min	240 min
Stomach	Control	58.3±8.5	35.3±5.9	9.1±1.4	1.1±0.3
	Asteracantha longifolia	53.8±9.5	40.2±7.3	11.1±2.1	1.4±0.4
Upper	Control	13.9±2.3	10.2±1.8	4.9±1.1	1.7±0.4
	Asteracantha longifolia	14.2±2.1	10.3±1.9	5.6±0.9	2.1±0.6
Middle	Control	20±4.3	16±4.2	7.5±1.8	1.3±0.3
	Asteracantha longifolia	21.3±6.6	16.4±4.1	6.8±0.9	1.5±0.5
Lower	Control	2±0.5	4.5±1.1	1.7±0.4	1±0.3
	Asteracantha longifolia	2.2±0.6	4.8±0.9	1.6±0.3	1.2±0.2
Caecum	Control	1.6±0.5	1.7±0.3	1.6±0.4	1.8±0.5
	Asteracantha longifolia	2.1±0.5	2.2±0.4	1.9±0.4	1.6±0.3
Large Intestine	Control	1±0.3	0.9±0.3	1.2±0.2	1.1±0.2
	Asteracantha longifolia	0.9±0.3	1.0±0.3	1.1±0.2	1.3±0.4

Table 3.1: unabsorbed sucrose content (mg) in the gastrointestinal tract after sucrose load

Data are presented as Mean±SEM; n=4. Data values are significantly different from the corresponding values of the CONTROL group at $p<0.05$

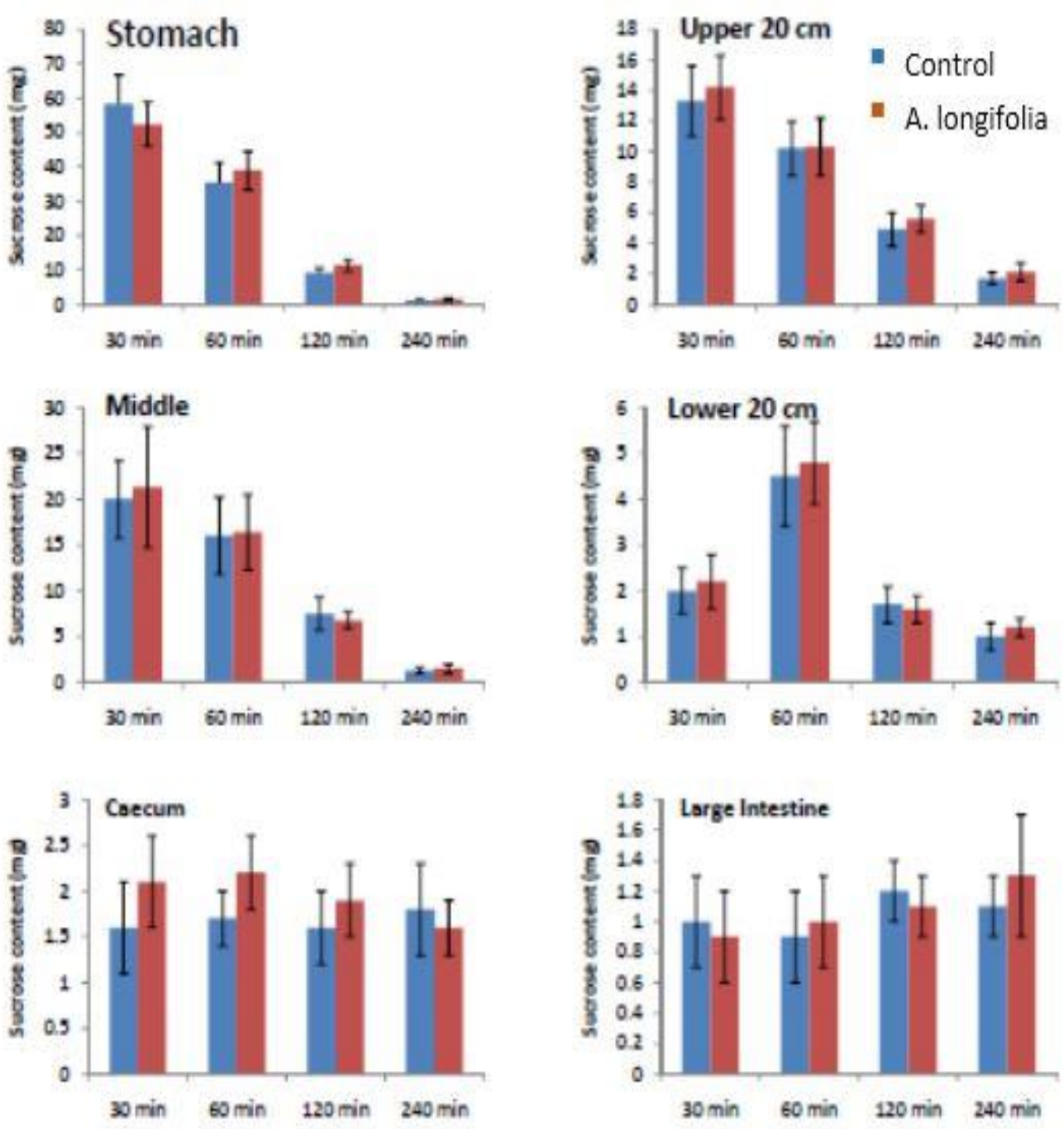

Figure 3.1: Effect of *Asteracantha longifolia* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

3.2 Effect of *A. longifolia* on Intestinal Disaccharidase Enzyme Activity

Asteracantha longifolia extract showed significant inhibition ($p<0.05$) of disaccharidase enzyme activity.

Groups	Disaccharidase activity (µmol/mg/h)	SEM
Control	1.6	.2
A. longifolia	1.06	.1
Acarbose	1.09	.1

Table 3.2: Disaccharidase enzyme activity of *Asteracantha longifolia*

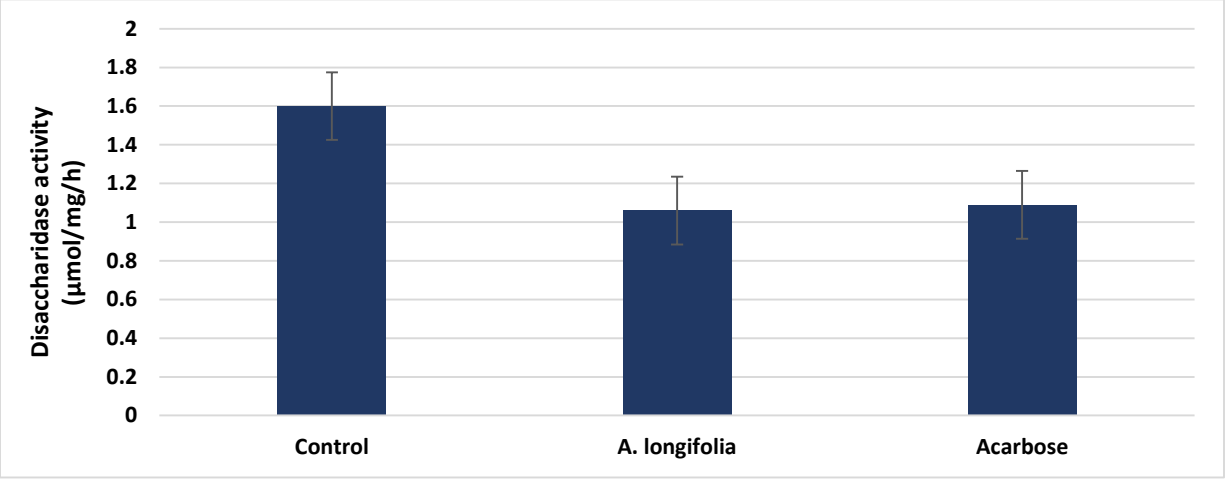

Figure 3.2: Effects of ethanol extract of ***A. longifolia*** on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of ***A.***

longifolia (100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 mg/Kg) was used as reference control for disaccharidase activity test. Values are means and standard deviations represented by vertical bars (n=12). It significantly decreased ($p<0.05$) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

CHAPTER 4

DISCUSSION

4. Discussion

Renewed attention in alternative medicines and natural therapies has led to a revived interest in the use of traditional plants for the treatment of diabetes. In this regard the screening of plant materials for hypoglycemic properties is important as it might provide a new lead(s) as antidiabetic agent(s). *Asteracantha longifolia* has been using as an antidiabetic agent for a long time. Efficacy of this plant in the treatment of diabetes has been studied in details. In the present study, this plant was selected to explore the mechanism of action in Long Evans rat.

In previous studies it has been found that *Annona squamosal* helped in total control of diabetes. In the present study we explored the extra pancreatic action of the plant in Long-Evans rats.

In six segment method, the sucrose extract solution was administered to the model rat, water and sucrose was administered to the control. Then after 30 minutes, 60 minutes, 180 minutes and 360 minutes the rats were sacrificed to observe the amount of sucrose remaining in the gastrointestinal tract. From the result we can deduce that the extract of the leaf of *Asteracantha longifolia* was capable to cause a decrease in the amount of unabsorbed sucrose from the gastrointestinal tract.

The results obtained from both six-segment method and Intestinal Disaccharidase Enzyme Activity test significantly demonstrates, more conclusively, that the ethanol extract of *Asteracantha longifolia* can be effective in diabetic treatment.

4.3 Conclusions

The present study has evaluated potential antidiabetic activity of *Asteracantha longifolia,* traditionally used in the treatment of DM. The experiment carried out showed positive hypoglycemic effects of the plant. Hopefully this will provide as a lead to carry out further investigation to assess whether or not *Asteracantha longifolia* extracts may be used commercially.

CHAPTER 5

REFERENCE

5. References

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