Evaluation of the Pharmacological Effect of Methanolic Extract of *Heritiera fomes* on Hyperglycemia in Laboratory Animals

"This dissertation is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy"

Submitted by

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December, 2017

DECLARATION BY THE CANDIDATE

I, Md. Iqbal Hossain Nayan, hereby declare that this dissertation, entitled **"Evaluation of the pharmacological effect of methanolic extract of *Heritiera fomes* on hyperglycemia in laboratory animals"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy, is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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CERTIFICATION BY THE SUPERVISOR

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Dedication

This research paper is dedicated to my respected Parents and loving Friends

Abstract

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes mellitus. Our aim was to explore the activity of the methanolic extract of *Heritiera fomes* on hyperglycemia in *Long Evans* rat and anti-diarrhoeal or laxative effect of the methanolic extract of *Heritiera fomes* in *swiss albino* mice model with a view to explore its use for the treatment of diabetes mellitus in humans. The present studies were focused on the probable anti-hyperglycemic activity and anti-diarrheal or laxative activity of the plant *Heritiera fomes.* The plant extract was subjected to anti-hyperglycemic study through assessing disaccharidase activity test, and carbohydrate absorption test which was performed to assess the amount of sucrose remaining at six different positions in the GIT. It seems to act by significantly ($p<0.05$, $p<0.01$, $p<0.001$) decreasing the digestion and absorption of carbohydrates in the stomach, brush border membrane of intestinal epithelial cells and flatten the prosprandial glucose rise without giving extra load on beta cells to secret insulin. The Gut motility activity was assessed by measuring the movement of $BaSO_4$ milk through the small intestine of *swiss albino* mice model. Bisacodyl was used as standard drug for GI motility test because of its potent action as anti-diarrheal drug, and acarbose was used as standard drug for disaccharidase activity test because of its potent inhibitory effect on disaccharidase which was recently proved by different research. In conclusion, we can say that our present findings suggest that methanolic extracts of *Heritiera fomes* root and bark contain anti-hyperglycemic principles and it is expected that this plant can be put to further investigation for the development of potent anti-diabetic drugs.

Keywords: *Heritiera fomes,* Diabetes, Anti-Hyperglycemic drug, Hyperglycemia, Insulin, Disaccharidase activity, Carbohydrate absorption, Six segment, GI motility, Constipation, Anti-diarrheal, Brush border membrane, Porsprandial hyperglycemia.

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Chapter 1

Introduction

1.1 Diabetes - An Inception

Diabetes, often referred to by physicians as Diabetes Mellitus, describes a group of metabolic diseases in which the person has high blood glucose level (blood sugar), either because insulin production is inadequate, or the body's cells do not respond to insulin properly, or both. Patients with high blood sugar level will typically experience frequent urination and they will become increasingly thirsty and hungry.

1.2 Diabetes Mellitus [American Diabetes Association, 2001; Perring et al., 1985; Clements & Bill, 1986; WHO, 2002]

Diabetes is a problem with our body that causes blood glucose (sugar) levels to rise higher than normal. This is also called hyperglycemia. When we eat, our body breaks food down into glucose and sends it into the blood. Insulin then helps in moving the glucose from the blood into our cells. When glucose enters our cells, it is either used as fuel for energy right away or stored for later use. In a person with diabetes, there is a problem with insulin. But, not everyone with diabetes has the same problem. (American Diabetes Association, 2001)

Diabetes Mellitus is a heterogeneous group of metabolic disorders characterized byhyperglycemia resulting from defects in insulin secretion, insulin action, or both.

A consequence of the disease is adverse effects on both the macrovascular and microvascular system. Diabetic complications associated with macrovascular diseases are atherosclerotic macrovascular disease and ischemic coronary heart disease. Diabetic complications related to microvascular disease include retinopathy, nephropathy, neuropathy, and peripheral vascular diseases. (Perring et al., 1985)

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)

1.2.1 Etymology [Dallas and John 2011; Harper and Douglas, 2010; Adams, 1972; www.wikipedia.org, Accessed on 2017]

The word diabetes comes from Latin diabētēs, which in turn comes from Ancient Greek ‘diabētēs’ which literally means "a passer through; a siphon, ultimately, the word comes from Greek ‘diabainein’, meaning "to pass through."Ancient Greek physician Aretaeus of Cappadocia (fl. 1st century CE) used that word, with the intended meaning "excessive discharge of urine", as the name for the disease (Adams, 1972).It is composed of dia, meaning "through" and bainein, meaning "to go" (Harper and Douglas, 2010).

The word "diabetes" is first recorded in English, in the form diabete, in a medical text written around 1425. The word mellitus comes from the classical Latin word mellītus, meaning "mellite" i.e. sweetened with honey; honey-sweet. It was Thomas Willis who in 1675 added "mellitus" to the word "diabetes" as a designation for the disease, when he noticed the urine of a diabetic had a sweet taste called glycosurea (Dallas and John, 2011).This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians, and Persians. (www.wikipedia.org, Accessed on 2017)

1.2.2 History of Diabetes Mellitus [Leutholtz and Ripoll, 2011; Leonid and Poretsky, 2009; Ahmed, 2002]

Clinical features similar to diabetes mellitus were described 3000 years ago by the ancient Egyptians. Diabetes is one of the first diseases described with an Egyptian manuscript from 1500 BCE mentioning too great emptying of the urine. The term "diabetes" was first coined by Araetus of Cappodocia (81-133AD). Later, the word mellitus (honey sweet) was added by Thomas Willis (Britain) in 1675 after rediscovering the sweetness of urine and blood of patients (first noticed by the ancient Indians). It was only in 1776 that Dobson (Britain) firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness. (Leutholtz and Ripoll, 2011)

In modern time, the history of diabetes coincided with the emergence of experimental medicine. An important milestone in the history of diabetes is the establishment of the role of the liver in glycogenesis, and the concept that diabetes is due to excess glucose production Claude Bernard (France) in 1857. The role of the pancreas in pathogenesis of diabetes was discovered by Mering and Minkowski (Austria) in 1889. Later, this discovery constituted the basis of insulin isolation and clinical use by Banting and Best (Canada) in 1921. Trials to

prepare an orally administrated hypoglycemic agent ended successfully by first marketing of tolbutamide and carbutamide in 1955. This report will also discuss the history of dietary management and acute and chronic complications of diabetes. (Leonid and Poretsky, 2009; Ahmed, 2002)

1.3 Types of Diabetes

The most common types of Diabetes Mellitus are type 1 and type 2 diabetes. There are some other types of Diabetes seen as well.

1.3.1 Type 1Diabetes Mellitus [American Diabetes Association, 2001; Eisenbarth, 1986, Rossini et al., 1993]

Type 1 diabetes, defined by an absolute requirement for administration of exogenous insulin, results from the autoimmune destruction of the insulin-secreting pancreatic cells.

In type 1 diabetes, our immune system mistakenly destroys the beta-cells, which are the cells in our pancreas that make insulin. Our body treats these beta-cells as foreign invaders and destroys them. The destruction can happen over a few weeks, months, or years. When enough beta cells are destroyed, our pancreas stops making insulin, or makes so little insulin that you need to take insulin to live. (American Diabetes Association, 2001; Eisenbarth, 1986).

Type 1 diabetes is a severe form associated with ketosis in the untreated state. It arises most commonly in juveniles but occasionally in non-obese adults and elderly. It is a catabolic disorder in which circulating insulin is virtually absent with elevated level of plasma glucagon. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis and reduce the elevated blood glucose level. It is thought to result from an infectious or toxic environmental-induced autoimmune disorder. Autoimmunity has been proposed to be the main reason for cell destruction associated with type 1 diabetes. (Rossini et al., 1993)

1.3.2 Type 2Diabetes Mellitus [American Diabetes Association, 2001; Rodger, 1991]

Type 2 or non-insulin-dependent diabetes mellitus is characterized by a relative insulin deficiency due to predominantly an insulin secretory defect with insulin resistance.

In this case, the pancreas retains some β-cell function but effective insulin response is inadequate for the glucose level. Actual insulin levels may be normal but it is ineffective (insulin resistance). Those diagnosed with Type 2 Diabetes are typically overweight or obese, have a positive family history of diabetes. (American Diabetes Association, 2001).

Type 2 diabetes represents a heterogeneous group of disorders comprising milder forms of diabetes that occur predominantly in adults but occasionally in adolescents. Circulating exogenous insulin is sufficient to prevent ketoacidosis but is often either subnormal or relatively inadequate because of tissue insensitivity.

Obesity, which generally results in an impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type 2 are obese. Genetic factors also underlie the disease. (Rodger, 1991).

1.3.3 Differences between Type 1 and Type 2 Diabetes Mellitus

[Barski, 2013; Cold et al., 2017]

Table 1.1: 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.

1.3.4 Other types of Diabetes [American Diabetes Association, 2001; Landon & Gabbe, 1988]

☙ Gestational Diabetes

Gestational diabetes (GDM) is the diabetes that develops during pregnancy. For most women, blood glucose levels will return to normal after giving birth. If a woman had GDM, she needs to be tested regularly since she will be at much higher risk for developing type 2 diabetes later in life. (American Diabetes Association, 2001)

☙ Secondary Diabetes

Occurs when the diagnosis of diabetes is a result of other disorders (e.g., Cushing syndrome, acromegaly, cystic fibrosis, Down's syndrome, pancreatic disorders) or treatments (e.g. administration of glucocorticoids, antipsychotics).

☙ Prediabetes

Individuals who have elevated blood glucose levels that do not meet diagnostic criteria for diabetes, but that are too high to be considered normal, are classified as having prediabetes. Prediabetes is a high-risk category for the future development of Type 2 Diabetes. (Landon & Gabbe, 1988)

1.4 Causing Factors ofDiabetes Mellitus [American Diabetes Association,2001; WHO, 1998]

Diabetes is presumed to be caused by some identifiable etiologies such as:

- → Genetic defects of beta cell function (e.g. MODY 1, 2,3)
- → Genetic defects in insulinaction
- → Diseases of the exocrine pancreas (e.g. cancer of the pancreas, cystic fibrosis,pancreatitis)
- → Endocrinopathies (e.g.Cushing Syndrome)
- → Drug or chemical induced (e.g.steroids)
- → Infection (e.g. rubella, Coxsackie,CMV)
- → Uncommon forms of immune-relateddiabetes
- → Other geneticsyndromes.

In 1985 fibrocalculus pancreatic diabetes (FCPD) was grouped as a subtype of malnutrition related diabetes mellitus (MRDM) by the WHO study group on diabetes mellitus (WHO, 1998). However, the ADA Expert Committee on diagnosis and classification of diabetes mellitus suggested it as secondary diabetes and termed it as fibrocalculous pancreatopathy.(American Diabetes Association,2001)

1.5 Epidemiology of Diabetes Mellitus [World Health Organization, 2001; Wareham, 2014]

WHO estimates that 347 million people worldwide suffer from diabetes. In the region of Africa, around 20 million are living with diabetes. This figure is expected to double by 2030. In comparison to other world regions, Africa has the highest percentage of undiagnosed diabetes cases reaching 62% and the lowest diabetes related health expenditure. (World Health Organization, 2001).

Globally, an estimated 422 million adults are living with diabetes mellitus, according to the latest 2016 data from the World Health Organization (WHO). Diabetes prevalence is increasing rapidly; previous 2013 estimates from the International Diabetes Federation put the number at 381 million people having diabetes. The number is projected to almost double by 2030. Type 2 diabetes makes up about 85-90% of all cases. Increases in the overall diabetes prevalence rates largely reflect an increase in risk factors for type 2, notably greater longevity and being overweight or obese. (World Health Organization, 2001)

Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, occurring in low- and middle-income countries including in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, including increasingly sedentary lifestyles, less physically demanding work and the global nutrition transition, marked by increased intake of foods that are high energy-dense but nutrient-poor (often high in sugar and saturated fats, sometimes referred to as the Western pattern diet). The risk of getting type 2 diabetes has been widely found to be associated with lower socio-economic position across countries. (Wareham, 2014)

The WHO declared that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death. However another 2.2 million deaths worldwide were attributable to high blood glucose and the increased risks of associated complications (e.g. heart disease, stroke, kidney failure), which often result in premature death and

are often listed as the underlying cause on death certificates rather than diabetes. (World Health Organization, 2001)

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.One is more likely to develop type 2 diabetes at the age 45 or older, have a family history of diabetes, or is overweight. Physical inactivity, race, and certain health problems such as high blood pressure also affect your chance of developing type 2 diabetes. You are also more likely to develop type 2 diabetes if you have prediabetes or had gestational you were pregnant. Learn more about risk factors for type 2 diabetes. (World Health Organization, 2001)

1.6 Signs and Symptoms of Diabetes Mellitus

[www.medicalnewstoday.com, Accessed on 2017]

- → Acute hyperglycemia that includes polyuria, polydipsia, polyphagia, weight loss, blurred vision, fatigue, headache, and poor wound healing
- → Chronic hyperglycemia that can lead to damage and potential failure of various organs, including the eyes, heart, kidneys, blood vessels, and nerves.
- → The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes.
- → Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes.

→ A number of skin rashes that can occur in diabetes are collectively known as Diabetic Dermadromes.

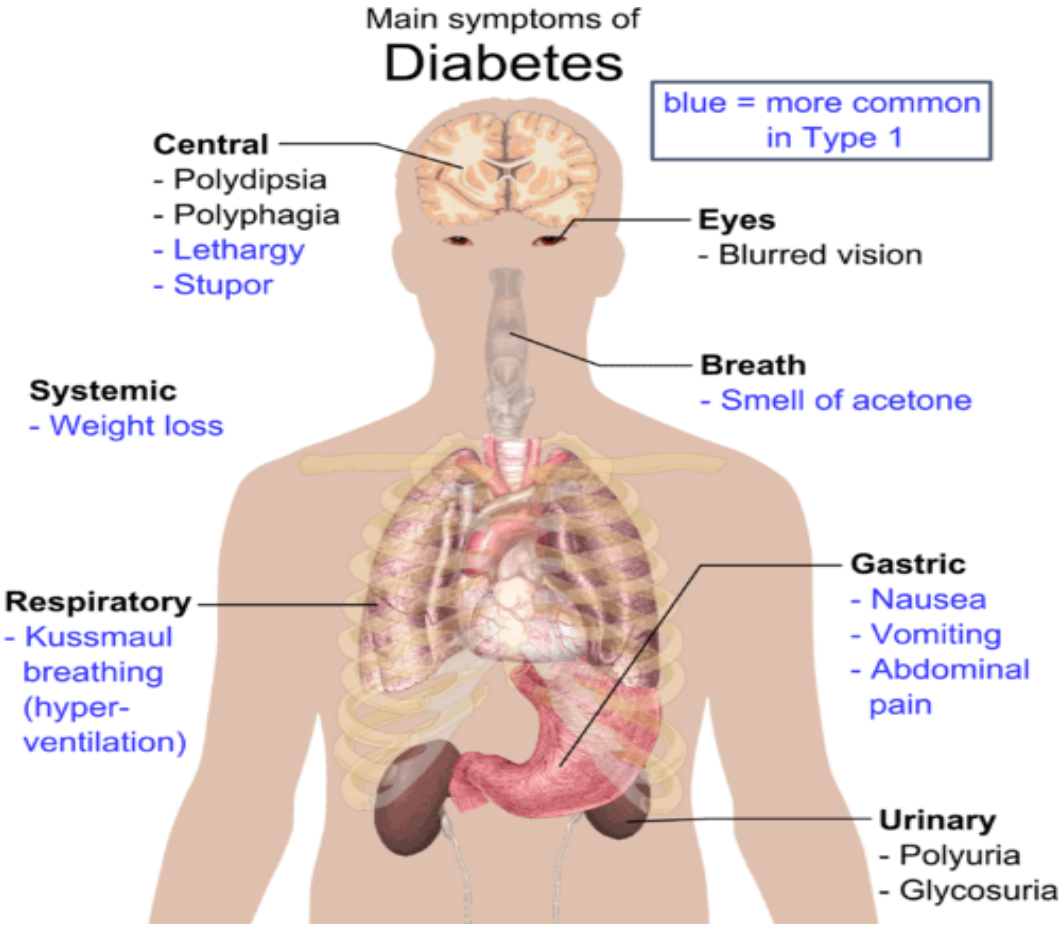

Figure-1.1 Symptoms of diabetes by Mikael Häggström

1.7 Complications linked to badly controlled Diabetes[Donnelly et al., 2000; Pessin & Saltiel,2000]

Diabetes is a complex heterogeneous disease where multiple levels of abnormalities are present in various tissues. Defects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The major long-term complications of diabetes mellitus are macrovascular diseases such as coronary and peripheral vascular diseases & microvascular diseases such as nephropathy, retinopathy and neuropathy.

Complications	Related Effects
Eye complications	Glaucoma, Cataracts, Diabetic retinopathy, and someothers.
Foot complications	Neuropathy, Ulcers, and sometimes gangrene which may require that the foot beamputated
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. Stroke canoccur.
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 mentaldisorders
Hearing loss	Diabetes patients have a higher risk of developing hearingproblems
Gum disease	There is a much higher prevalence of gum disease among diabetespatients
Gastroparesis	The muscles of the stomach stop workingproperly
Ketoacidosis	A combination of ketosis and acidosis; accumulation of ketone bodies and acidity in theblood.
Neuropathy	Diabetic neuropathy is a type of nerve damage which can lead to several differentproblems.
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 kidneydisease
PAD (peripheral arterial disease)	Symptoms may include pain in the leg, tingling and sometimes problems walkingproperly
Erectile dysfunction	Male impotence.
Infections	People with badly controlled diabetes are much more susceptible toinfections
Healing of wounds	Cuts and Lesions take much longer to heal.

1.8 Glucose Homeostasis[www.endocrineweb.com, Accessed on 2017]

Glucose is the primary source of energy for the body as well as the only source of energy for the brain. Glucose homeostasis is maintained by a number of hormones:

✍ Insulin

It is secreted by pancreatic β-cells, when blood glucose concentration rises. Insulin reduces blood glucose levels either by inhibition of hepatic glucose production (Glycogenolysis and Gluconeogenesis) or by increasing glucose uptake into liver, muscle, and fat tissue.

✍ Glucagon

It is secreted by pancreatic α-cells in response to low concentrations of glucose. It acts principally at the liver and antagonizes the effects of insulin by increasing Glycogenolysis and Gluconeogenesis.

✍ Other hormones

These include Amylin, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Amylin is actually co-secreted with insulin from β-cells and functions by slowing gastric emptying and promoting satiety. GLP-1 and

GIP are incretins or gut-derived peptides and primary function is to promote the synthesis and secretion of insulin.

1.9 Glucose Transporters[Stuart and Paul, 2003]

Glucose transporters are a wide group of membrane proteins that facilitate the transport of glucose over a plasma membrane. Because glucose is a vital source of energy for all life, these transporters are present in all phyla. The glucose transporters are a family of membrane-bound Glycoproteins divided into two main types:

∅ Sodium-Glucose Co-Transporters (SGLT)

It is Present in the absorptive epithelial cells of the intestines and the brush border membrane of the kidney. It transports glucose against its concentration gradient. (Stuart and Paul, 2003)

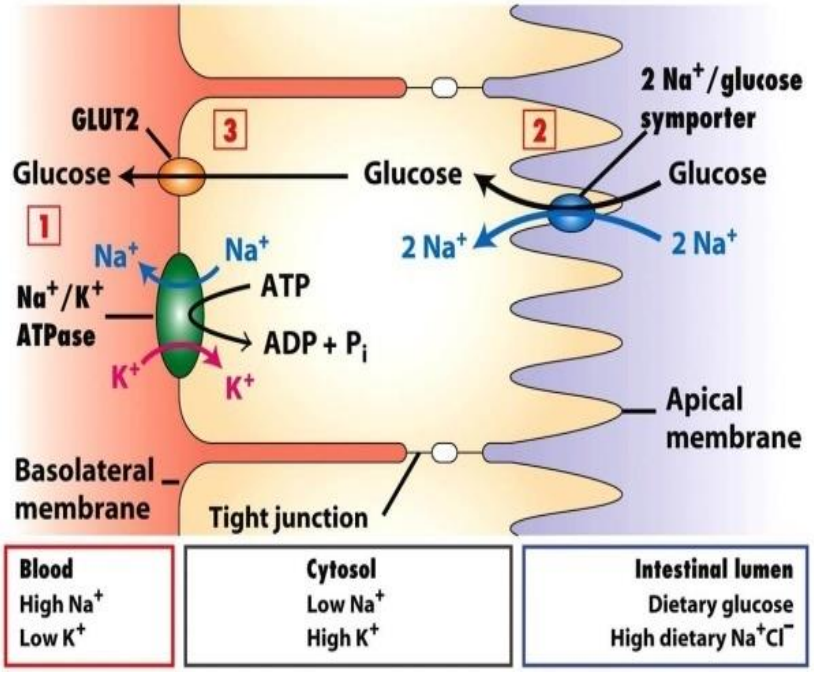

Figure 1.2: Sodium Glucose Co-Transporter

☙ Facilitative Glucose Transporters (GLUT)

GLUT4 transporter is by far the most abundant type, is expressed in adipose and muscle and is responsible for insulin-stimulated transport of glucose. When insulin binds to and activates its cell surface receptors, the intracellular vesicles, migrate toward the plasma membrane. These vesicles fuse with the membrane, and the transporters orient themselves such that they become channels through which glucose can enter the cell. (Stuart and Paul, 2003)

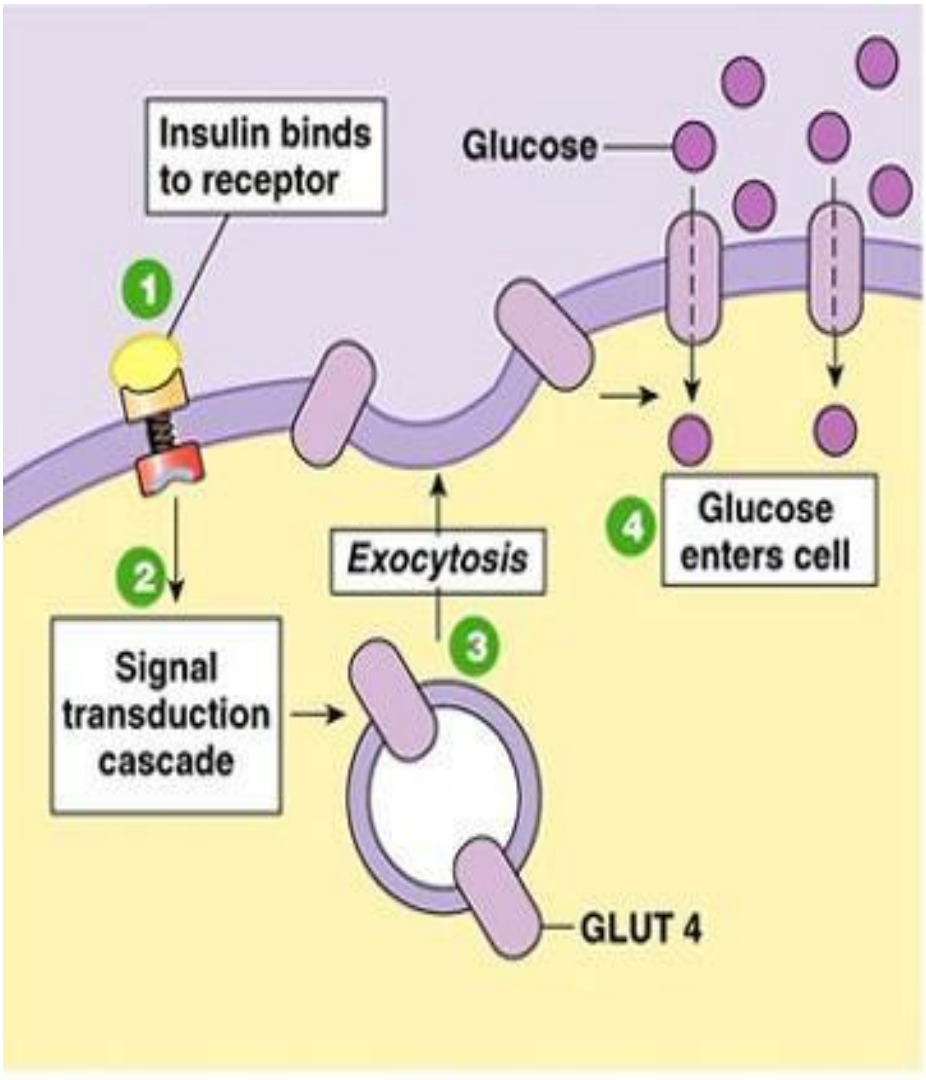

Figure 1.3: Facilitative Glucose Transporter

1.10 Different types of GLUT and their locations [www.ehow.com, Accessed on 2017]

Table 1.2: Different types of GLUT

Transporter	Tissues	Function
GLUT 1	All tissues, especially red cells and brain	Basal uptake of glucose; transport across the blood brain barrier
GLUT 2	B-cells ofpancreas, liver, kidney, gut	Regulation of insulin release and other aspects of glucose homeostasis
GLUT 3	Brain, kidney, placenta and other tissues	Uptake into neurons and other tissues
GLUT 4	Muscle, adipose tissue	Insulin mediated uptake of glucose
GLUT 5	Gut, kidney	Absorption of fructose

1. 11 Physiology of Insulin Secretion and action [Pessin & Saltiel, 2000]

Insulin is the most potent anabolic hormone promoting the synthesis and storage of carbohydrates, lipids and proteins, and inhibiting their degradation and release back into the circulation. Insulin regulates glucose homeostasis by inhibiting gluconeogenesis and the breakdown of glycogen in the liver and by stimulating glucose uptake, utilization and storage in insulin-sensitive tissues, such as adipose tissue, skeletal muscle and cardiac muscle. In muscle and liver, insulin increases

glycogen synthesis.

1.11.1 Biosynthesis of Insulin [www.vivo.colostate.edu, Accessed on 2017]

Human insulin consists of 51 amino acids in two chains connected by disulfide bridges. $T_{1/2}$ ~5-10 minutes; degraded by glutathione-insulin trans-hydrogenase (Insulinase). Bovine insulin differs by 3 amino acids, pork insulin differs by 1 amino acid. Insulin is stored in a complex with Zn^{2+} ions.

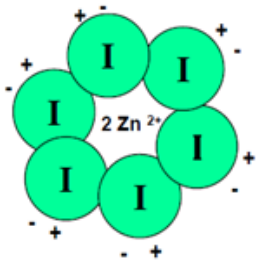

Figure 1.4: Insulin Hexamer

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.

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. (www.vivo.colostate.edu, Accessed on 2017)

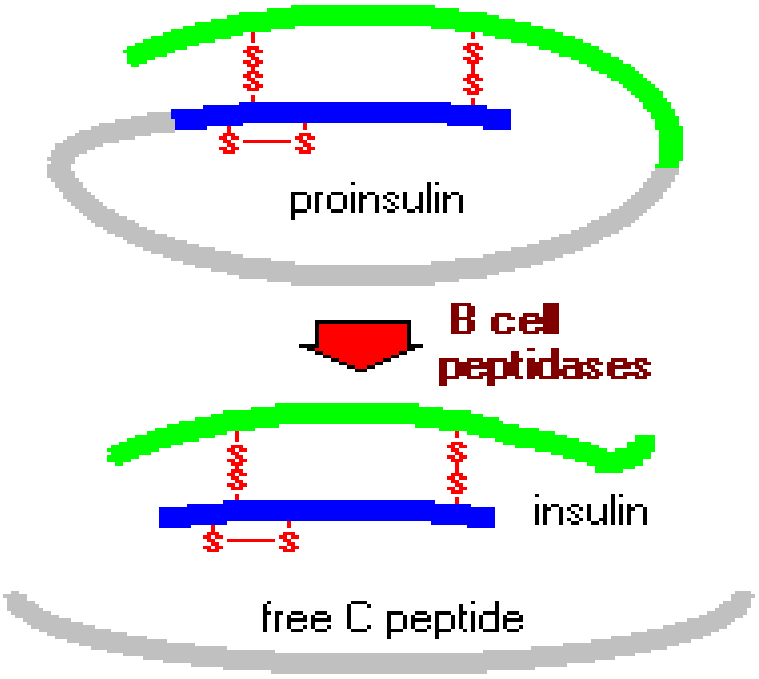

Figure 1.5: Biosynthesis of Insulin(1)

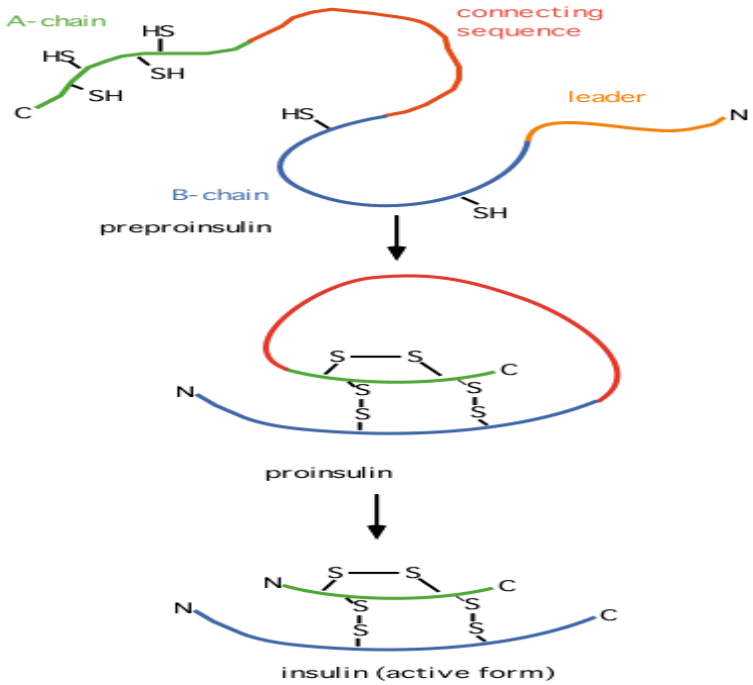

Figure 1.6: Biosynthesis of Insulin (2)

1.11.2 Mechanism of Insulin Secretion [Fu et al., 2013]

The biologically active form of insulin is the monomer. The storage form of insulin in the granules of the β-cells is hexamer. Hexamer is formed by its coordination with two zinc ions. When released from the granules, the hexamer gets diluted in the plasma and dissociates into monomers.

Secretion of insulin is primarily regulated by glucose. Glucose enters the β-cell facilitated by GLUT2 transport and enters glycolysis ultimately generating ATP. The increase in ATP changes the ratio of ATP to ADP and prevents an ATP-sensitive K^+ channel from functioning, which in turn leads to depolarization of the β-cells.

This prompts activation of a voltage-gated calcium channel, and calcium flows into the β-cells. Increased intracellular Ca^{2+} stimulates translocation of insulin-containing granules to the plasma membrane and the exocytotic release of insulin.

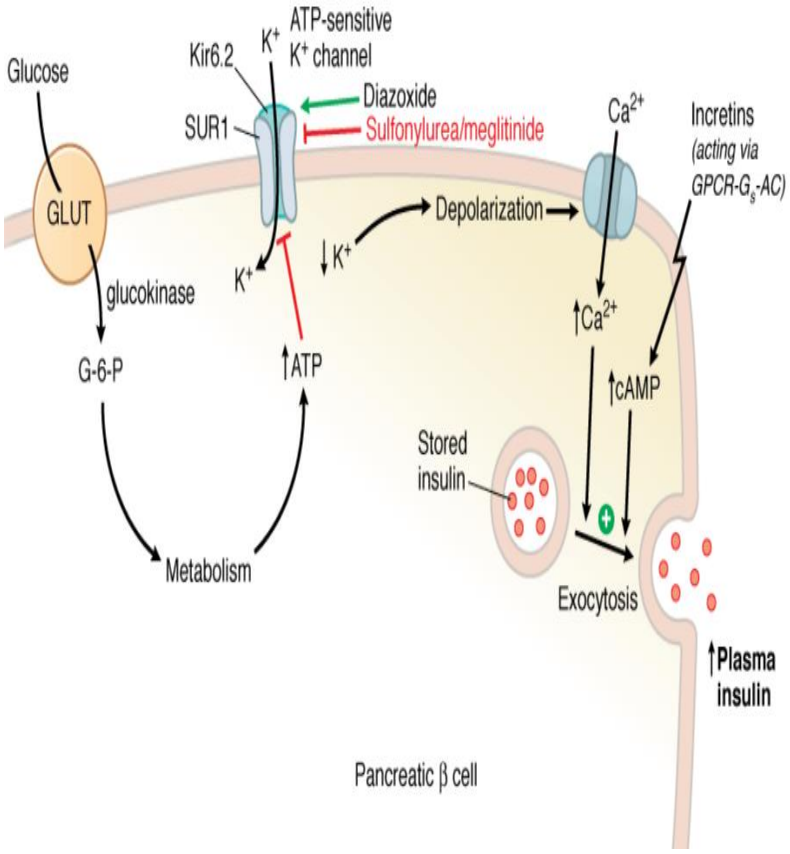

Figure 1.7: Insulin Secretion

1.11.3 Pathways responsible for Insulin Secretion

1.11.3.1 ATP-sensitive K+ channels (K_{ATP} channels)– Membrane Depolarization – Voltage Dependent Calcium Channel (VDCC) Pathway [Leung et al.,(2007); MacDonald et al., (2002)]

★ Glucose is the main stimulator of insulin secretion and utilizes this pathway.

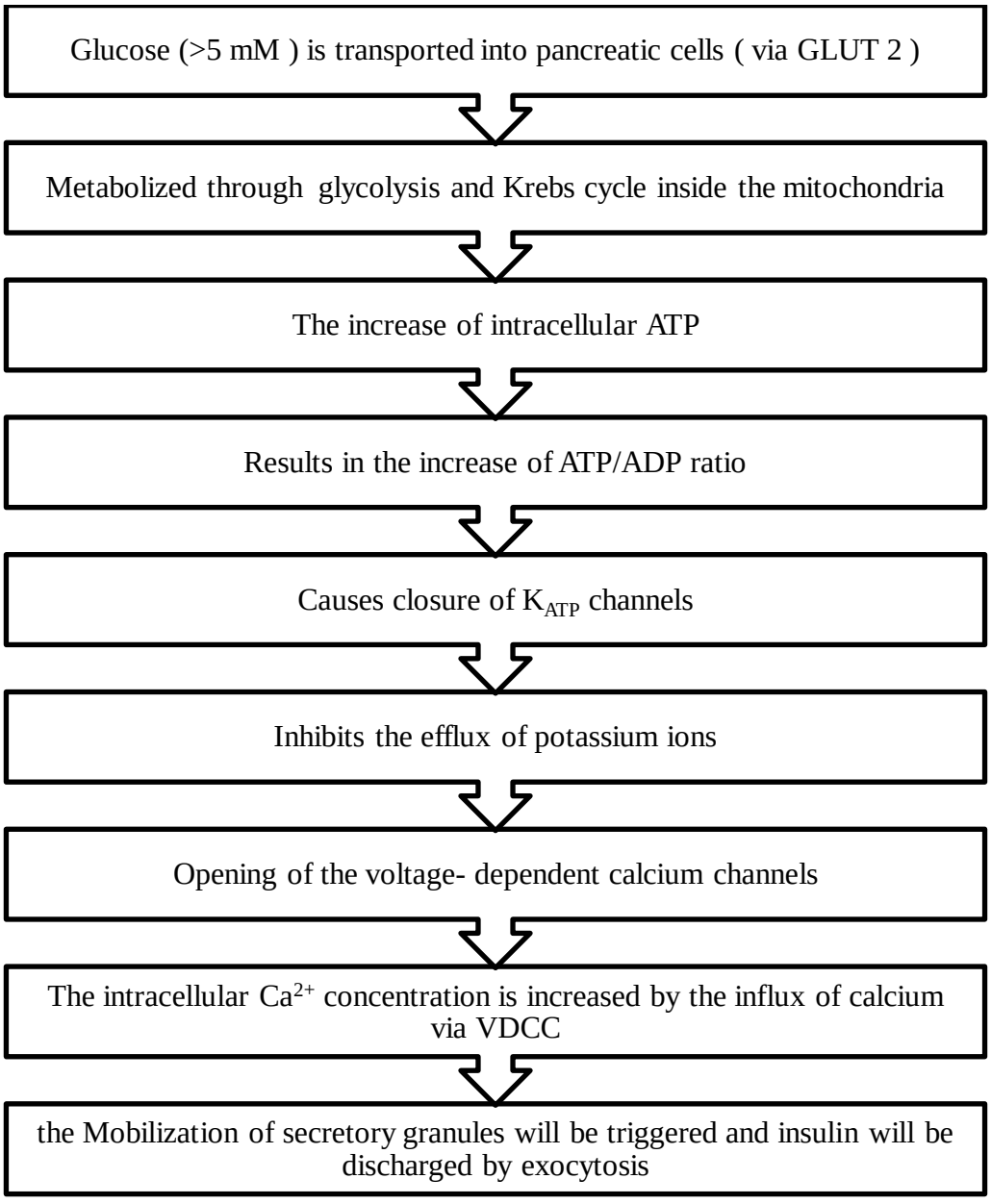

Figure 1.8: Flow Chart showing Insulin Secretion by ATP-sensitive K+ channels (KATP channels) mediated pathway

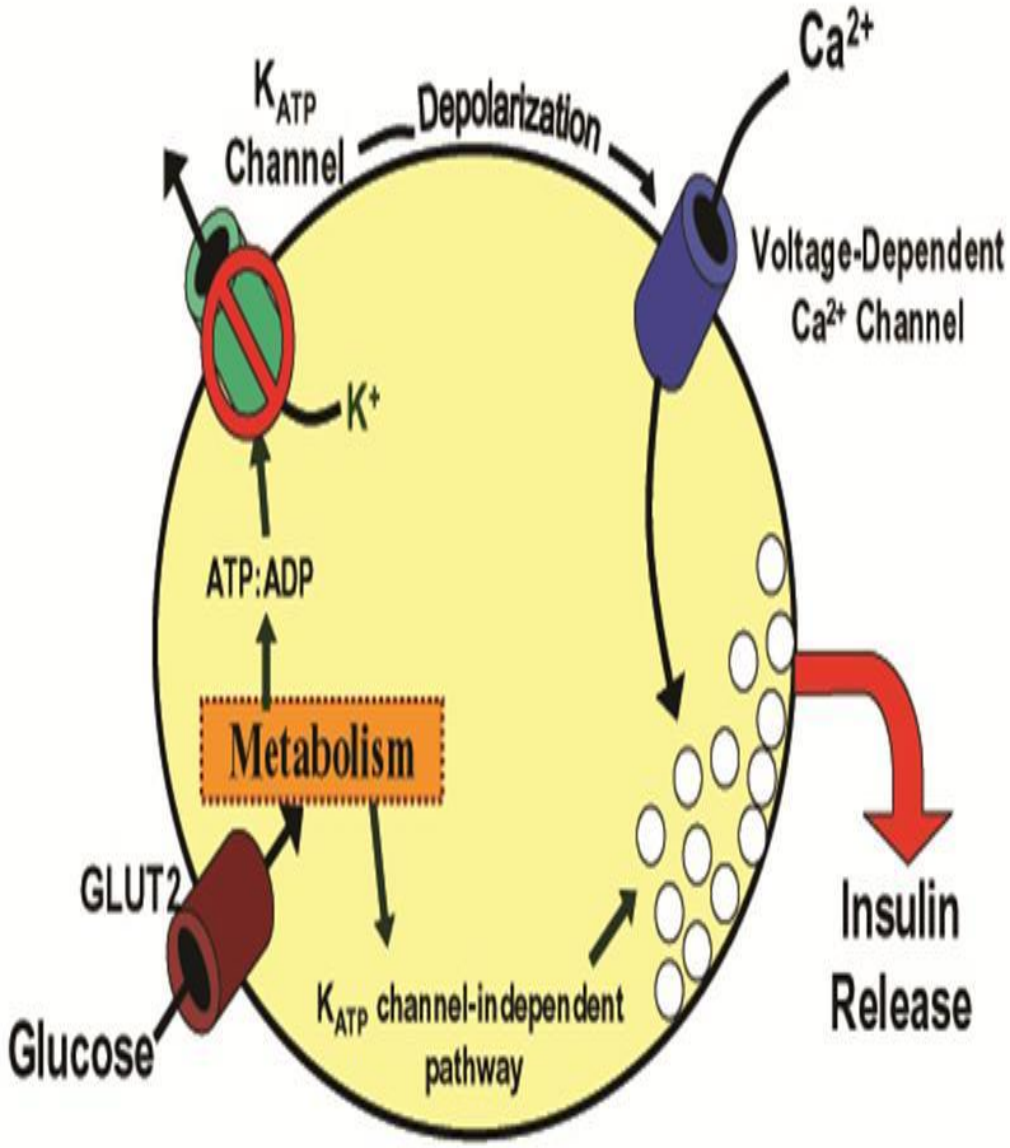

Figure 1.9: Mechanism of Insulin Secretion by K_{ATP} Channel Dependent Pathway

1.11.3.2 Potentiation of Insulin Secretion via Regulation of Second Messengers[Susumu et al., 2010; Liao and Carpenter, 2010]

℘ **cAMP – Protein kinase Apathway**[Susumu et al., 2010]

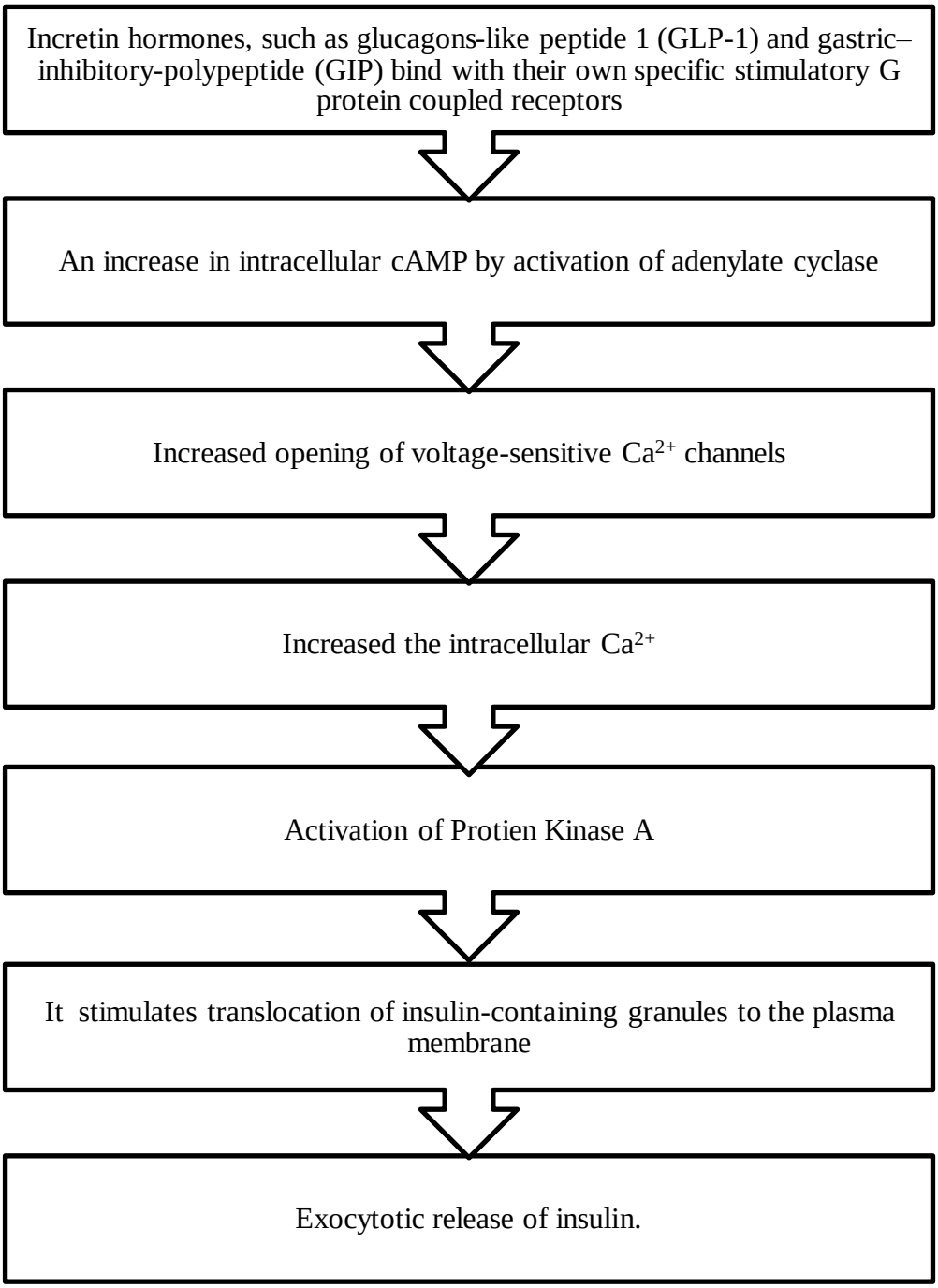

Figure 1.10: Flow Chart showing Protein Kinase A Pathway of Insulin Secretion

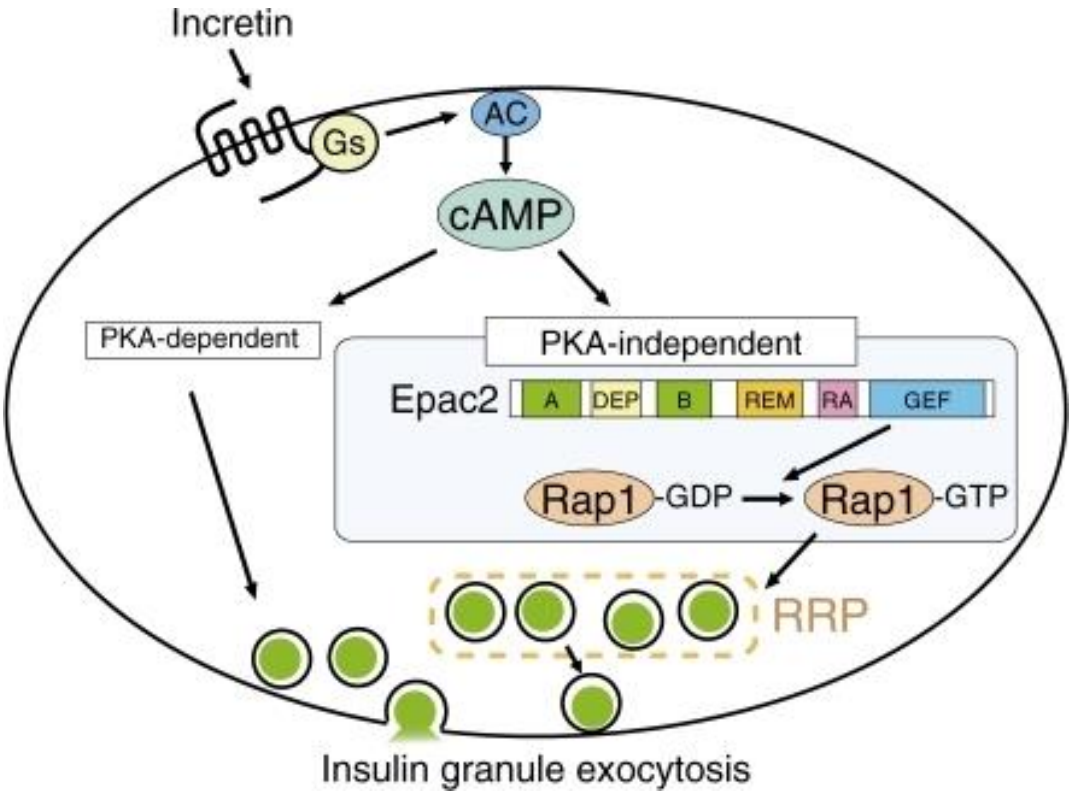

Figure 1.11: Secretion of Insulin via cAMP Pathway

- **Phospholipase C- Protein Kinase CPathway**[Liao and Carpenter, 2010]

Phospholipase C (PLC) is a key component of activation of the calcium-calmodulin and protein kinase C system. This activation is via hydrolysis of $PtdInsP_2$ into $InsP_3$ and diacylglycerol (DAG). As a result, IP_3 increases intracellular calcium via mobilazation of intracellular calcium stores in ER or microsomes. Elevation of intracellular calcium is directly associated with insulin exocytosis and along with DAG activates PKC which has been suggested to contribute in K_{ATP} channel independent pathways for insulin release. Neurotransmitters, such as acetylcholine, and the gastrointestinal hormone, cholecystokinin-8 (CCK-8), enhance glucose induced insulin secretion by activation of the PLC-PKC pathway following binding to specific muscarinic and CCK-8 receptors, respectively. Direct activation of PKC with the phorbol ester, phorbol 12-myristate 13 acetate (PMA) stimulates insulin secretion. However, down-regulation of PKC activity by chronic culture with phorbol esters has little effect on glucose-stimulated insulin secretion. (Liao and Carpenter, 2010)

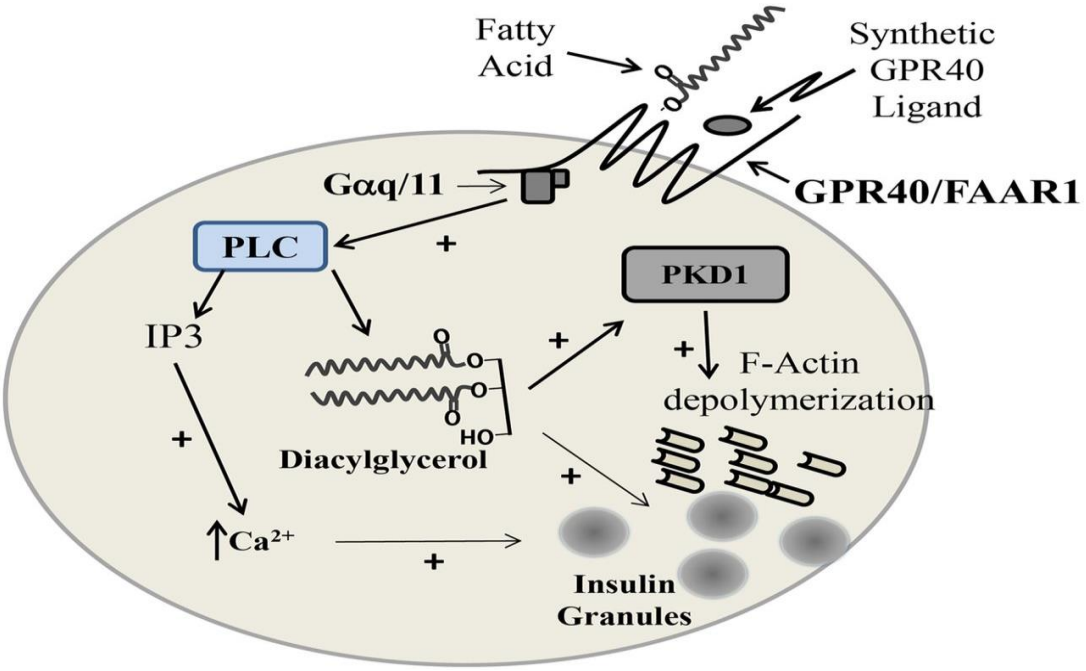

Figure 1.12: Secretion of Insulin via PLC pathway

1.12 Insulin Receptor [www.vivo.colostate.edu, Accessed on 2017]

The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into and subunits, which are then assembled into a tetramer linked by disulfide bonds. A hydrophobic domain in each subunit spans the plasma membrane.The insulin receptor is composed of two alpha subunits and two beta subunits linked by disulfide bonds. The alpha chains are entirely extracellular and house insulin binding domains, while the linked beta chains penetrate through the plasma membrane. The extracellular subunit contains the insulin-binding site. The cytosolic domain of the subunit is a tyrosine kinase, which is activated by insulin.

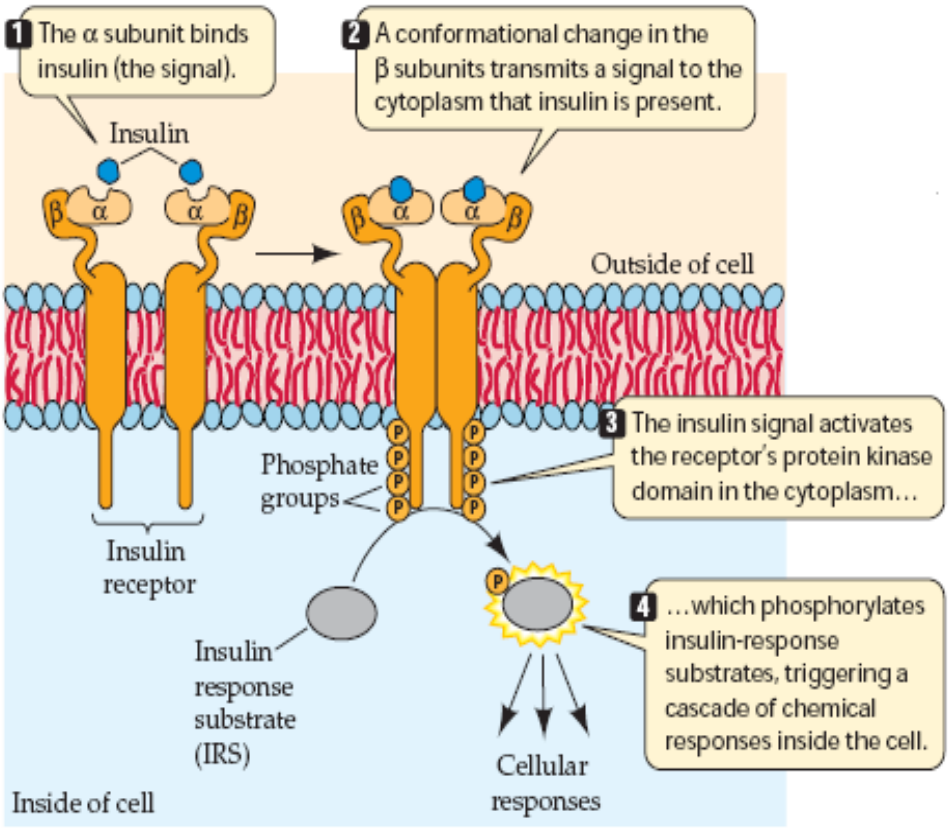

Figure 1.13: Insulin Receptor

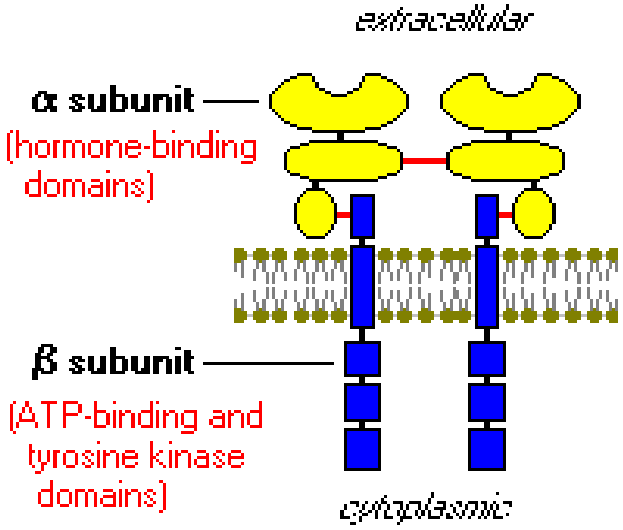

Figure 1.14: Subunits of Insulin Receptor

1.13 Effects of Insulin on Glucose Uptake [Satake et al., 2002]

Insulin stimulates glucose uptake in muscle and adipose tissue by translocating intracellular glucose transporter protein-4 (GLUT4) units to the plasma membrane. Basal glucose uptake is mediated primarily by GLUT1 and GLUT3. Any increase in the plasma glucose levels will enhance glucose uptake into peripheral tissues by these transporters.

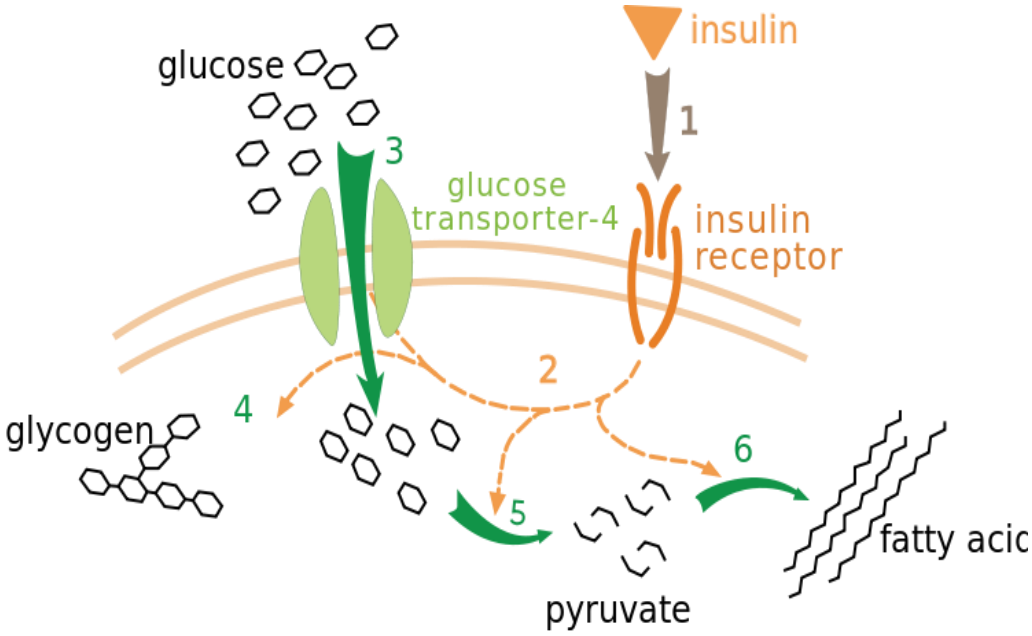

Figure 1.15: Effects of Insulin on Glucose Uptake

1.14 Insulin Action on Various Tissues [Lambadiari et al.,2015]

Table 1.3: Action of insulin on various tissues

Liver	**Muscle**	**Adipose**
↓ glucose production	↑ Glucose transport	↑ glucose transport
↑ glycolysis	↑ glycolysis	↑ lipogenesis& lipoprotein lipase activity
↑ TG synthesis	↑ glycogen deposition	↓ intracellular lipolysis
↑ Protein synthesis	↑ protein synthesis	

1.15 Diagnosis of Diabetes[Donnelly et al., 2000]

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

- **The HbA1ctest**
  - ✓ At least 6.5% meansdiabetes
  - ✓ Between 5.7% and 5.99% meansprediabetes
  - ✓ Less than 5.7% meansnormal
- **The FPG (Fasting Plasma Glucose level)test**
  - ✓ At least 126 mg/dl meansdiabetes
  - ✓ Between 100 mg/dl and 125.99 mg/dl meansprediabetes
  - ✓ Less than 100 mg/dl meansnormal

- ✓ An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG).

- **The OGTT (Oral Glucose ToleranceTest)**

  - ✓ At least 200 mg/dl meansdiabetes
  - ✓ Between 140 and 199.9 mg/dl meansprediabetes
  - ✓ Less than 140 mg/dl meansnormal
  - ✓ An abnormal reading following the OGTT means the patient has impaired glucosetolerance.

1.16 Current Treatment Proceduresfor Diabetes Mellitus [Campbell,2009]

Since diabetes conditions encompass a multiplicity of endocrine and metabolic disturbance, it is necessary to consider a wide range of pharmacological approaches to manage these. These may be required individually or in combinations to treat different features of the disease process.

Currently glycaemic control is achieved by dietary manipulation, oral hypoglycemic agents (for example - Sulphonylureas or Biguanides) or insulin injections. Approximately 75% of diabetic patients in UK achieve glycaemic control without exogenous insulin treatment.

1.17 Dietary Discipline [Fowler,2007; www.bda.uk.com, Accessed on 2017]

The regulation of food intake is central to the treatment of diabetes mellitus and various dietary regimes have been considered to assist in the control of hyperglycemia. The control of diet should be the first treatment offered to type 2 patients before drugs are considered. The main goal of nutritional management is to correct obesity as weight loss will improve glucose control, lower blood pressure and lipid concentration, all of which may help in preventing or diminishing long term complications. (Fowler,2007)

Various dietary regimes have been considered to assist in the control of

hyperglycemia. However, in most cases the dietary recommendations for type 2 diabetic patients are identical to those for the general population. (bda.uk.com, Accessed on 2017)

1.18 Insulin as Drug [Hartman, 2008]

The discovery of insulin by Banting, Best and co-workers in 1922 dramatically improved the prospects of individuals with diabetes mellitus. As type 1 is characterized by insulin insufficiency caused by partial or total destruction of insulin releasing pancreatic beta cells, patients with this condition required exogenous insulin replacement for treatment. The last decade has seen increasing refinement of exogenous insulin delivery in type 1 diabetes. In an attempt to reinstate normoglycemia, efforts have been made to match exogenous insulin delivery with the 24 h glucose profile. These have led to the introduction of continuous subcutaneous insulin infusion (CSII) and practice of multiple (4/d) subcutaneous insulin injections. Although intensive insulin regimes have unquestionably improved the control of diabetes they have not consistently achieved normoglycemia in clinical practice. In certain cases of type 2, exogenous insulin is required to achieve glycemic control.A number of insulin preparations have been developed since its discovery based on the duration of action. Although various procedures were attempted to prolong the duration of insulin action, the two forms endured; the production of neutral protamine hagedorn (NPH) insulin, where absorption is retarded by protamine and development of the lente series by the use of zinc-insulin complexes. Insulin can be broadly classified as having short, medium, or long duration of action, however their effects vary considerably from one patient to another and in the same patient from time to time.

1.18.1 Structure of Insulin [Medscape, Accessed on 2017]

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.

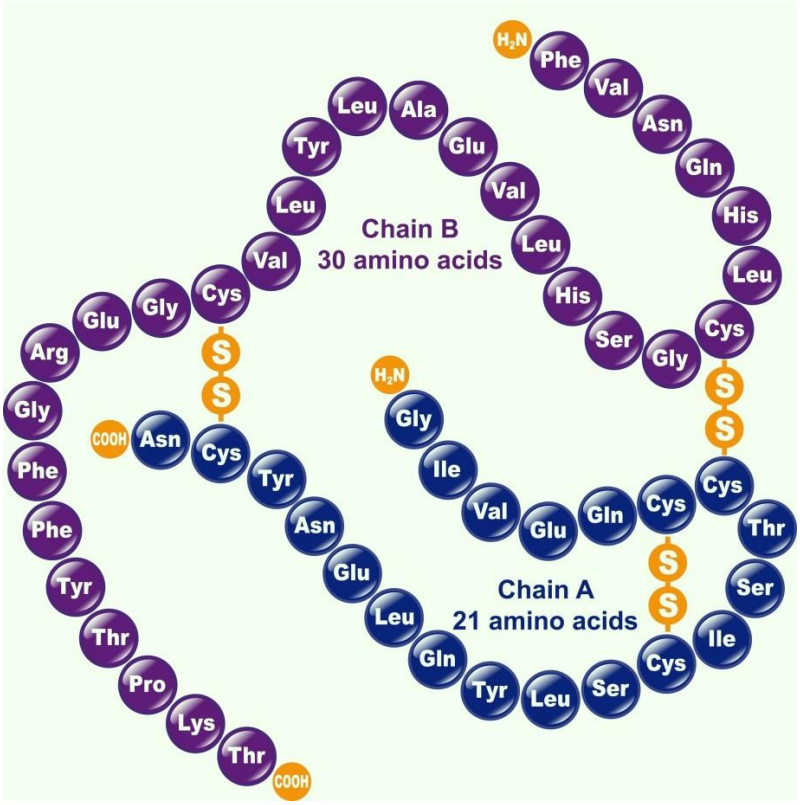

Figure 1.16: Structure of Insulin

1.18.2 Insulin Analogues [Hartman,2008;www.diabetes.co.uk, Accessed on 2017; www.medscape.com, Accessed on 2017; www.diapedia.org, Accessed on 2017]

☙ Rapid acting insulin include

1. Regular insulin (Humulin R, Novolin R)
2. Insulin lispro (Humalog)
3. Insulin aspart (Novolog)
4. Insulin glulisine (Apidra)
5. Prompt insulin zinc (Semilente, Slightly slower acting)

- **Intermediate acting insulin**

1. Isophane insulin, neutral protamine Hagedorn (NPH) (Humulin N, Novolin N)
2. Insulin zinc (Lente)

- **Long acting insulin**

1. Extended insulin zinc insulin (Ultralente)
2. Insulin glargine (Lantus)
3. Insulin detemir (Levemir)

1.19 Anti-Diabetic Drugs [www.healthline.com, Accessed on 2017; Diabetes Wiki, Accessed on 2017; Chaudhury et al.,2017; Leahy, Clark & Cefalu, 2000; Florence,1999]

Those patients who fail to achieve glycemic control through dietary intervention measures require oral hypoglycemic agents. Approximately 50% of type 2 patients in the UK are treated with oral hypoglycemic agents. Although there are new oral hypoglycemic agents on the horizon, the choice at the present is primarily between sulphonylureas and biguanide (metformin). Some anti-diabetic drugs are described below,

1.19.1 Sulfonylureas

With the labeling of tolbutamide (Orinase) by the U.S. Food and Drug Administration in 1962, the sulfonylurea class of drugs quickly became the mainstay of treatment for type 2 diabetes. Although newer agents have recently entered the marketplace, sulfonylureas still play a primary role in pharmacologic management of type 2 diabetes. Patients who respond best to treatment with sulfonylureas include those with a diagnosis of type 2 diabetes before 40 years of age, duration of disease less than five years before initiation of drug therapy and a fasting blood glucose level of less than 300 mg per dL (16.7 mmol per L). (Chaudhury et al.,2017).

Approximately two thirds of patients who begin therapy with a sulfonylurea respond, although up to 20 percent of them eventually require additional medication. Few

patients with uncontrolled diabetes receive clinical benefit when switched from one sulfonylurea agent to another. The use of agents with a longer half-life (e.g., chlorpropamide [Diabinese]) in the elderly and in patients with renal impairment is discouraged because the risk of hypoglycemia is increased. (www.healthline.com, Accessed on 2017)

1.19.2 Metformin

Metformin is a biguanide agent that lowers blood glucose primarily by decreasing hepatic glucose output and reducing insulin resistance. Metformin is used as monotherapy or in combination with sulfonylureas for management of type 2 diabetes. When used as monotherapy, metformin does not cause hypoglycemia and is thus termed an "antihyperglycemic." The use of metformin is contraindicated in patients with renal insufficiency (i.e., a serum creatinine level of 1.5 mg per dL [130 μmol per L] in men and 1.4 mg per dL [120 μmol] in women, or abnormal creatinine clearance) or acute or chronic metabolic acidosis. Metformin should be temporarily withheld before any procedure involving intravascular administration of iodinated contrast media. Normal renal function should be confirmed 48 hours after the procedure before restarting metformin therapy. There is no known reason to discontinue metformin therapy during other parenteral contrast studies. (Chaudhury et al.,2017)

Extreme caution should be used in patients with severe hepatic dysfunction, hypoxemic states (e.g., severe chronic obstructive pulmonary disease, congestive heart failure), moderate to severe illness and excessive alcohol intake. In these patients, the use of metformin may contribute to the development of lactic acidosis, a condition that is fatal in about 50 percent of patients who develop it (one episode per 100,000 patient-years). Cimetidine (Tagamet) decreases the renal clearance of metformin and may potentiate its effects. Patients receiving oral anticoagulant therapy and metformin may require a higher dosage of warfarin (Coumadin) to achieve a therapeutic antithrombotic effect. Hemoglobin, hematocrit, red blood cell indexes and renal function should be monitored at least annually in patients taking metformin. (Chaudhury et al., 2017)

1.19.3 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors, such as acarbose (Precose) and miglitol (Glyset), are indicated as monotherapy or in combination with sulfonylureas for management of type 2 diabetes. These agents inhibit the breakdown of complex carbohydrates and delay the absorption of monosaccharides from the gastrointestinal tract. Acarbose and miglitol should be titrated over two to three weeks to minimize flatulence and other gastrointestinal side effects that commonly lead to discontinuation of these agents. Alpha-glucosidase inhibitors are contraindicated in patients with inflammatory bowel disease, partial intestinal obstruction, a predisposition to intestinal obstruction, colonic ulceration and other gastrointestinal disorders. (Chaudhury et al.,2017)

Dose-dependent hepatotoxicity is associated with this drug class, so liver function tests should be carefully monitored in patients receiving higher dosages of these medications (e.g., more than 50 mg three times daily). Transaminase elevations are reversible with discontinuation of the drug and are often asymptomatic. Serum transaminase levels should be checked every three months for the first year patients take the medication and periodically thereafter. Drugs that are susceptible to binding with other agents (e.g., cholestyramine [Questran]) should be taken two to four hours apart from alpha-glucosidase inhibitors to avoid drug interactions. Intestinal absorbents and digestive enzyme preparations should not be administered with acarbose. (Chaudhury et al.,2017).

1.19.4 Troglitazone

The thiazolidinediones are a unique drug class of “insulin sensitizers” that promote skeletal muscle glucose uptake. Troglitazone is the first agent of this drug class to be introduced in the U.S. market and, like metformin, it reduces insulin resistance. Troglitazone is beneficial in patients requiring large daily amounts of insulin (more than 30 units per day) whose diabetes is still uncontrolled. A reduction of up to 50 percent in total daily insulin dosage is possible with drug titration. Troglitazone is also effective when used in combination with other oral agents, thereby potentially delaying the need to start insulin therapy. (Leahy, Clark & Cefalu, 2000)

The U.S. Food and Drug Administration recently ruled that troglitazone should only be used in combination with other diabetic therapies. The effectiveness of oral

contraceptives may be decreased with troglitazone administration. Over 150 case reports of hepatotoxicity have been reported with troglitazone, so liver function must be monitored every month for the first eight months of treatment and every other month for four months thereafter. Periodic transaminase measurements should be obtained as long as the patient is taking troglitazone. (Leahy, Clark & Cefalu, 2000)

1.19.5 Repaglinide

Repaglinide (Prandin) is a benzoic acid derivative and the first of the non-sulfonylurea meglitinides introduced in early 1998. The mechanism of action and side effect profile of repaglinide are similar to those of the sulfonylureas. This agent has a rapid onset of action and should be taken with meals two to four times daily. Repaglinide is a suitable option for patients with severe sulfa allergy who are not candidates for sulfonylurea therapy. The drug is used as monotherapy or in combination with metformin. It should be titrated cautiously in elderly patients and in those with renal or hepatic dysfunction. (Leahy, Clark & Cefalu, 2000)

1.20 Herbal medicines

The natural medications with antidiabetic activity are widely formulated because they are better compatibility with human body, easily available and less side effects when compared with the synthetic antidiabetic medications. Antidiabetic herbal formulations are considered to be more effective for treatment of diabetes. More number of plant and plant products have been scientifically tested and reported to possess anti diabetic activity. (www.healthline.com, Accessed on 2017).

1.20.1 Traditional Herbal Treatment for Diabetes [Chikezie, 2015]

Herbal medicines involve the integration of several therapeutic experiences and practices of indigenous systems of medicine that may span many previous generations, which often provide valuable guidelines to the selection, preparation and application of herbal formulation with a view to providing therapeutic benefits. Treatment of illness and maintenance of health/well-being using herbal medicines is the oldest and most popular form of healthcare practice known to humanity that has been practiced by all cultures in all ages throughout the history of civilization.Medicinal plants have been used since ancient times for the treatment and

management of diabetic mellitus (DM) in traditional medicine systems of many cultures throughout the world.

Currently, medicinal plants continue to play an important role in the management of DM, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies. In developed countries, the use of anti-diabetic herbal remedies has been on the decline since the introduction of insulin and synthetic oral hypoglycemic drugs during the early part of the 20th century.

However, recently in the developed countries, there has been the resurgence of interest in medicinal plants that exhibit hypoglycemic property. The renewed interest in herbal anti-diabetic remedies in developed countries is believed to be motivated by several factors that include: adverse reactions, high secondary failure rates and cost of conventional synthetic anti-diabetic remedies. Recently, the World Health Organization (WHO) recommended the use medicinal plants for the management of DM and further encouraged the expansion of the frontiers of scientific evaluation of hypoglycemic properties of diverse plant species. Consequently, current estimates showed that over 70% of the global population applies resources derived from traditional medicine for the management and alleviation of DM and its complications.

Ethno-pharmacological surveys indicate that more than 1,200 plants are used in traditional medicine systems following claims of their hypoglycemic properties. The hypoglycemic activity of a large number of plant products have been evaluated and confirmed in animal models as well as in human beings. In some cases, the bioactive principles of the medical plants have been isolated and identified. Nevertheless, the mechanisms of action of most of these anti-diabetic bioactive principles are not well defined and remain largely speculative. However, reports suggest that the array of anti-diabetic bioactive principles in medicinal plants may act in synergy to exert glycemic control through interference with one or more processes involved in glucose metabolism and homeostasis**.**

The present review sought to highlight some experimentally confirmed anti-diabetic medicinal plants and their bioactive principles that have been implicated in exerting glycemic control. By extension, the outlook and regular form in which scientific investigations on some Nigerian indigenous anti-diabetic medicinal plants

are designed and carried out, coupled with the obvious limitations of these research findings were also considered for review.

1.20.2 Use of Medicinal Plants in Bangladesh to treat Diabetes [Ashraf et al. 1982; Ocvirk et al., 2013; Claquin,1981; Bhardwaj et al., 1986; Ahmed,2000; Rahmatullah et al., 2010; Kadir et al., 2012; www.niport.gov.bd, Accessed on 2017]

The usage of medicinal plants is traditionally rooted in Bangladesh and still an essential part of public healthcare. Recently, a dramatically increasing prevalence brought diabetes mellitus and its therapy to the focus of public health interests in Bangladesh. We conducted an ethno-botanical survey to identify the traditional medicinal plants being used to treat diabetes in Bangladesh and to critically assess their anti-diabetic potentials with focus on evidence-based criteria. Bangladesh features a sub-tropical climate and low-lying landmass largely adjacent to extensive river deltas. (Ashraf et al., 1982; Bhardwaj et al., 1986)

The country comprises very fertile soils and is home to some rare ecosystems such as the Sundarbans mangrove forests. Given the fertile plains and high population density, the indigenous vegetation has mostly given way to cropland and extensive cultivation.

Today, almost 60% of the landmass is used for farming, which is a global maximum value. However, originally large parts of Bangladesh featured tropical forests and marshy jungle with highly bio-diverse flora - being also an excellent source for medicinal plants. The Bangladeshi traditional medicine is a unique conglomerate of different ethno-medical influences. (Ocvirk et al., 2013)

Due to the geographic location and socio-cultural characteristics of the country, it involves traditionally rooted elements influenced by local indigenous people and close-by Indian Ayurveda and Unani medicine. Given its inexpensive, easily accessible and well-established health services, the use of traditional medicine is an integral part of public health services in Bangladesh with its providers being deeply embedded within the local community. Recent data suggest that the utilization of traditional medicine health services in Bangladesh is widespread and plays a crucial

role in providing health care for poor people, people in rural areas and for tribal people. (Kadir et al., 2012)

In the context of using traditional medicinal plants for treating diabetes, extensive screening has been performed in many ethno-medical systems within the Indian subcontinent. However, in Bangladesh the traditional medicinal plants that are used for the treatment of diabetes have not yet been studied in great detail. Therefore, these herbal remedies are important objects of research, especially in context of the virtually exploding prevalence of diabetes mellitus in Bangladesh. Although diabetes is more prevalent in urban areas in rural communities prevalence rates for diabetes rose from 2.3% to 6.8% in between 1999 to 2004. (Rahmatullah et al., 2010).

A recent survey in Bangladesh demonstrated that in slum areas, 86% of female and 78% of male diabetic patients use either inadequate medical treatment or none. In non-slum areas only 34% of female and male diabetic patients undergo adequate medical treatment. (www.niport.gov.bd, Accessed on 2017)

1.20.3 Common Anti-Diabetic Medicinal Plants [Chikezie, 2015]

It is worthwhile to note here that there is yet no effective cure for DM and available drugs and insulin therapy used for the management of the disease are associated with several undesirable side effects. The undesirable side effects and high cost of anti-diabetic drugs led to the search for medicinal plants that exhibit hypoglycemic property, with a view to applying them for the management of DM. Several species of medicinal plants used for the management of DM worldwide have been evaluated. Some of theplants include*Allium cepa*, *Allium sativum* , *Aloe vera*, *Cinnamomum cassie*, *Coccinia indica*, *Gymmema slyvestre*, *Momordica charantia*, *Catharanthus roseus*, *Muurrayi komingii*, *Ocimum sanctum*, *Panax ginseng*, *Trigonella foemum-graecum*, *Pterocarpus marsupium* and *Syzigium cumini*.

A survey of several medicinal plant research findings showed that the polysaccharides, sterols, terpenoids, alkaloids, saponins, flavonoids, amino acids and their derivatives are the most encountered bioactive principles that exhibited glycemic control in experimental animals.

The anti-diabetic bioactive principles and possible mechanism of action of these medicinal plants are summarized in the table given below: (Chikezie, 2015)

Table 1.4: Overview of anti-diabetic medicinal plants

Plant	**Family**	**Usable Parts**	**Effect**
Allium cepa (Onion)	Alliaceae	Onion bulbs.	Stimulate insulin secretion. Compete with insulin for insulin inactivating sites in the liver.
Allium sativum (Garlic)	Alliaceae	Garlic gloves	Stimulate insulin secretion. Inhibit glucose production by the liver
Aloe vera	Aspholedeceae	Leaf, pulp and gel.	Stimulate synthesis and/or release of insulin. Alter the activity of carbohydrate metabolizing enzymes.
Catharanthusroseus	Apocynaceae	Fresh leaf juice.	Increase hepatic utilization of glucose. Suppress activities

			of gluconeogenic enzymes.
Cinnamomumcassie (Chinese cinnamon)	Lauraceae	Bark.	Enhance insulin action. Increase glucose uptake and glycogen synthesis.
Cocciniaindica;	Cucurbitaceae	Leaves.	Suppress glucose-6-phosphatase activity.
Fiscusbengalensis	Moraceae	Leaves and bark.	Increase insulin secretion. Inhibit insulinase activity.
Gymmemaslyvestre (Gurnar)	Asclepiadaceae	Leaves.	Stimulate insulin secretion from rat Islets. Decrease the activity of gluconeogenic enzymes. Induce β-cell regeneration.
Momordicacymbalaria (Bitter Melon)	Cucurbiteae	Fruit pulp, seeds, leaves and whole plant.	Stimulate insulin secretion. Suppress the activity of gluconeogenic enzymes. Increase β-cells in diabetic rats.

Muurrayikomingii (Cury leaf	Rutaceae.	Leaves.	Stimulate insulin secretion. Increase glycogenesis and decrease glycogenolysis and gluconeogenesis.
Ocimum sanctum	Lamiaceae	Leaves.	Stimulate insulin secretion.

1.21 Plant Profile

1.21.1 Botanical Name

Heritiera fomes Buch. & Ham.

1.21.2 Synonym

Heritiera minor Roxb. (Wikipedia)

1.21.3 Local Name

Sundori Tree

1.21.4 Scientific Classification

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Malvales

Family : Sterculiaceae

Genus : *Heritiera*

Species : *fomes*

(Wikipedia)

1.21.5 Plant Pictures:

Figure 1.17: Leaf and Seed of *Heritiera fomes*

Figure 1.18: Root of *Heritiera fomes*

1.21.6 Habitat [Wikipedia]

Mangrove forests in the upstream estuarine zone of the high intertidal region, preferring freshwater. Plants can be fast-growing in low-saline environments.

(Wikipedia)

1.22 Plant Morphology [Banerjee and Rao,1990; Mukherjee et al., 2003; Bangladesh Bureau of Statistics,1983; Asianplant.net]

H. fomes, Sundari is a mangrove buttressed tree of 10 to 25 m tall with dense, robust pneumatophores about 50 cm height. It is the only *Heritiera* species that produces pneumatophores. The roots do not penetrate deep into the soil, but spread on the surface with numerous stout offshoots and often with narrow ridges forming plant like projections above the soil and also form flat narrow buttress to the basal trunk. (Banerjee and Rao,1990)

It prefers freshwater and is fast-growing in low-saline environments. The species is commonly found along the tidal creeks and channels of the coastal swamps, and regenerate naturally through seeds. The species is found in the upstream estuarine zone in the high intertidal region and adapted best along the seashore. Cluster analysis using the AFLP (Amplified Fragment Length Polymorphism) banding patterns of all the primer combination reveals that *H. fomes* in the due course of evolutionary process might have migrated to land and evolved as a new species. (Banerjee and Rao,1990)

The leaves are simple, shortly petiolate, dark green on top while lower leaf surface silvery white. Mature leaf 15 to 20 cm. Long and 5 to 7 cm wide. The flowers are simple, unisexual yellow orange in colour and organized in panicles. H. fomes flowers in March to April. The fruit carpel ripens in July-August. Individual fruit is 5 to 7 cm. Long and possesses a ridge on the outer edge. Germination hypogeal and takes place very soon after the fruits fall. (Asianplant.net)

Sundari contains 0.25% and 0.09% (dry weight) of chl-*a* and chl-*b*, respectively. Studies have reported that the carbon, polyphenol, tannin and protein content are 0.11%, 39.45%, 21.12% and 29.22% of dry weight, respectively for the species. The chemicals produced from the species can be used for gastro-intestinal disorders (including dysentery, diarrhea, indigestion, colic, acidity, constipation, bloating, lack of appetite, stomachache). Besides this, it can be used to treat hepatic disorders (including jaundice and hepatitis), insect repellent and skin diseases (including eczema, abscess, acne, boils, scabies, itch, infections, dermatitis, rash, sores, scar,

warts, etc.). Sundari has been the main timber species and is the primary resource base for 221 small saw mills and 350 pitsaw units in the region. (Bangladesh Bureau of Statistics,1983)

The species is now on the extinction threat in West Bengal due to overcutting and increased salinity. In the highly populated Bengal (India and Bangladesh) the dry season demand for freshwater has increased dramatically; major rivers have been dammed and the downstream effects are becoming apparent with increasing soil salinities and unexplained 'top dying' disease is threatening the *H. fomes* population. The first factor is clearly anthropogenic; the second, although aggravated by upstream diversions of Ganga water, is largely due to long-term geomorphic processes. (Mukherjee et al., 2003).

Figure 1.19: Leaves, Fruits and flowers of *Heritiera fomes*

1.23 General Information on Sundarbans: [Blasco, 1975; Hazra et al. 2002; Chaudhuri and Choudhury, 1994; Lal and Aggarwal, 2000; Kathiresan and Ravi, 1990; Robertson et al., 1990]

The term "Sundarbans" has been coined from the

- forests of Sundari (*Heritiera fomes*
- forests of beautiful plants
- forests of *Samudra* (i.e., Ocean). (Lal and Aggarwal, 2000)

Sundarbans extend over *ca* 14,600 km^2 distributed over both Bangladesh and India, with the latter occupying *ca* 4266.6 km^2 in West Bengal state (Plate 1). In comparison to the Bangladesh part, the Indian component of the Sundarbans has poor forest formation due to higher salinity and biotic interactions leading to different growth pattern and ecological succession. (Hazra et al. 2002)

Figure 1.20: Area of Sundarbans (Hazra et al. 2002)

Being on the land sea interface, mangroves are always associated with and subjected to saline seawater. However, saline condition is not a prerequisite for their development; rather mangroves choose saline conditions to avoid the competition with the more vigorous terrestrial plants. (Kathiresan and Ravi, 1990)

The Indian Sundarbans can be divided in to three parts *i.e.*, central and eastern, based on their salinity level (Lal and Aggarwal, 2000). The western part is the least saline due to the freshwater discharge from the Ganga-Bhagirathi-Hooghly rivers; whereas, the central part is most saline due to non-receipt of fresh water from the Ganges owing to heavy siltation since the late 15th century and the rising sea level. The rate of sea level rise is 3.14 mm/yr, which is higher than the global and Indian coastline averages of 2.12 mm/yr and 2.50 mm/yr, respectively. The sea level rise and subsequent saline water intrusion into the islands of Sundarbans, they are also vulnerable to extreme climatic events owing to their location below the average Mean Sea Level. Since, the Sundarbans is located in a low-lying floodplain, most of the silt carried out by the Gangetic rivers are lost in the trench of the Bay of Bengal. A large portion of the silt are deposited on the eastern side causing land accretion, particularly in the south-eastern region; and compensatory erosion in the southwestern part, thereby pushing the coastline towards the sea. (Chaudhuri and Choudhury, 1994)

Figure 1.21: Sundarbans (Picture was taken during visit)

Mangroves are rich in polyphenols and tannins. Phenols and flavonoids present in mangrove leaves serve as UV-screen compounds. Flavonoids increase during pre-

monsoon period. Pigment concentrations may vary with environmental conditions and seasons. Oswin and Kathiresan (1994) found high level of chlorophyll and carotenoids during the summer but highest level of anthocyanin in the monsoon months. However, depletion of growing stock, post-dispersal predation of seeds by crabs, sporadic flowering, and poor seed set in the remnant mangrove forests have been reported. The primary threats to all mangrove species including *H. fomes* are habitat destruction and removal of mangrove areas for conversion to aquaculture, agriculture, urban and coastal development, and overexploitation. (Blasco, 1975)

1.24 Edible Uses of *Heritiera fomes* [tropical.theferns.info]

- The seed is rich in starch, it is eaten in times of food shortage. The seed contains tannins and is very astringent unless these are first removed by leeching the seeds in cold water. Either the whole seed can be used, or the seed can be dried and ground it into a powder. Two common methods around the world for removing tannins are:
  - ✓ A quick method of removing them is to cook the seeds in several changes of water until the cook water is either free of bitterness, or the bitterness has been reduced to acceptable levels.
  - ✓ A more traditional, and slower, method is to leach the tannins by thoroughly washing the seed in running water, though many minerals will also be lost.
- The wood is very hard, heavy, elastic and durable. It is used for construction. The wood is a good source of fuel, and makes a good quality charcoal.

1.25 Plant Collection

Figure 1.22: Bark and leaf collection from plant

Chapter 2

Aim & Objective

The objective of this study is to evaluate the pharmacological effect of different parts (Bark & Root) of *Heritiera fomes* on hyperglycemia in laboratory animals by performing following experiments:

- Effect of the plant material on Barium Sulphate Milk aided GI Motility in *swiss albino* mice model.
- Screening for the possible inhibition of carbohydrate absorption in *Long Evans* rat model by plant material.
- Assessment of the effect of plant materials on intestinal disaccharidase activity in *Long Evans* rat model.

Chapter 3

Literature Review

At the year of 2004, a study was done by systematic screening of the extracts of Bangladeshi plants for free radical scavenging activity using DPPH assay. Bangladesh flora is a rich source of a range of plant species , many of which are medicinal plants, and have been used in the preparation of the Unani and Ayurvedic traditional medicines and some of have been developed as natural antioxidant formulations for food, cosmetic and other applications. Different Bangladeshi plants including *Heritiera fomes* had been selected for the assessment and studies on the contents of alkaloids, anthraquinone, flavonoids and tannins. Most of these species have been used in traditional medicine in Bangladesh and other countries for the treatment of various illnesses ranging from common cold to cancer. (Uddin et al. 2004)

In 2008, MONDAL S et al had a study on in vitro antibacterial activity of the pneumatophores of *Heritiera fomes* and *Xylocarpus moluccensis*. They have done the disc diffusion assay by using ethanol extracts of those plants and found similar in vitro antibacterial activities against a number of bacterial strains. And the minimum inhibitory concentration (MIC) was determined by the broth dilution method. From their research It was assumed that *X. moluccensis* and *H. fomes* are potential sources for novel 'lead' discovery for antibacterial drug development. (Mondal et al, 2008)

In 2009, a systematic ethnobotanical survery was done of the 64 districts of Bangladesh to obtain information on the plant species and ailments for which these were used. For hundreds of years, the population of jessore and the surrounding areas like Magura, Jhenaidaha, Satkhira, Khulna, Narail used to rely on traditional medicinal practitioners who used medicinal plants and floral species for treatment of various ailments. On the survey a semi-structured questionnaire was followed by extensive questioning and taking of notes as to plant species being used, formulations, dosage, and side-effects, if any. Plant species as pointed out by the traditional medicinal practitioners were photographed and plant specimens were brought to Bamgladesh National Herbarium for identification. Information on 26 plant species belonging to 22 families was obtained from practitioners in which *Heriteria fomes* was included which has side effects of sterculiaceae, goiter. (Nawaz et al. 2009)

In 2009, Helle Wangensteen et al had a study on Antioxidant and antimicrobial effects of the mangrove tree *Heritiera fomes*. They have used EtOH extract of stem bark from H. fomes. They have found that the extract was rich in procyanidins in Trimeric, pentameric and hexameric forms along with highly polymeric material. Bioactivity studies have shown that the bark extracts which contain procyanidins having high DPPH radical scavenging and 15-lipoxygenase inhibiting activities and have antibacterial activities against *K. rhizophilia, S. aureus, B. subtilis* and *P. aeruginosa.* And in the brine shrimp assay no toxicity was found. (Wangensteen et al, 2009)

In 2010, an evaluation of phosphate solubilizing ability of five "Streptomyces" isolated from the phyllosphere of *"Heritiera fomes"* was done by using tricalcium phosphate (TCP) in both plates and broth culture conditions as well as with and without NaCl. The requirement of NaCl for better solubilization of TCP was observed in all *"Streptomyces"* . It was seen that the solubilization potential varies among different isolates of *"Streptomyces"*. It also differed according to incubation period. Over all, the best solubilization ability of all test "Streptomyces" could be observed in the presence of 0.2% NaCl. (Gupta, Sahoo, 2010)

In 2010, a study was done in which twenty three Bangladeshi traditional medicinal plants were evaluated for brine shrimp lethality toxicity. Different solvent extracts of these plants including Heritera fomes were used in this experiment. Of the 23 plants tested, about 80% were toxic to brine shrimp. (Rahmatullah et al. 2010)

In the year of 2011, A study was presented to evaluate the anti-hyperglycemic activity of methanol extract of barks in oral glucose tolerance tests in glucose-loaded Swiss albino mice. By anti-hyperglycemic activity tests, the extract demonstrated a dose-dependent and significant reduction in serum glucose levels, both at 60 as well as 120 minutes after glucose loading. At a dose of 250 mg extract/kg body weight, the level of serum glucose fell by 49.2% in mice following 60 minutes of glucose loading, which was higher than that obtained with the standard drug, glibenclamide at a dose of 10 mg/kg body weight (43.5%). When serum glucose levels were evaluated after

120 minutes following glucose loading, the percent reductions in serum glucose concentrations with glibenclamide, and 250 and 500 mg extract/per kg body weight were respectively, 30.1, 35.6, and 44.7. Overall, the results showed that methanol extract of bark of the plant possess good anti-hyperglycemic activities, which validate the folk medicinal uses of the plant in diabetes. (Ali et al, 2011)

In the year 2013, a study was done which was themed to phytochemical and pharmacological investigation to determine anti-nociceptive, antioxidant and analgesic activity to give an appropriate guide for future exploration. Standard test methods were used to explore phytochemical constituents. The acetic acid-induced writhing model was applied to inspect chemical anti-nociceptive effect, hot plate model for thermal nociception, DPPH assay for antioxidant activity, and disc diffusion assay method for antimicrobial activity. It had been seen thay phytochemical exploration of leaves extract of *"Heritiera fomes"* demonstrated the presence of reducing sugar, saponins, alkaloids, glycosides, tannins, flavonoids amd gums. It can be said that, the leaves extract of *"Heritiera fomes"* possess significant anti-nociceptive, antioxidant and antimicrobial activity. (Dhivya et al. 2013)

In 2013, Jayanta Kumar Patra and Hrudayanath Thatoi had a study on Heritiera fomes. In methanol extract of both the leaf and stem powder of H. fomes has showed strong anticancer property with 40% inhibition against B16 mouse melanoma (in vitro system) and Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice (in vivo system). They also have done partial characterizations of the methanol extract and found phenolic as the lead compounds by TLC, HPLC, 1H-NMR and FTIR spectral analysis. (Patra and Thatoi, 2013)

In 2014, A report was published on medicinal and other purposes of *Heritiera fomes* by doing a survey on the coastal people over a year. From the survey, they found the applications in traditional folk medicine as evidenced by its extensive use for treating diabetes, hepatic disorders, gastrointestinal disorders, goiter, and skin diseases by the local people and traditional health practitioners. A number of investigations indicated that the plant possesses significant antioxidant, antinociceptive, antihyperglycemic, antimicrobial, and anticancer activities. (Mahmud et al, 2014)

In 2016, a study was done where the researcher found some medicinal activity of *Heritiera fomes* and anti-diabetic activity was one of them. They formed silver and zinc oxide nanoparticles by using aqueous solution of *H. fomes.* By fourier transform infrared spectroscopy (FT-IR) they confirmed the presence of Oxime and other heterocyclic compounds. responsible for the reduction and stability of nanoparticles in the solutions. The synthesized NPs displayed moderate free radical scavenging properties. Then they found the Ag-NPs with unique properties of inhibiting α-amylase (91.14% and 89.16%) which indicate significant anti-diabetic activity of *Heritiera fomes.* (Thatoi et al, 2016)

In 2017, a study was done to evaluate the anti-obesity potential of the methanolic extract of leaves of *Heritera fomes*. In this experiment wister strain of albino rats were used by dividing then into 6 groups. Group 1 rats were served normal pellet chow, group 2 rates were served high fat cafeteria, group 3,4,5 rats were provided MEHfF and group 6 rats were given HFCD. Different results were seen on the test which varried on the rat's weight, serum total cholesterol LDL cholesterol, VLDL cholesterol, glucose level etc. These results demonstrate clearly that repeatedbpral administration of Heritera fomes methanolic extract can evoke a potent anti-obesity activity. (Mirza, Ali, Sanghvi, 2017)

Chapter 4

Materials

4.1 Sample Collection

To observe the anti-hyperglycemic activity of *Heritiera fomes*, different parts such as bark, root and leaf of this plant was collected from Sundarbans. After the collection of the plant materials, their identities were confirmed from the forest officer and also from botanical garden. Then the sample keep in a dry place and bring into home for washing.

Figure 4.1: Bark collection from tree

4.2 Sample

- Bark of *Heritiera fomes*
- Root of *Heritiera fomes*
- Leaf of *Heritiera fomes*

Figure 4.2: Plant materials

4.3 Reagents

Table 4.1: Reagents using for Extraction and Experiment purpose

Reagent	Source	Purpose
Methanol		Solvent for extraction
$BaSO_4$	EWU laboratory	To prepare $BaSO_4$ milk
Na-Carboxy methyl cellulose	EWU laboratory	To prepare $BaSO_4$ milk
H_2SO_4	EWU laboratory	Research purposes
NaOH	EWU laboratory	Research purposes
Saline	Local Pharma shop	Research purposes
Distilled water	EWU laboratory	

4.4 Equipments and Instruments

Table 4.2: Equipments and Instruments

Equipments/Instruments	Source	Origin	Purpose
Rotary Evaporator	Labtech	Korea	Extraction
Dissecting box	Research instructor	USA	Animal sacrifice
Electronic balance	Shimadzu AY220	Japan	To prepare $BaSO_4$ milk
Distil water plant	Bibby scientific	USA	Make distil water
Feeding needle	Research Instructor	USA	Drug administration to the animal

Figure 4.3: Rotary evaporator, Balance , Dissecting box and feeding needle

4.5 Apparatus

Some technical equipment or machinery needed for a particular activity or research work. Apparatus may to machine, equipment and critical apparatus. Some apparatus are listed in the following table those were widely used throughout the experiments and research work.

Table 4.3: Other apparatus used for research work

1	Beaker
2	Test tube
3	Filter paper
4	Filter cloth
5	Funnel
6	Volumetric flask (250 ml, 1000 ml)
7	Pipette (10 ml, 2 ml)
8	Spatula
9	Electric heater
10	Aluminium Foil paper
11	Test tube holder
12	Thermometer

4.6 Animal Model

Table 4.4: Animal Model	
Animal	**Source**
Swiss Mice	Icddr,b animal resource centre
Long Evans rat	Icddr,b animal resource centre

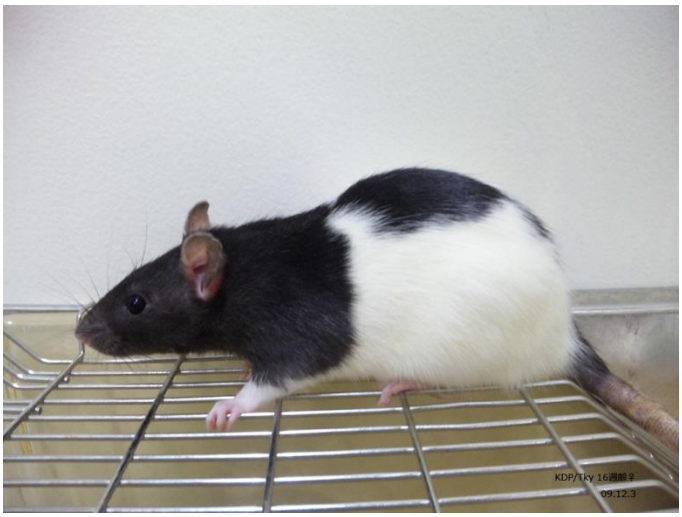

Figure 4.4: Long Evans Rat

Figure 4.5: Swiss Mice

Chapter 5

Methodology

5.1 Extraction of Chemical Constituents from Plant Materials

5.1.1 Extraction [www.chemicool.com, Accessed on 2017]

Extraction is a way to separate a desired substance when it is mixed with others. The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble.

Extraction uses two immiscible phases (these are phases that do not mix, like oil and water) to separate the substance from one phase into the other.

The solvent used for extraction is known as Menstrum and the inert insoluble material that remains after extraction is called Marc.

The extracts from plant tissue are a rich source of lead compounds for nutraceutical or pharmaceutical applications. (www.chemicool.com, Accessed on 2017)

5.1.2 Different Types of Extraction Processes [www.medicinalplants-pharmacognosy.com, Accessed on 2017;www.satveda.com, Accessed on 2017; Lee, 2017]

Chemical constituents can be extracted from plant materials by using various methods such as maceration, infusion, digestion, decoction, percolation etc.

5.1.2.1 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. (Lee, 2017)

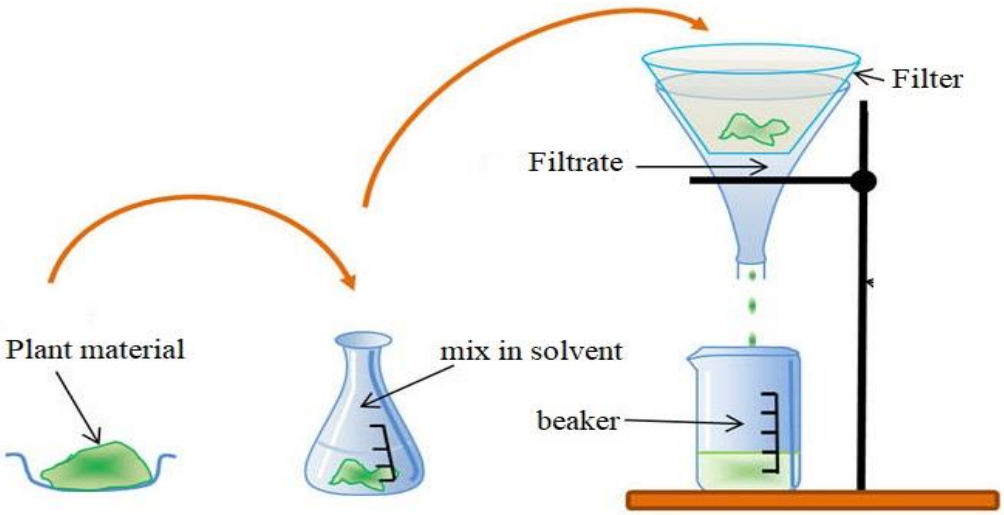

Figure 5.1: Maceration process

5.1.2.2 Infusion

Fresh infusions are prepared by sokaing the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs. (Lee, 2017)

Figure 5.2: Herbal Infusion

5.1.2.3 Digestion

This is a form of extraction in which gentle heat is used during the process. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased. (Lee, 2017)

5.1.2.4 Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, thermostable constituents. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one - fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further. (Lee, 2017)

5.1.2.5 Percolation

Percolation is a continuous flow of the solvent through the bed of the crude drug material to get the extract.In this process, the powdered drug is moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 hours in a well-closed container, after which the mass is packed and the top of the percolator is closed.Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to soak in the closed percolator for 24 hours. (www.satveda.com, Accessed on 2017)

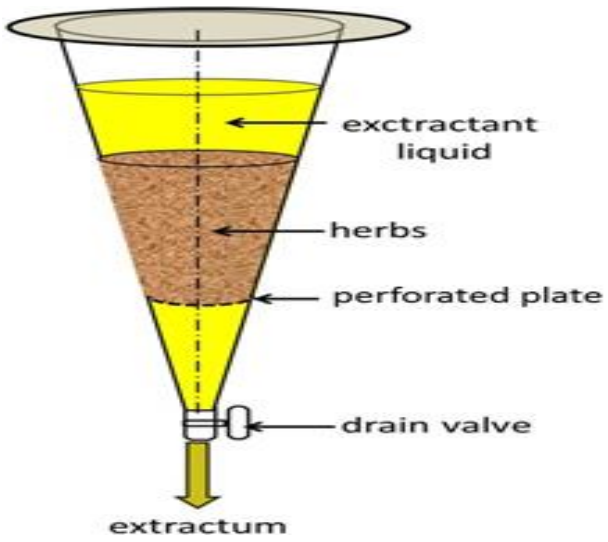

Figure 5.3: Percolation Method of Extraction

5.1.3 Extraction of Chemical Constituents from Different Parts of *Heritiera fomes*

Maceration method is used for the extraction ofdifferent parts of *Heritiera fomes* (Bark and Root)

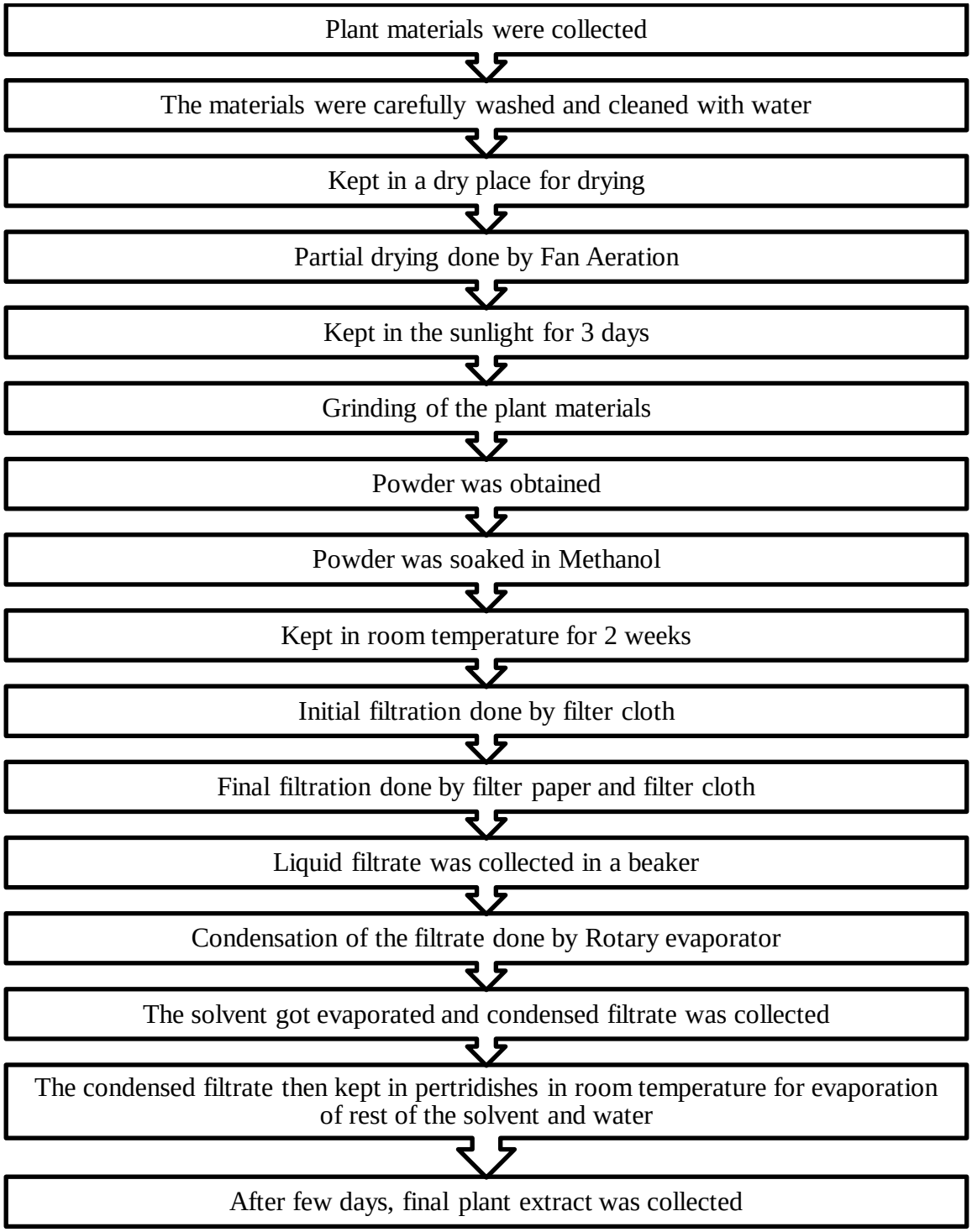

Figure 5.4: Flow Chart of preparation of methanolic extracts of Root and Bark of *Heritiera fomes*

Figure 5.5: Sunlight drying of plant part

Figure 5.6: Soaking of powdered plant materials in Methanol

Figure 5.7: Powdered Bark and Root of *Heritiera fomes* after grinding

Figure 5.8: Final Extract obtained from powdered Bark and Root of *Heritiera fomes*

5.2 Method to measure the Effect of *Heritiera fomes* on GI Motility

5.2.1 Principle

One of the pivotal tasks of GI tract is its ability to organize and co-ordinate transport of luminal content which is perfectly adjusted to the digestive needs of the body. To achieve this GI tract exhibits a wide repertoire of motor patters that are based on spatiotemporal co-ordination of muscle activity. The GI tract is able to monitor caloric density; osmolarity and pH of the luminal content react with the initiation of the appropriate motility pattern.

The fascinating variety of motility patterns is best appreciated by imagining gut motility and transit of luminal content by video fluoroscopy.

Motility disorders in the gut are major causes and concomitant phenomena of various functional, structural and inflammatory bowel disease; one of the most prominent example is Irritable Bowel Disease (IBS).

5.2.2 Research Animal

- ✓ *Swiss albino* mice collected from Icddr,b

5.2.3 Method Design

- For the experiments, 24 mice were selected randomly for each extract (MEHfR & MEHfB) and then divided into 3 groups. Each group consisted of 8 mice and they were termed Group 1 to Group 3.
  - ✓ Group 1 – Control (Distilled Water)
  - ✓ Group 2 – Test (Plant Extract)
  - ✓ Group 3 – Standard (Bisacodyl)
- Before the experiment, the mice were weighed and marked accordingly. The dose of the sample and the standard drug were administered as per body weight. A specific treatment was set for each group.

5.2.4 Plant Extract (Sample)

- ✓ Methanolic extract of *Heritiera fomes* root. (MEHfR)
- ✓ Methanolic extract of *Heritiera fomes* bark. (MEHfB)

5.2.5 Standard Drug

- ✓ Bisacodyl (Duralax - 5mg) collected from Opsonin Pharma Ltd.

5.2.6 Dose

- ✓ 500 mg/kg per body weight of the mice

5.2.7 Preparation of Standard and Sample Solution

For the preparation of MEHfR & MEHfB solution at doses 500 mg/kg per body weight of mice, the extract was weighed based on the weight of the experimented mice and sonicated in a unidirectional way by the addition of distilled water. A small amount of CMC was slowly added as a suspending agent for proper mixing. To stabilize the suspension, it was stirred adequately.

For the preparation of positive control group, Bisacodyl (1mg/kg) was taken and required volume of suspension was prepared.

5.2.8 Preparation of $BaSO_4$ Milk

$BaSO_4$ milk was prepared by dissolving 10% (w/v) $BaSO_4$ milk in 0.5% (w/v) CMC suspention.

5.2.9 Experiment plan

Table 5.1: Test samples used in the estimation of effect of MEHfR & MEHfB on GI motility

Group	Treatment	Dose	Route of Administration
Group 1 (Control)	Distilled Water	10 ml/kg	Orally
Group 2 (Extract)	MEHfR & MEHfB	500 mg/kg	Orally
Group 3 (Standard)	Bisacodyl	1mg/kg	Orally

5.2.10 Procedure

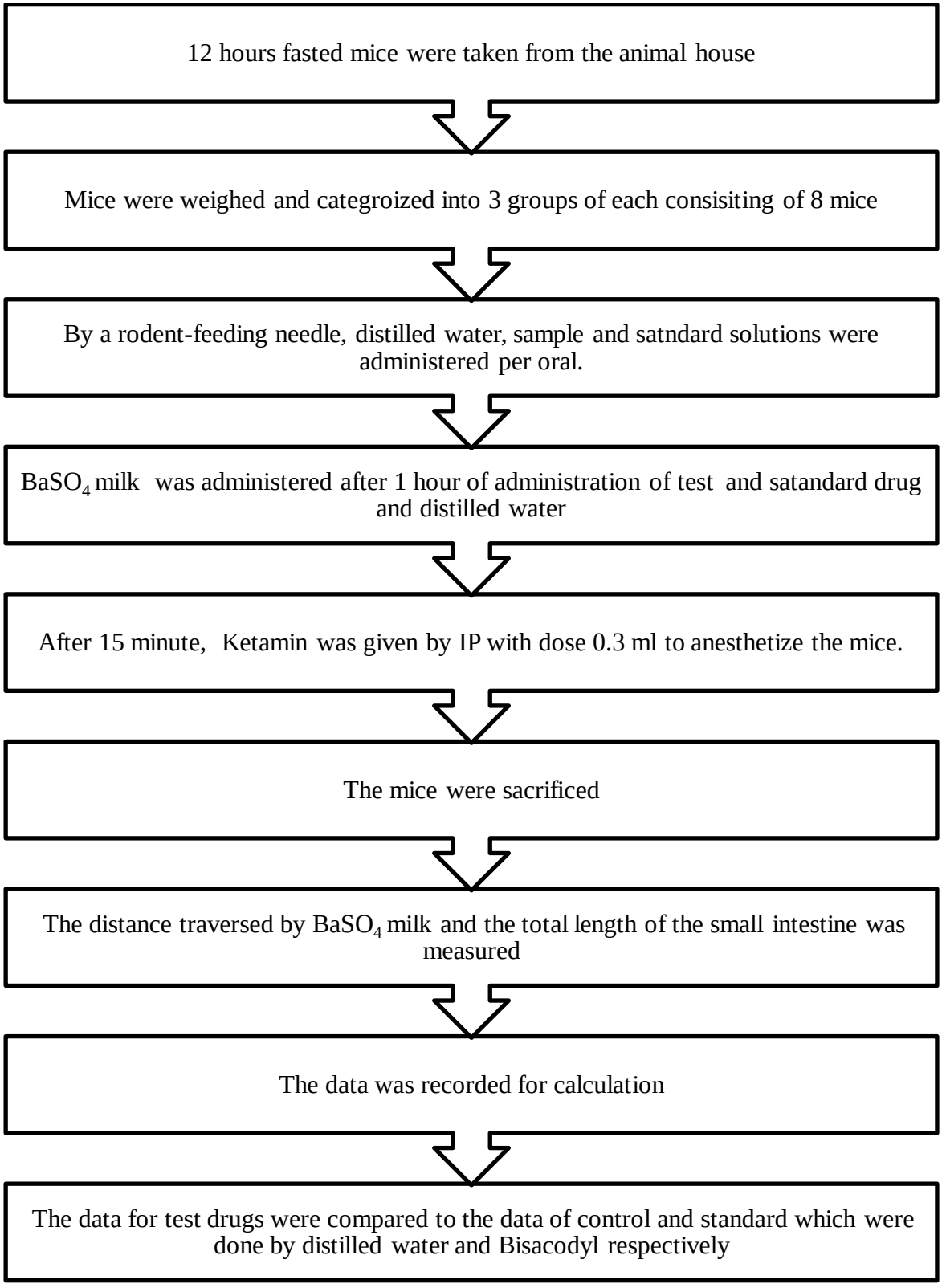

Figure 5.9: Flow chart of $BaSO_4$ aided GI Motility Test

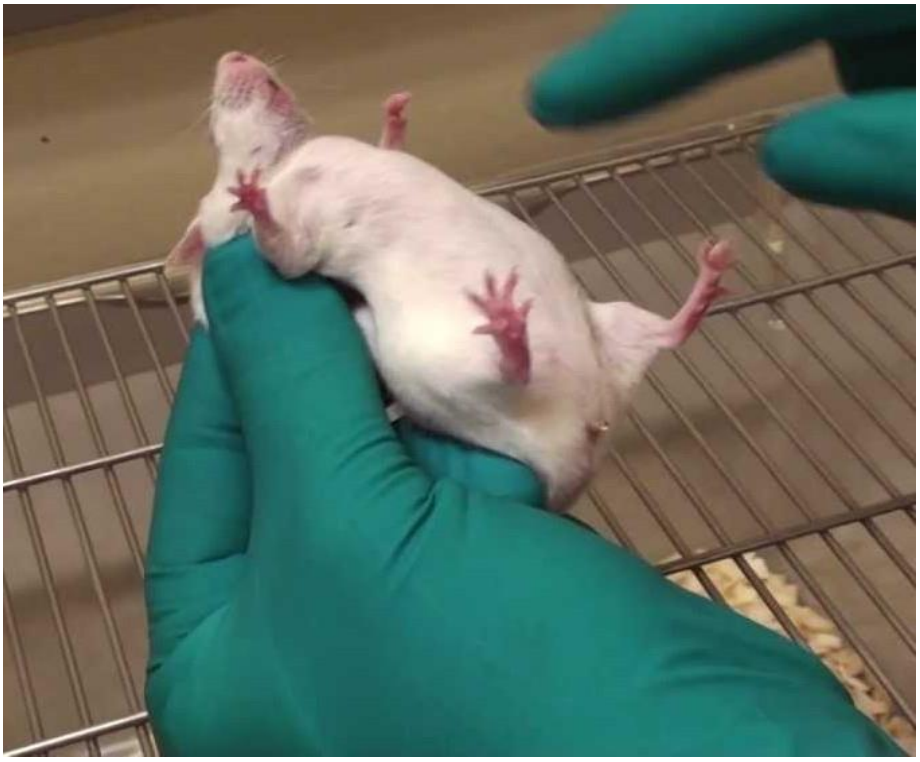

Figure 5.10:Mice handling

Figure 5.11: Sacrifice of Mice

Figure 5.12: Excision of Small Intestine of Mice

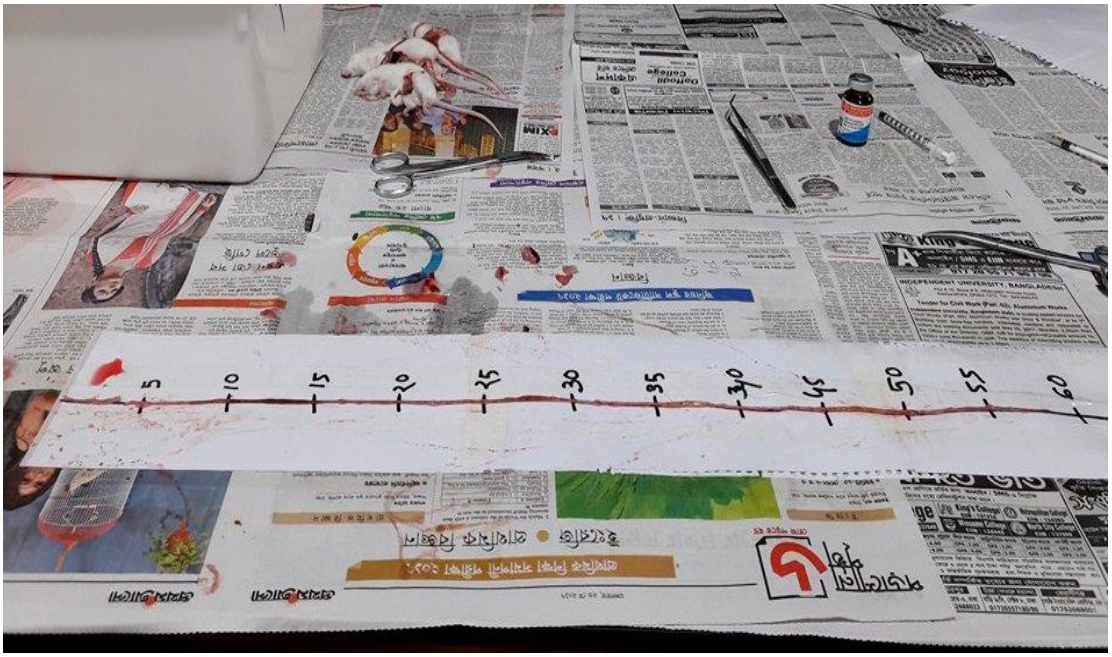

Figure 5.13: Measuring of the traversed $BaSO_4$ Milk in the Small Intestine of the Mice

5.3 Method to measure the Effect of *Heritiera fomes* plant parts on Carbohydrate Absorption

5.3.1 Principle

The postprandial hyperglycemia is common among diabetic patients. The control of postprandial hyperglycemia is very difficult because an excessively high blood glucose level may cause the induction and progression of diabetic complications through activation of the polyol pathway, increased glycation of protein and promotion of hyperinsulinemia. Therefore, the retardation of carbohydrate absorption offers a potential therapeutic approach for the management of diabetes mellitus.

5.3.2 Research Animal

- ✓ *Long Evans* rat collected from Icddr,b

5.3.3 Method Design

- For the experiments, 16 rats were selected randomly for each extract (MEHfR & MEHfB) and then divided into 2 groups. Each group consisted of 8 rats and they were termed Group 1 to Group 2.
    - ✓ Group 1 – Control (Sugar solution)
    - ✓ Group 2 – Test (Plant Extract)
- Each group than divided into 4 sub-group based on the sacrifice time.
    - ✓ 30 minute
    - ✓ 60 minute
    - ✓ 120 minute
    - ✓ 240 minute
- Before the experiment, the rats were weighed and marked accordingly. The dose of the sample and the control were administered per body weight. A specific treatment was set for each group.

5.3.4 Plant Extract (Sample)

- ✓ Methanolic extract of *Heritiera fomes* root. (MEHfR)
- ✓ Methanolic extract of *Heritiera fomes* bark. (MEHfB)

5.3.5 Dose

- ✓ 500 mg/kg per body weight of the rat.

5.3.6 Preparation of Sucrose Solution

2.5 gm sucrose per kg body weight of rat was dissolved in 5ml distilled water.

5.3.7 Preparation of Sample Solution

For the preparation of MEHfR & MEHfB solution at doses 500 mg/kg per body weight of rat, the extract was weighed based on the weight of the experimented rat and sonicated in a unidirectional way by the addition of required volume of sucrose solution.

5.3.8 Other Chemicals

- ✓ 2N H_2SO_4
- ✓ 1N NaOH
- ✓ Normal saline
- ✓ Glucose kit

5.3.9 Experiment Plan

Table 5.2: Test samples used in the estimation of effect of MEHfR & MEHfB on carbohydrate absorption

Group	Treatment	Dose	Route of Administration
Group 1 (Control)	Sucrose solution	2.5 g/kg	Orally
Group 2 (Extract)	MEHfR & MEHfB	500 mg/kg	Orally

5.3.10 Procedure

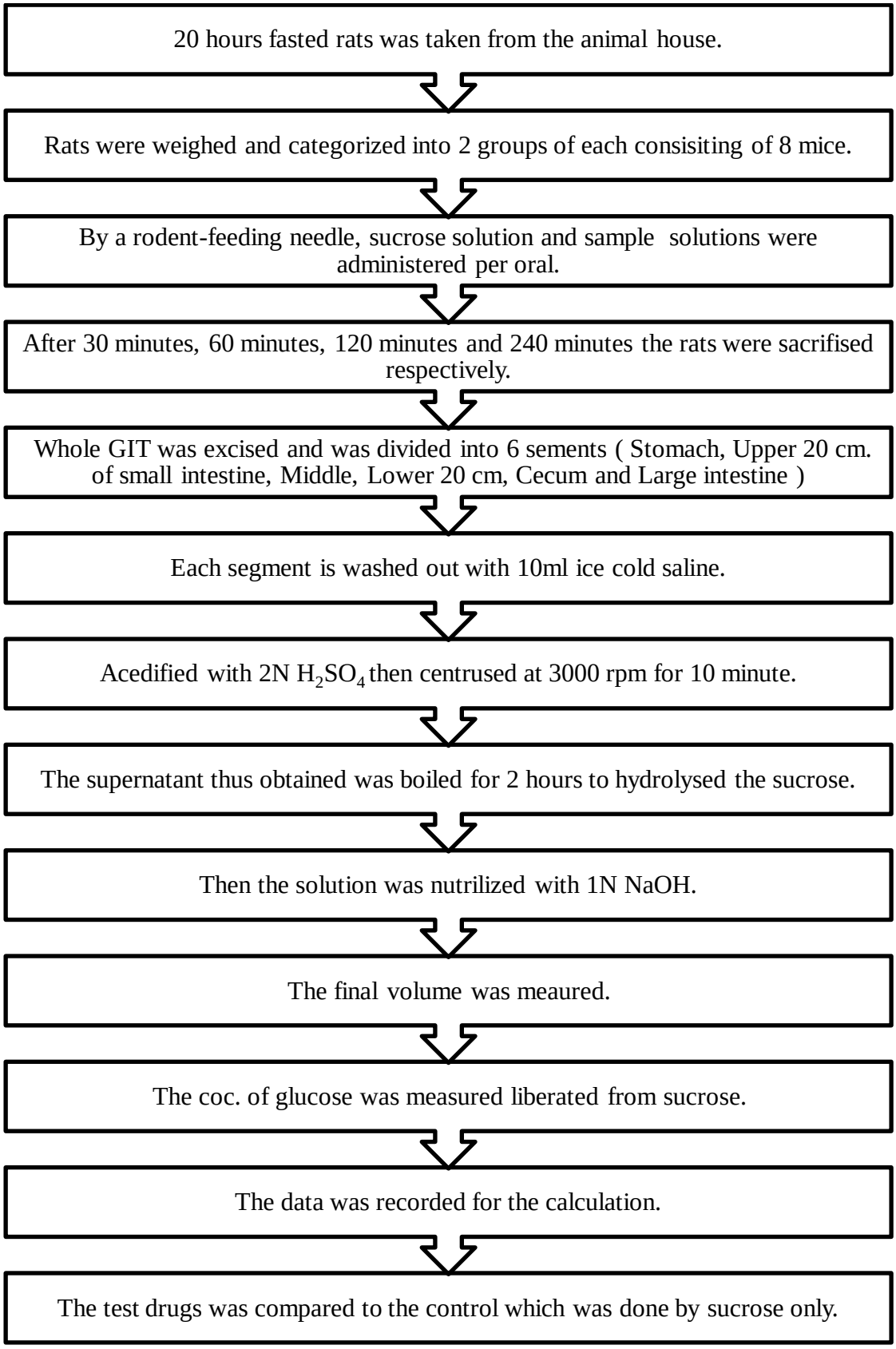

Figure 5.14: Flow chart of Carbohydrate absorption study in *Long evans* rats

Figure 5.15: Rat handling

Figure 5.16: Rat sacrifice

Figure 5.17: Sacrificed rat

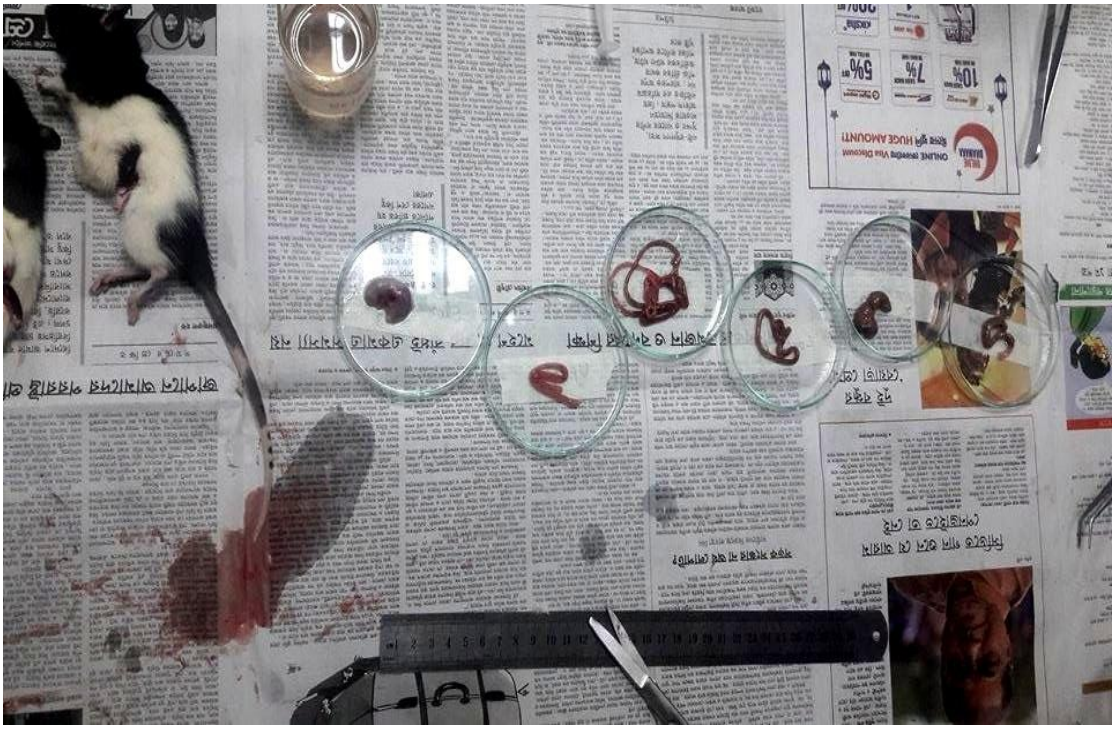

Figure 5.18: Six segments of GI tract

5.4 Assessment of the Effect of *Heritiera fomes*plant partson Intestinal Disaccharidase Activity

5.4.1 Principle

Carbohydrate is a vitally important component and disaccharidases are an essential subset of digestive enzymes required for the terminal step of carbohydrate digestion. The discovery of substances that inhibit the enzyme responsible for the digestion and absorption of carbohydrates in brush border membrane of the intestinal epithelial cells and flatten the postprandial glucose rise without giving extra load on the beta cells to secret insulin may introduce a new therapeutic approach for diabetic patient.

5.4.2 Research Animal

- ✓ Long Evans rat collected from Icddr,b

5.4.3 Method Design

- For the experiments, 12 rats were selected randomly for each extract (MEHfR & MEHfB) and then divided into 3 groups. Each group consisted of 4 rats and they were termed Group 1 to Group 3.
    - ✓ Group 1 - Control (Distilled Water)
    - ✓ Group 2 - Test (Plant Extract)
    - ✓ Group 3 - Standard (Acarbose)
- Before the experiment, the rats were weighed and marked accordingly. The dose of the sample and the control were administered per body weight. A specific treatment was set for each group.

5.4.4 Plant Extract (Sample)

- ✓ Methanolic extract of *Heritiera fomes* root. (MEHfR)
- ✓ Methanolic extract of *Heritiera fomes* bark. (MEHfB)

5.4.5 Dose

- ✓ 500 mg/kg per body weight of the rat.

5.4.6 Preparation of Sucrose Solution

0.137 gm sucrose was dissolved in 10 ml distilled water to prepare 40mM sucrose solution.

5.4.7 Preparation of Sample and Standard Solution

For the preparation of MEHfR & MEHfB solution at doses 500 mg/kg per body weight of rat, the extract and standard were weighed based on the weight of the experimented rat and sonicated in a unidirectional way by the addition of required volume of distilled water to make suspension.

5.4.8 Preparation of Nomal Saline

9 gm NaCl was dissolved in 1000 ml distilled water.

5.4.9 Experiment plan

Table 5.3: Test samples used in the estimation of effect of MEHfR & MEHfB on Disaccharidase activity

Group	Treatment	Dose	Route of Administration
Group 1 (Control)	Distilled water	10 ml/kg	Orally
Group 2 (Extract)	MEHfR & MEHfB	500 mg/kg	Orally
Group 3 (Standard)	Acarbose	500mg/kg	Orally

5.4.10 Procedure

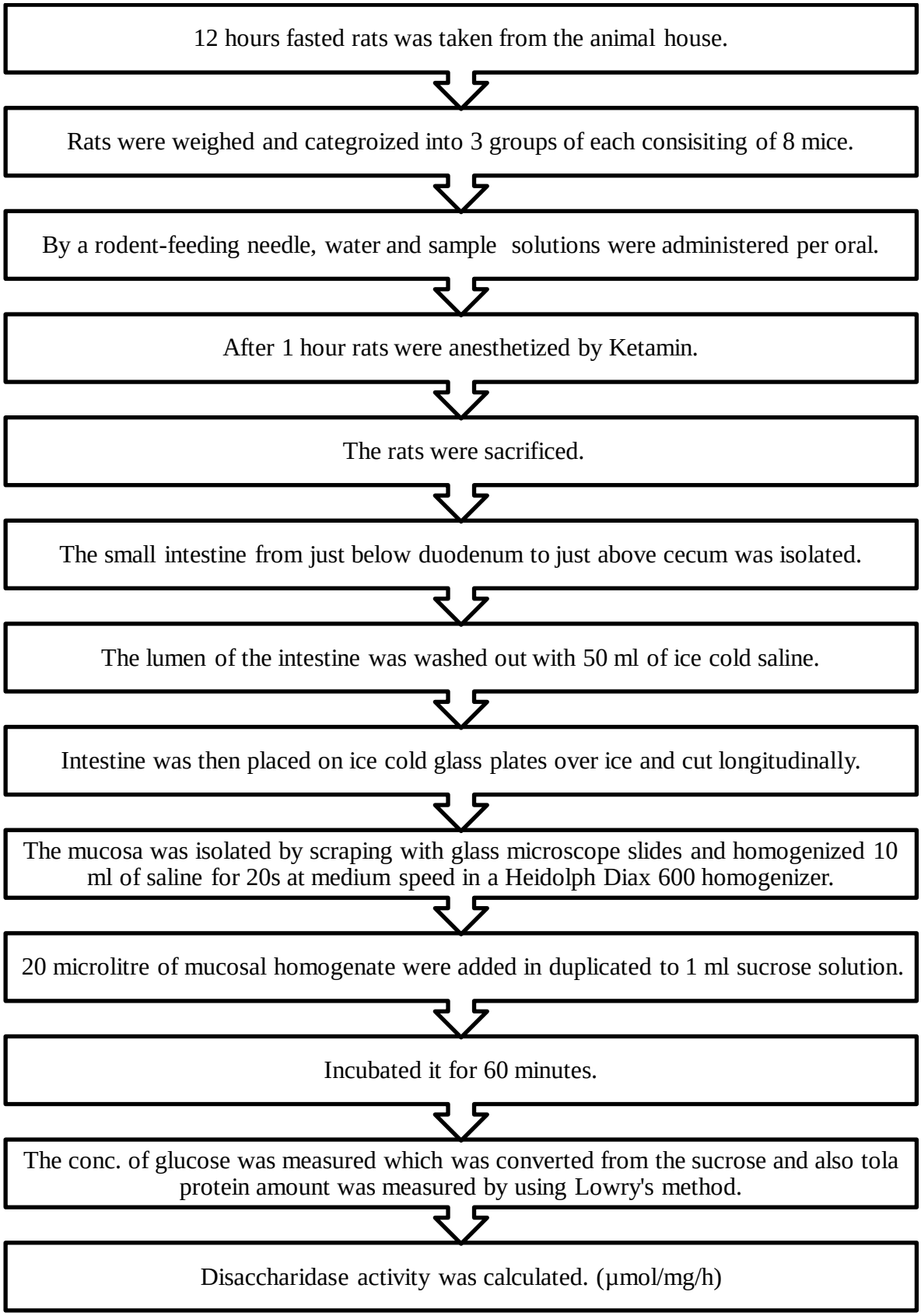

Figure 5.19: Flow chart of Disaccharidase Activity study in *Long evans* rats

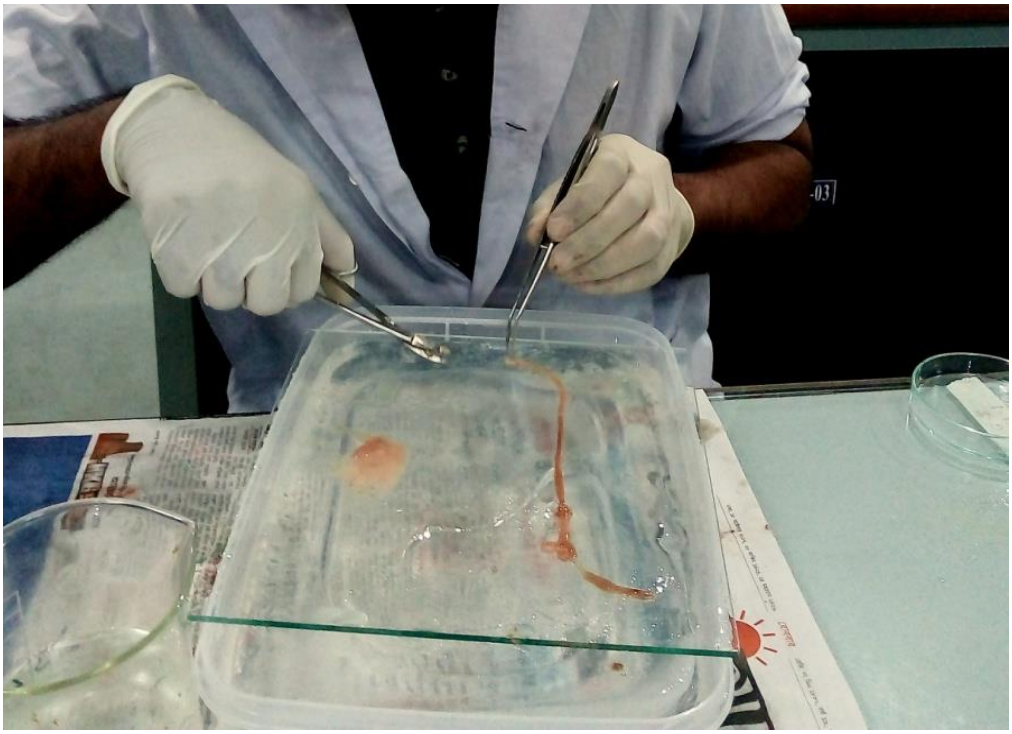

Figure 5.20: Longitudinally cutting of the small intestine

Figure 5.21: Separation of mucosa from the small intestine

Chapter 6

Result

&

Discussion

6.1 Result

6.1.1 Effect of the *Heritiera fomes* on GI Motility

At doses 500 mg/kg, experimental root and bark extracts were administered to mice. As a result, %GI motility got increased significantly ($p < 0.05/ 0.01/ 0.001$). From Table 6.1, significant levels of increase in %GI motility of mice after 1 hour of root extract solution administration can be observed. Table 6.2, significant levels of increase in %GI motility of mice after 1 hour of bark extract solution administration can be observed. The standard drug, Bisacodyl, also exhibited a significant increase in %GI motility in the mice model after 1 hour of administration.

Table 6.1: Data of the GI motility test to determine the effect of MEHfR

Group	**%GI Motility**
Control	57.637 ± 2.27
Heritiera fomes	67.962 ± 1.14 ***
Standard (Bisacodyl)	84.062 ± 0.51 ***

Table 6.2: Data of the GI motility test to determine the effect of MEHfB

Group	**%GI Motility**
Control	57.987±1.38
Heritiera fomes	60.262±1.67***
Standard (Bisacodyl)	84.062 ± 0.51***

Here, MEHfR refers to methanolic extract of *Heritiera fomes* root and MEHfB refers to methanolic extract of *Heritiera fomes* bark. Data are presented as Mean ± SEM; n=8. Data values marked with (***) significantly different from the corresponding values of the CONTROL group at $p<0.001$

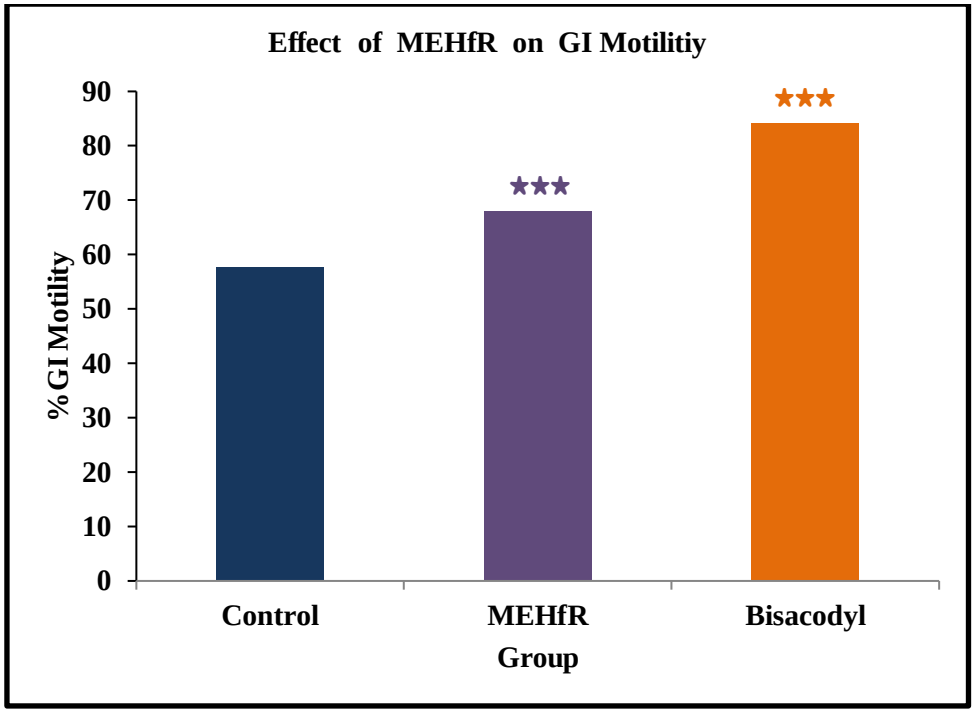

Figure 6.1: Graph for GI Motility test for MEHfR

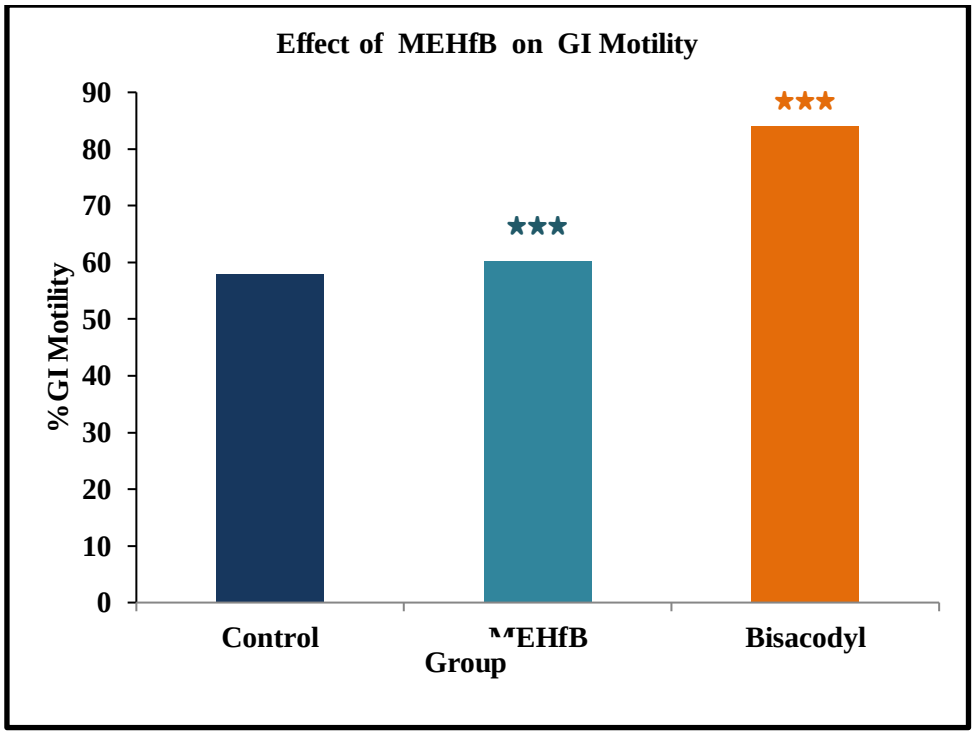

Figure 6.2: Graph for GI Motility test for MEHfB

6.1.2 Effect of *Heritiera fomes* on Carbohydrate Absorption

At doses 500 mg/kg, experimental root and bark extracts were administered to rat. As a result, carbohydrate absorption got decreased significantly ($p < 0.05/ 0.01/ 0.001$). From Table 6.3, significant levels of decrease in carbohydrate absorption of rat after 30 minutes, 60 minutes, 120 minutes and 240 minutes of root extract solution administration can be observed. Table 6.4, significant levels of decrease in carbohydrate absorption of rat after 30 minutes, 60 minutes, 120 minutes and 240 minutes of bark extract solution administration can be observed.

Table 6.3: Data of the carbohydrate absorption test to determine the effect of MEHfR

Segment	Group	30 min	60 min	120 min	240 min
Stomach	**Control**	54.13±1.7	34.04±3.94	8.50±0.60	1.32±0.32
	MEHfR	65.35±1.85*	45.98±2.02	15.76±0.44*	2.77±0.33
Upper	**Control**	14.69±0.89	11.68±0.66	4.56±1.08	0.95±0.15
	MEHfR	22.14±1.42*	16.92±0.11*	6.65±0.86	1.47±0.02
Middle	**Control**	20.17±1.95	17.48±0.72	7.99±0.05	1.26±0.08
	MEHfR	30.48±0.08*	22.53±0.03*	10.08±0.18**	1.71±0.02*
Lower	**Control**	5.57±0.7	3.24±0.73	1.26±0.56	0.98±0.02
	MEHfR	19.4±1.2**	9.28±0.32*	3.49±0.48**	1.18±0.03*
Cecum	**Control**	2.7±0.4	2.01±0.0	1.76±0.04	0.74±0.08
	MEHfR	6.04±0.63*	4.36±0.05***	3.52±0.39*	0.93±0.07
Large Instestine	**Control**	1.32±0.22	0.94±0.06	0.96±0.15	0.48±0.01
	MEHfR	4.88±0.23**	3.36±0.52*	2.20±0.21*	0.62±0.0***

Here, MEHfR refers to methanolic extract of *Heritiera fomes* root. Data are presented as Mean ± SEM; n=2. Data values marked with (***), (**) and (*) significantly different from the corresponding values of the CONTROL group at p<0.001, p<0.01 and p<0.005 respectively.

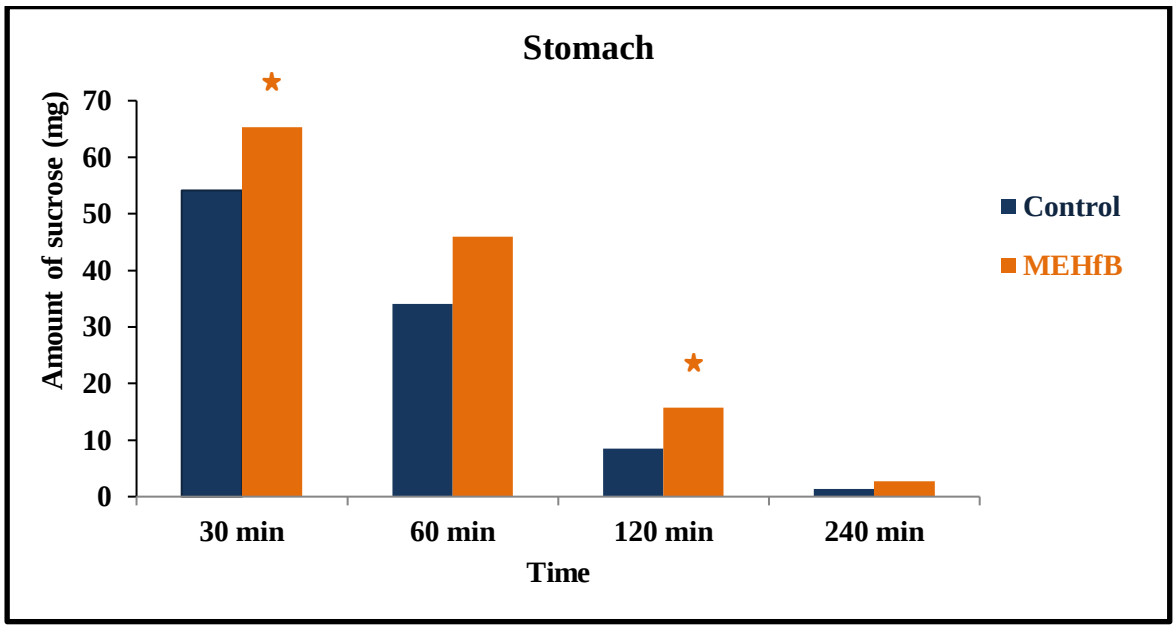

Figure 6.3: Graph of carbohydrate absorption test for MEHfR in stomach of rat.

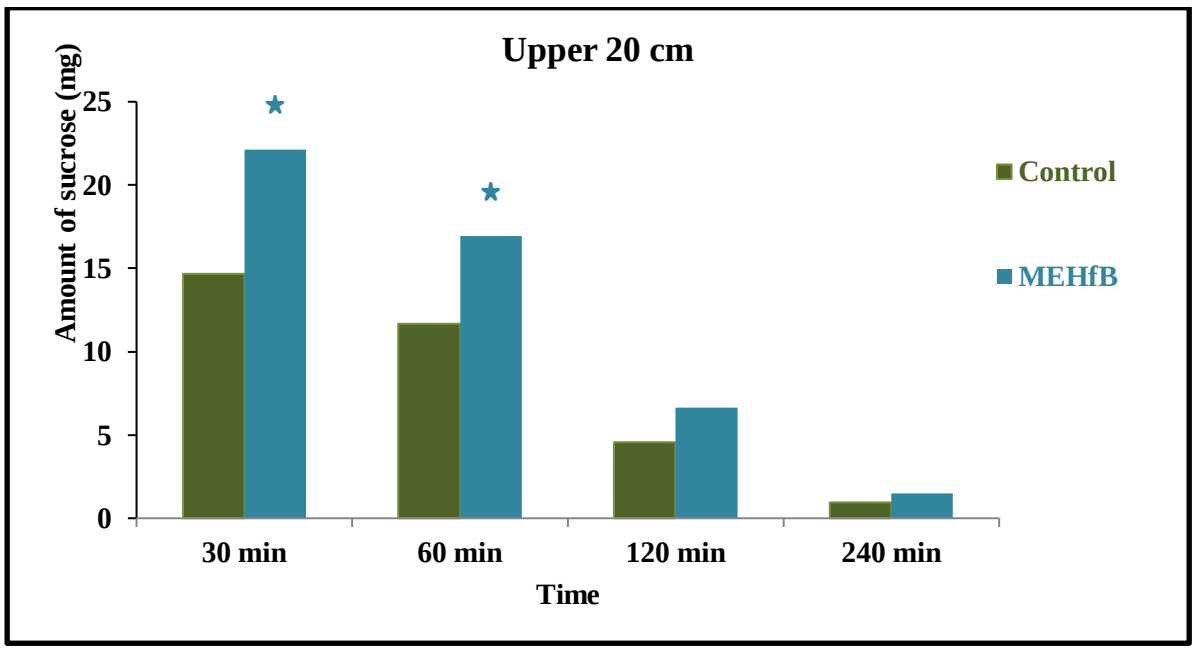

Figure 6.4: Graph of carbohydrate absorption test for MEHfR in upper 20 cm of rat small intestine.

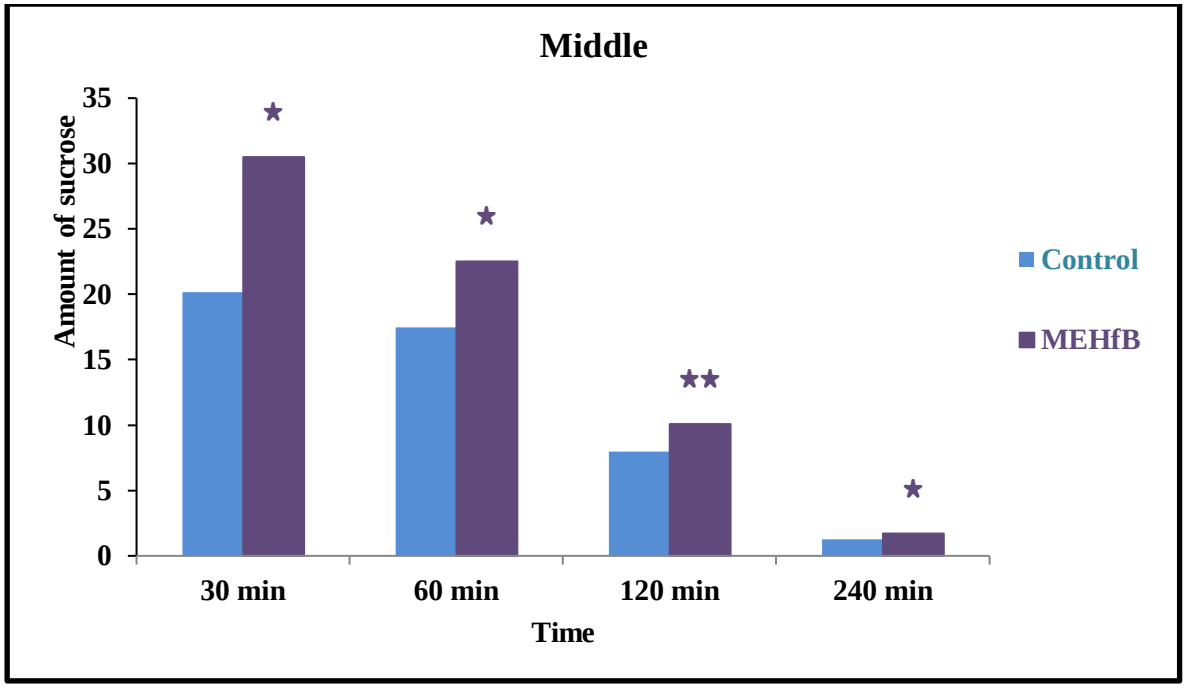

Figure 6.5: Graph of carbohydrate absorption test for MEHfR in middle of rat small intestine.

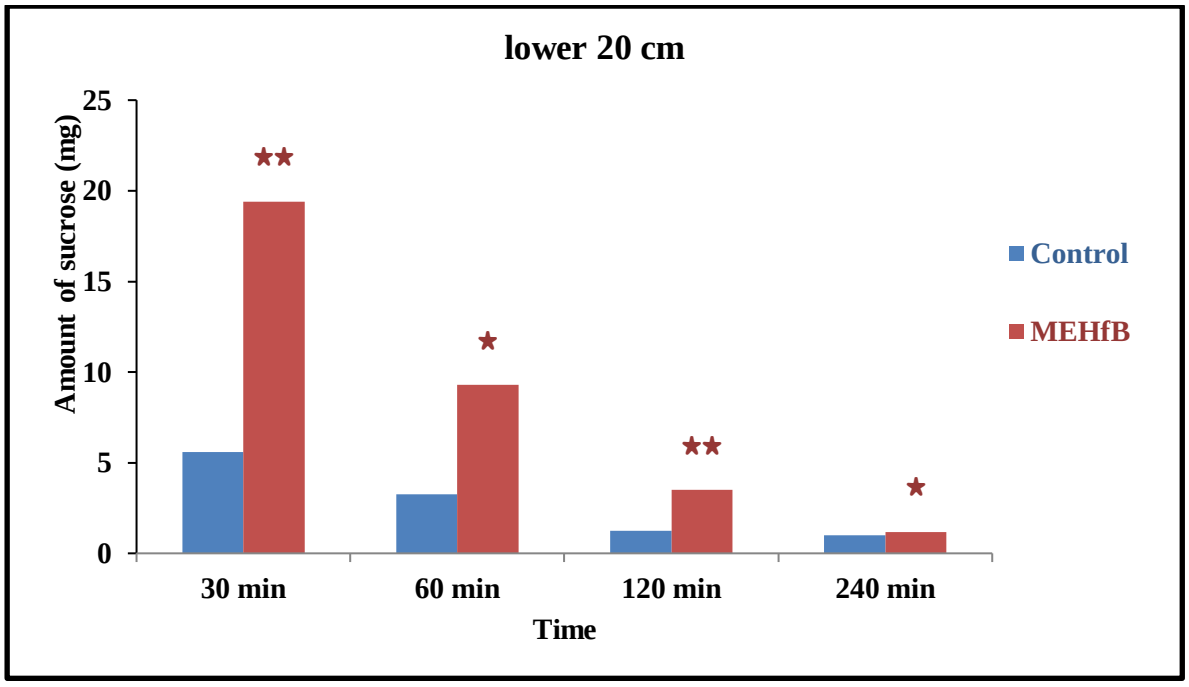

Figure 6.6: Graph of carbohydrate absorption test for MEHfR in lower 20 cm of rat small intestine.

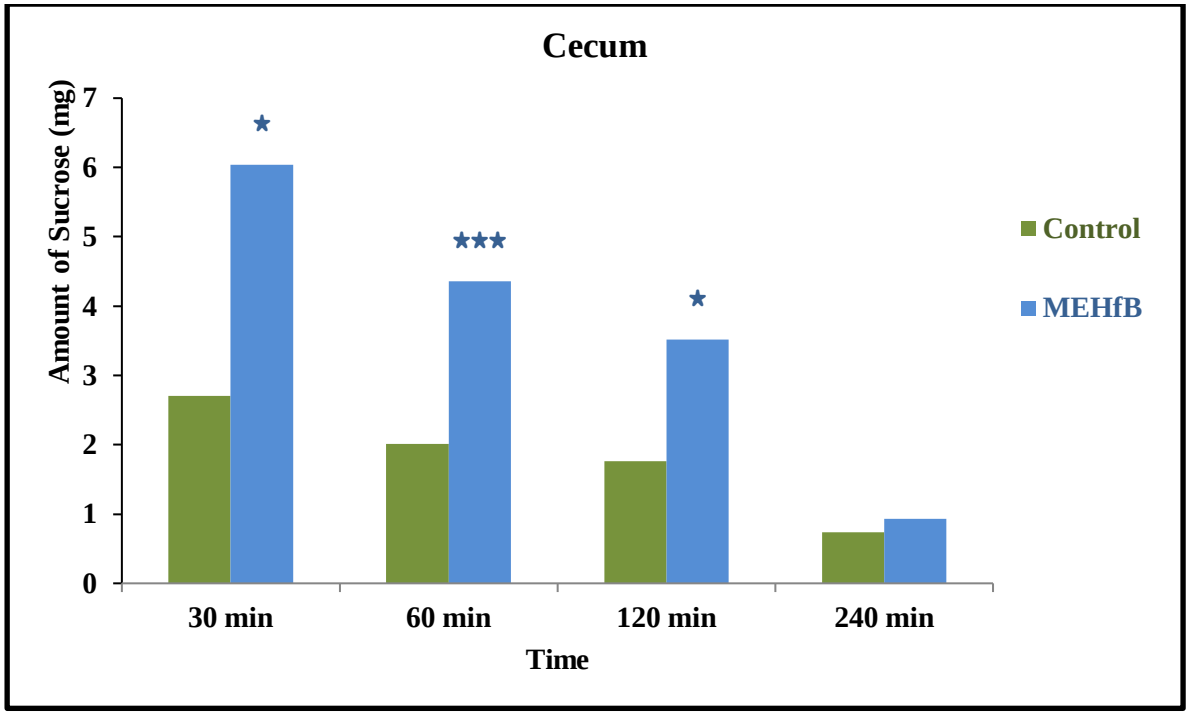

Figure 6.7: Graph of carbohydrate absorption test for MEHfR in cecum of rat.

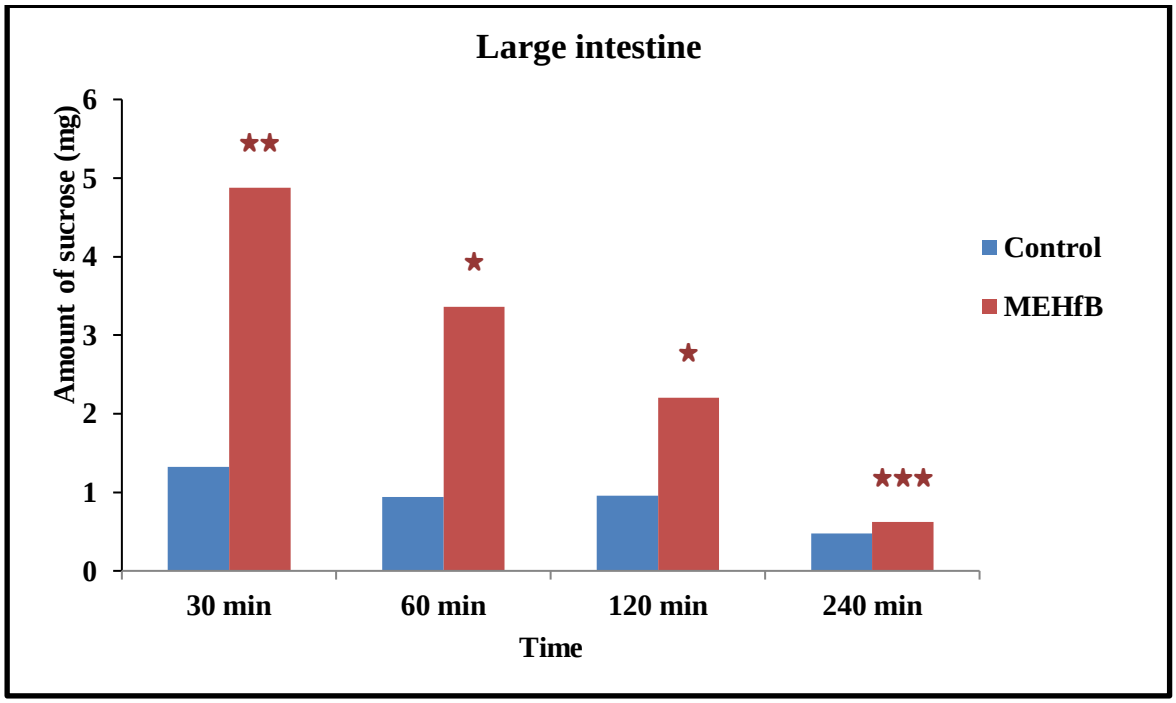

Figure 6.8: Graph of carbohydrate absorption test for MEHfR in large intestine of rat.

Table 6.4: Data of the carbohydrate absorption test to determine the effect of MEHfB					
Segment	**Group**	**30 min**	**60 min**	**120 min**	**240 min**
Stomach	**Control**	54.13±1.7	34.04±3.94	8.50±0.60	1.32±0.32
	MEHfB	58.85±2.55	43.91±7.89	10.82±0.29	2.90±0.10*****
Upper 20 cm	**Control**	14.69±0.89	11.68±0.66	4.56±1.08	0.95±0.15
	MEHfB	20.35±0.85*****	13.30±1.00	7.64±1.56	1.49±0.06
Middle	**Control**	20.17±1.95	17.48±0.72	7.99±0.05	1.26±0.08
	MEHfB	21.76±2.36	16.94±0.56	9.23±1.10	2.27±0.17*****
Lower 20 cm	**Control**	5.57±0.7	3.24±0.73	1.26±0.56	0.98±0.02
	MEHfB	8.26±0.16	8.00±0.55*****	3.38±0.02	1.98±0.17*****
Cecum	**Control**	2.7±0.4	2.01±0.0	1.76±0.04	0.74±0.08
	MEHfB	5.07±0.07*****	2.65±0.13*****	2.20±0.38*****	0.98±0.12
Large Instestine	**Control**	1.32±0.22	0.94±0.06	0.96±0.15	0.48±0.01
	MEHfB	3.57±0.97	2.45±0.05******	1.68±0.08*****	0.73±0.02******

Here, MEHfB refers to methanolic extract of *Heritiera fomes* bark. Data are presented as Mean ± SEM; n=2. Data values marked with (*******), (******) and (*****) significantly different from the corresponding values of the CONTROL group at $p<0.001$, $p<0.01$ and $p<0.005$ respectively.

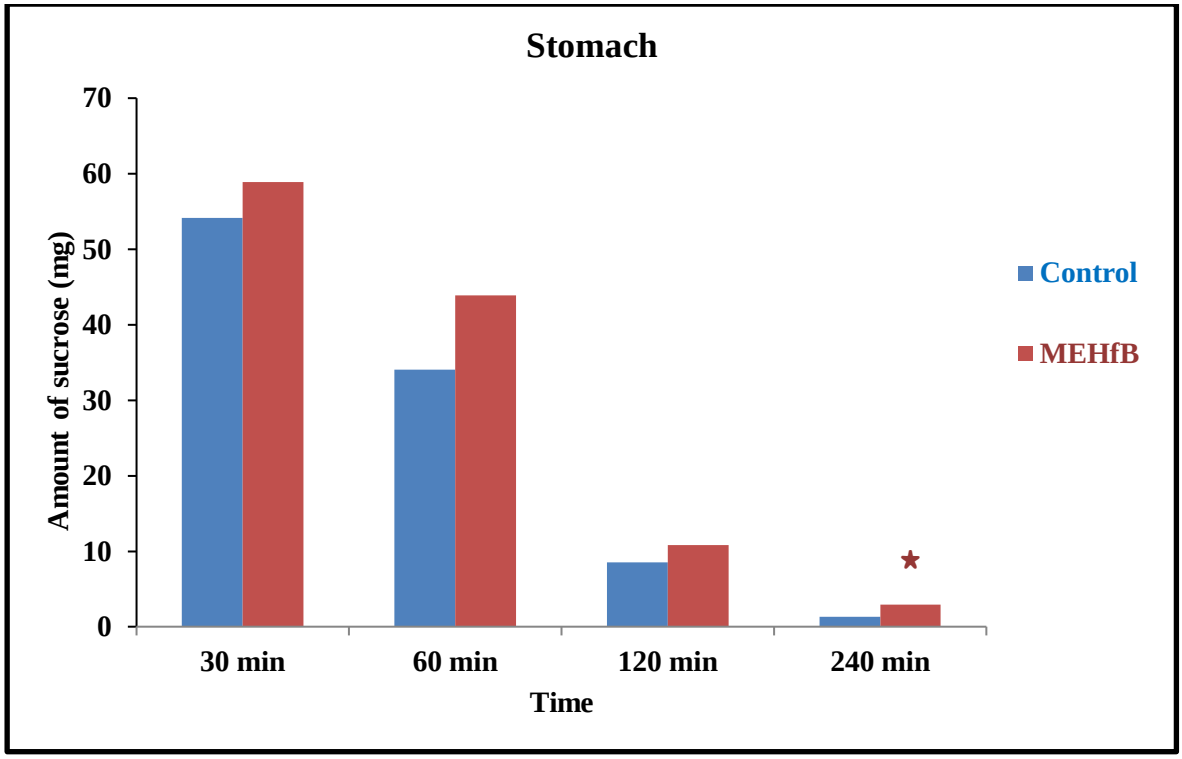

Figure 6.9: Graph of carbohydrate absorption test for MEHfB in stomach of rat.

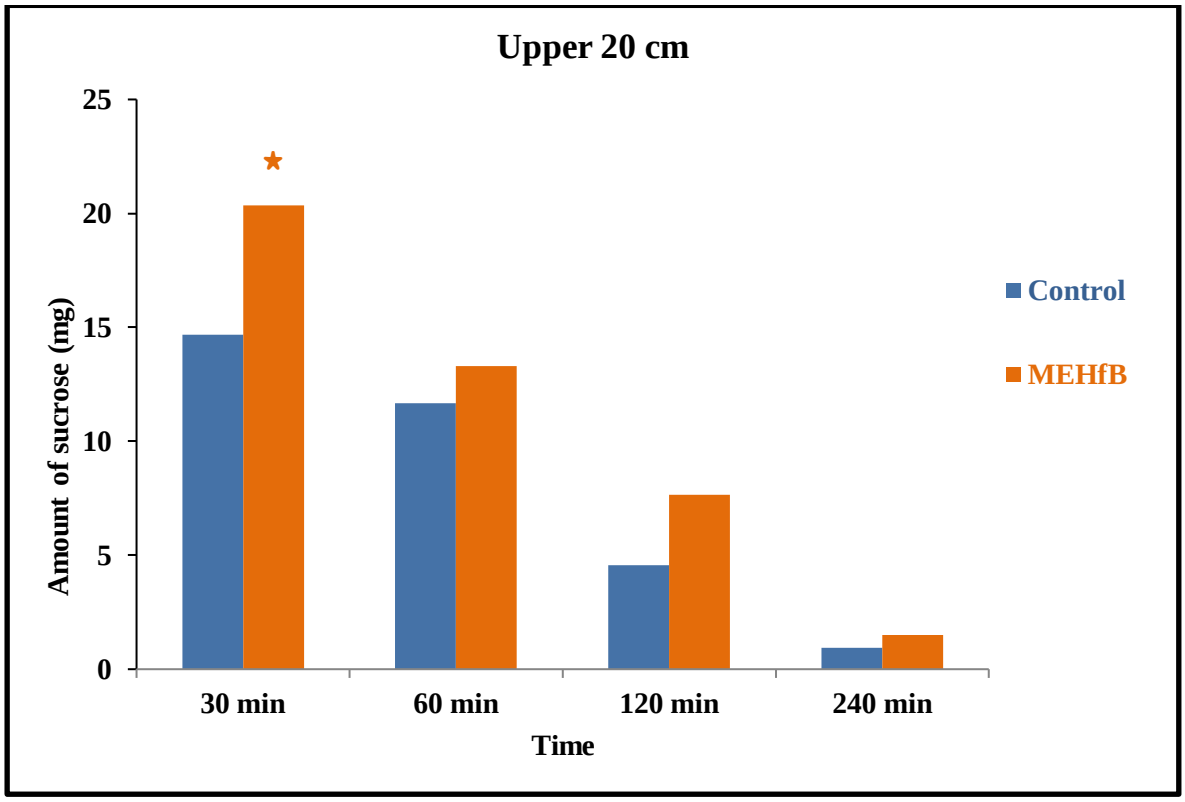

Figure 6.10: Graph of carbohydrate absorption test for MEHfB in upper 20 cm of rat small intestine.

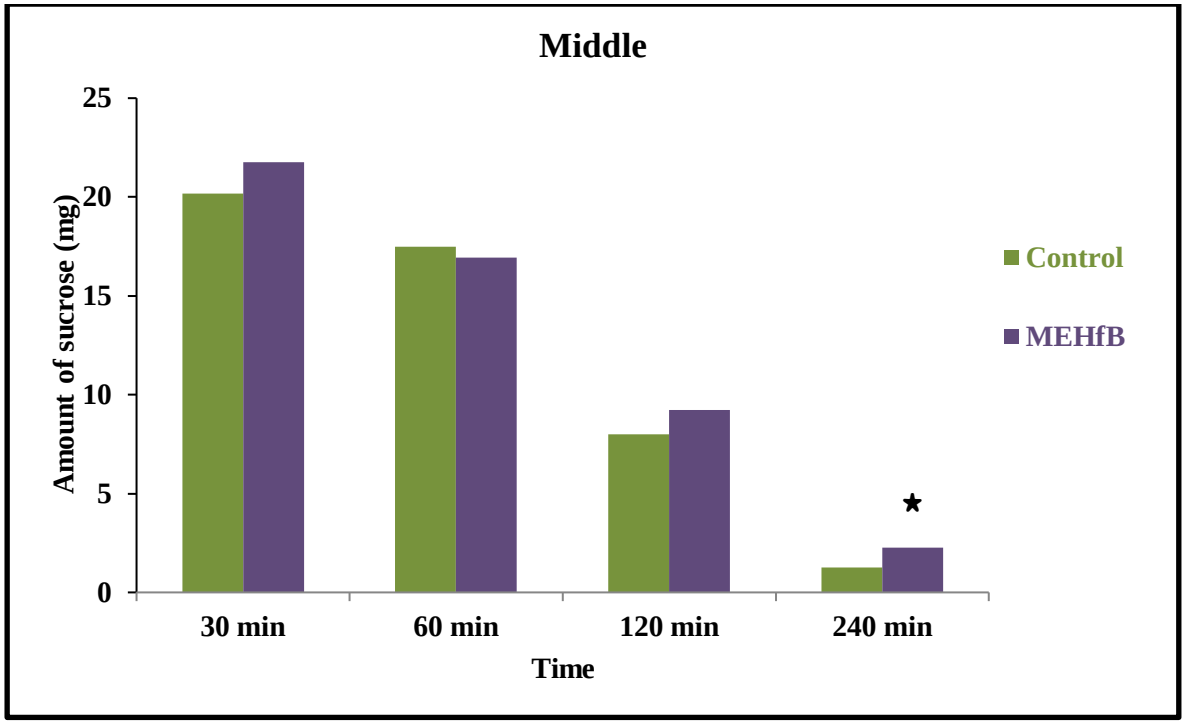

Figure 6.11: Graph of carbohydrate absorption test for MEHfB in middle of rat small intestine.

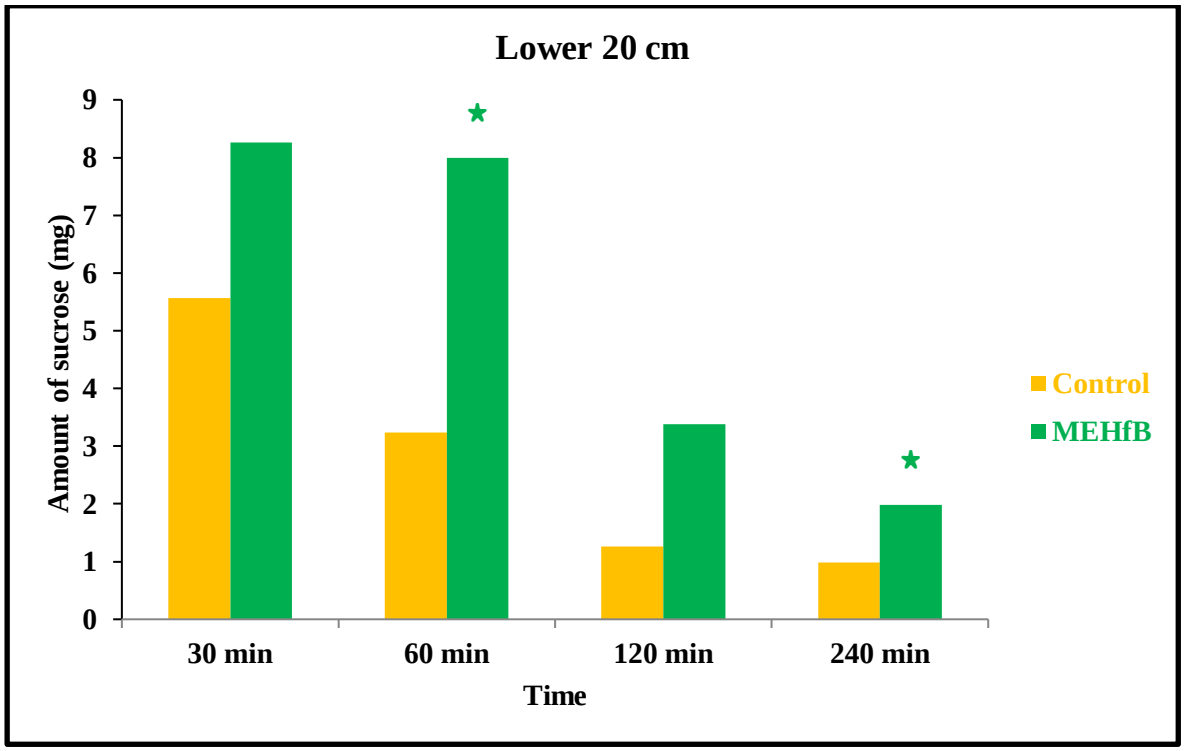

Figure 6.12: Graph of carbohydrate absorption test for MEHfB in lower 20 cm of rat small intestine.

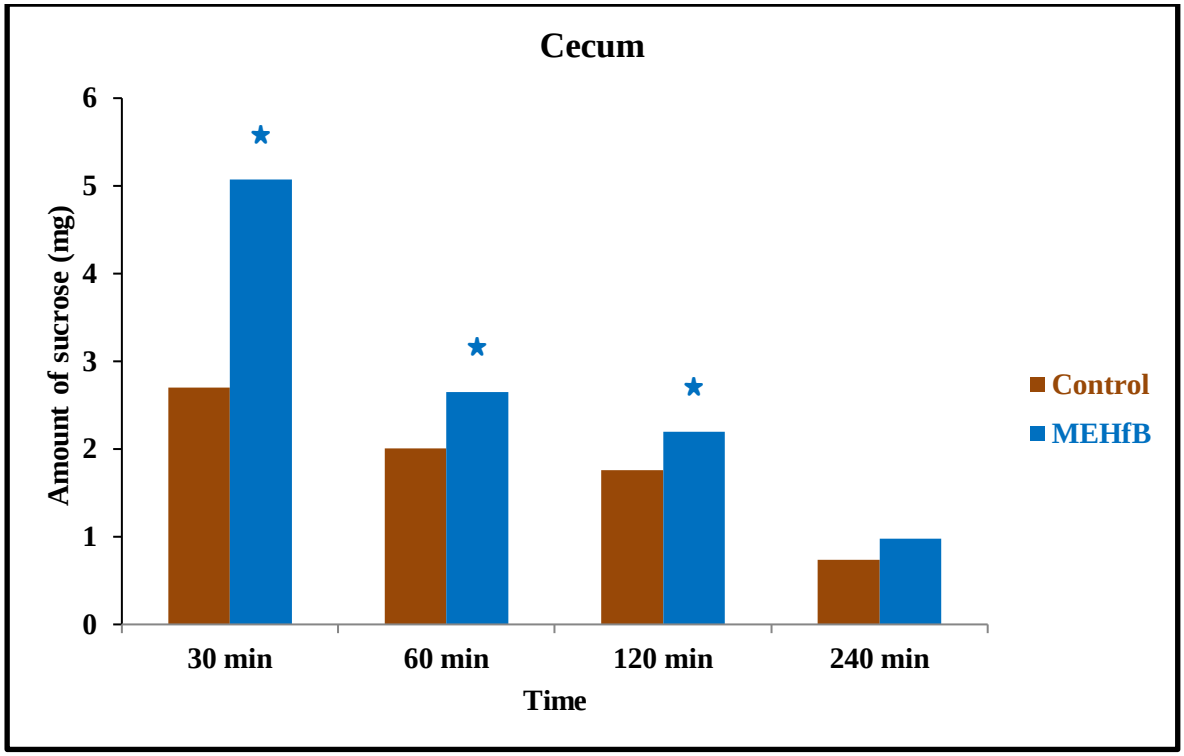

Figure 6.13: Graph of carbohydrate absorption test for MEHfB in cecum of rat.

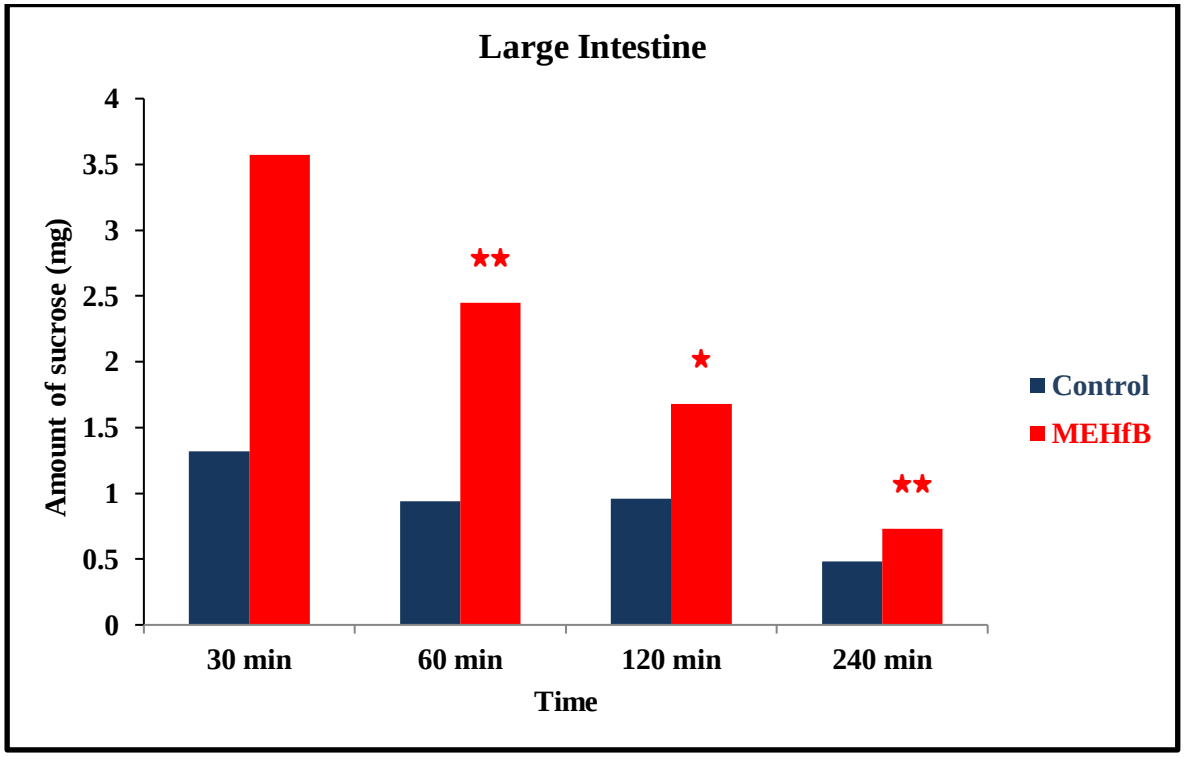

Figure 6.14: Graph of carbohydrate absorption test for MEHfB in large intestine of rat.

6.1.3 Effect of the *Heritiera fomes* on Disaccharidase Activity

At doses 500 mg/kg, experimental root and bark extracts were administered to rat. As a result, disaccharidase enzyme activity got decreased significantly ($p < 0.05/ 0.01/ 0.001$). From Table 6.5, significant levels of decrease in disaccharidase enzyme activity in rat model after one hour of root extract solution administration can be observed. Table 6.6, significant levels of decrease in disaccharidase enzyme activity in rat model after one hour of bark extract solution administration can be observed.

The standard drug, Acarbose, also exhibited a significant decrease in disaccharidase enzyme activity in the rat model after one hour of administration.

Table 6.5: Data of the disaccharidase activity test to determine the effect of MEHfR

Group	**Disaccharidase activity ± SEM**
Control	1.545 ± 0.026
MEHfR	1.010 ± 0.014 ***
Acarbose	1.065 ± 0.020 ***

Table 6.6: Data of the disaccharidase activity test to determine the effect of MEHfB

Group	**Disaccharidase activity ± SEM**
Control	1.545±0.026
MEHfB	1.060±0.021***
Acarbose	1.065 ± 0.020 ***

Here, MEHfR refers to methanolic extract of *Heritiera fomes* root and MEHfB refers to methanolic extract of *Heritiera fomes* bark. Data are presented as Mean ± SEM; n=4. Data values marked with (***) significantly different from the corresponding values of the CONTROL group at $p<0.001$

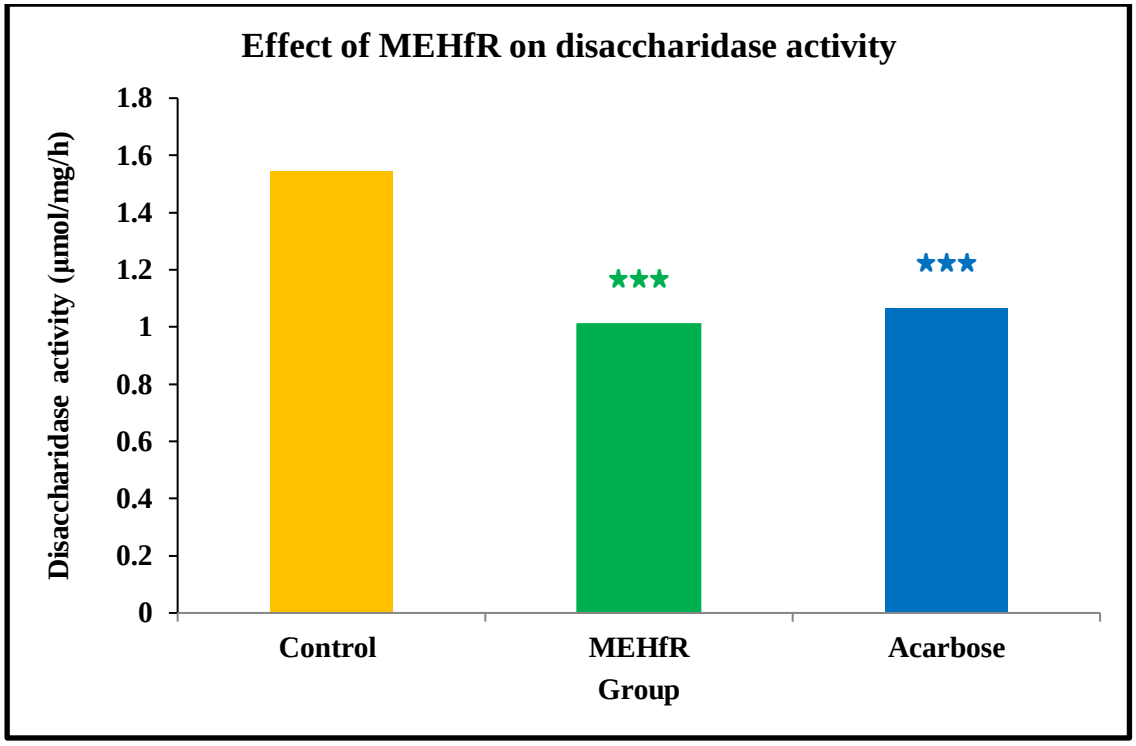

Figure 6.15: Graph of disaccharidase activity test for MEHfR in rat.

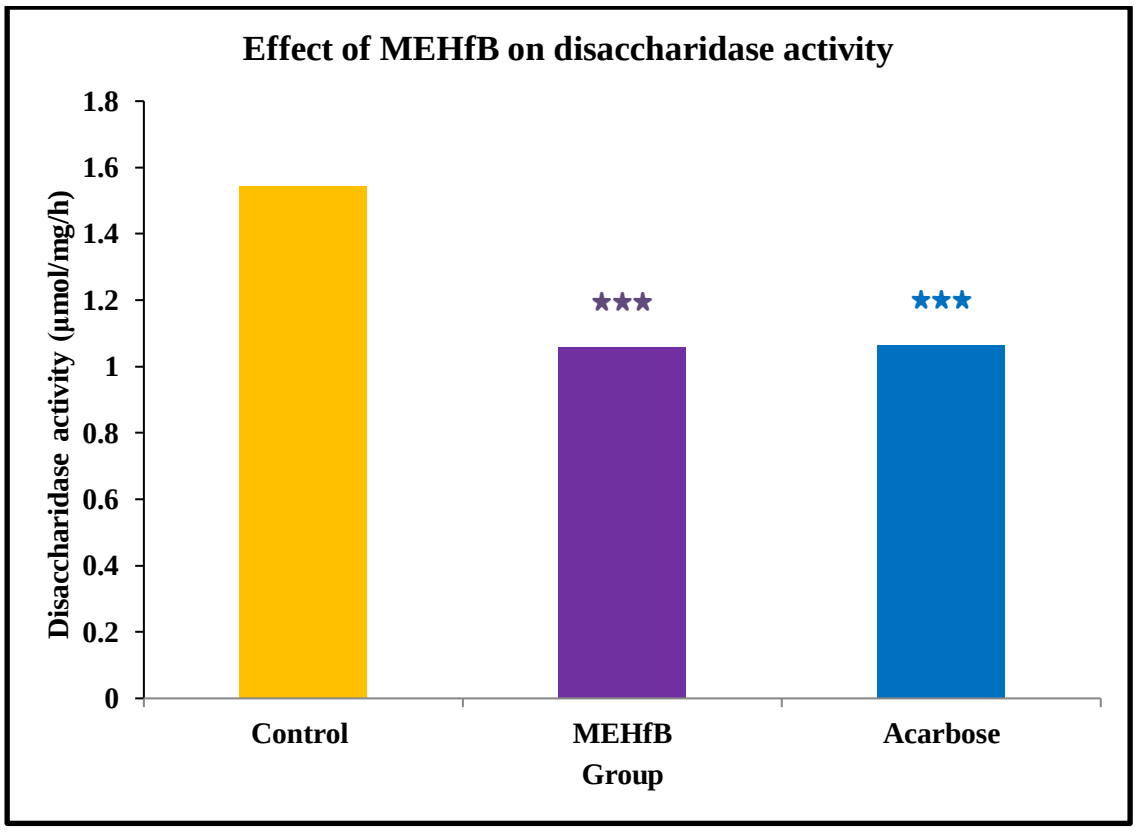

Figure 6.16: Graph of disaccharidase activity test for MEHfB in rat.

6.2 Discussion

This Studies were performed to evaluate the effect of methanolic extract of *Heritiera fomes* on three types of biological activity in mice and rat model.

1. GI motility
2. Carbohydrate absorption
3. Disaccharidase enzyme activity.

Our main target is to evaluate the anti-hyperglycemic activity by evaluating the pharmacological activity of methanolic extract of *Heritiera fomes* on those biological activity which can be inhibitory effect or can be stimulatory effect.

6.2.1 Discussion for the GI Motility Test

Gastrointestinal (GI) motility is defined by the movements of the digestive system, and the transit of the contents within it. When nerves or muscles in any portion of the digestive tract do not function with their normal strength and coordination, a person develops symptoms related to motility problems. The stomach is an organ located between the esophagus and small intestine. It's responsible for grinding food down and mixing it with stomach acids so that nutrients from food can be absorbed in the small intestine. Under normal conditions, the stomach empties its contents at a controlled rate. (health.ucsd.edu, accessed 2017)

There are several types of motility disorder. Such as,

- ✓ Gastroparesis or delayed gastric emptying
- ✓ Rapid gastric emptying (dumping syndrome)
- ✓ Idiopathic vomiting
- ✓ Functional dyspepsia
- ✓ Cyclic vomiting syndrome

For this test we use *swiss albino* mice model to observe the %GI motility by the help of barium sulphate milk. We got significant increase in %GI motility than the control category, where the control category was treated with water and the test category was treated with methanolic extract of *Heritiera fomes.* One of the most important reason

for diabetes is prosprandial hyperglycemia. After taking meal about 80% glucose absorbed in upper portion of small intestine beacuse of the availability of enzyme and vessels. So, If we inhibit the absorption we can be said that the drug have anti-hyperglycemic effect. Fast GI motility indicate the less absorption, from this study we got significant effect of *Heritiera fomes* to increase the GI motility. That means we can say that, the extract can be used for further test to evaluate the effect on hyperglycemia. Here, we use bisacodyl as the standard drug. It also give a significant effect on GI motility than control group. The plant extract give the almost 60% to 70% movement of food in the GI tract , bisacodyl give the result at 80% to 90%. Finnaly, We can come to a decision that the methanolic extract of *Heritiera fomes* increase GI motility and the extract can be used for further test to evaluate the effect on hyperglycemia .

6.2.2 Discussion for the Carbohydrate Absorption Test

In previous study, it has been found that *Heritiera fomes* helped in laxative effect which can help us to go to the next step to evaluate anti-hyperglycemic activty of *Heritiera fomes.*

Carbohydrates are nutrients that provide your body with energy. In our body, when we eat carbohydrates, it will break down to monosaccharides, such as sucrose converted into glucose. Glucose is absorbed into the bloodstream, and with the help of a hormone called insulin it travels into the cells of the body where it can be used for energy.

People with diabetes have problems with insulin that can cause blood sugar levels to rise. For people with type 1 diabetes, the pancreas loses the ability to make insulin. For people with type 2 diabetes, the body can't respond normally to the insulin that is made.

One of the objectives of the present study was to investigate whether the anti-hyperglycemic effect is related to the inhibition of carbohydrate absorption in stomach, small intestine, cecum and large intestine. In order to confirm this hypothesis, we examined sucrose content in six segments of the rat gastrointestinal tract after simultaneous administration of sucrose. Methanolic extract of *Heritiera fomes* significantly suppressed postprandial hyperglycemia after sucrose ingestion.

The extract increased the residual sucrose content throughout the gut at up to 4h after sucrose administration with the extract. This result suggest that the extract can delay the sucrose absorption and the reduction of hyperglycemia by the extract, at least partly, related to the retardation of carbohydrate absorption in the gut. This was also confirmed is gut perfusion experiment with glucose where the extract significantly reduced intestinal glucose absorption.

6.2.3 Discussion for the Disaccharidase Activity Test

Disaccharidases are enzymes the break down complex sugars (like lactose) into simple sugars (like glucose) so that the intestine can absorb the nutrients. Alpha glucosidase is a glucosidase enzyme, a type of disaccharidase enzyme located in the brush border of the small intestine that acts upon α(1→4) bonds. Alpha glucosidase breaks down starch and disaccharides to glucose. Maltase, a similar enzyme that cleaves maltose, is nearly functionally equivalent.

Absorption of carbohydrates requires the eventual breakdown of disaccharides to form single sugars by the enzymes in the brush border of the small intestine. Type 2 diabetes results from resistance to insulin effects coupled with a relative deficiency of insulin secretion. The most characteristic abnormality of insulin production is a reduction in the early-phase release of insulin from the pancreas.

Disaccharidase inhibitors, effectively compensate for defective early-phase insulin release by inhibiting the breakdown of disaccharides to monosaccharides in the intestinal epithelium. Consequently, there is delayed and decreased absorption of these sugars. Thus, there is a lower glycemic peak, permitting the diminished early-phase insulin secretion to cope more effectively with glucose disposal. The result is a decrease in postmeal glucose peaks in diabetic patients. (medscape.org,2017)

In the previous study we ensure the inhibition effect on the carbohydrate absorption of methanolic extract of *Heritiera fomes.* Now in the present study we explore the significant action on the carbohydrate absorption by inhibiting the disaccharidase enzyme activity, especially alpha glucosidase.

This result suggest that the methanolic extract of *Heritiera fomes* root have the significant inhibitory effect of disaccharidase enzyme which is better than the

standard drug acarbose (Table 6.5) and the methanolic extract of *Heritiera fomes* bark show almost same activity with acarbose.

By some recent studies it was already proved that Acarbose is a potent alpha-glycosidase inhibitor which decreases postprandial hyperglycemia when administered with a carbohydrate-containing meal.

6.2.4 Final decision

By analyzing the discussion that we already made from the results from GI motility test, Carbohydrate absorption test and Disaccharidase activity test we can come to some decisions, such as,

- The methanolic extract of *Heritiera fomes* have good laxative effect which can be opened a new era in anti-diarrhoeal research in future.
- The methanolic extract of *Heritiera fomes* root have significant inhibitory activity on carbohydrate absorption which suggest us the anti-hyperglycemic effect of the extract.
- The methanolic extract of *Heritiera fomes* have significant inhibitory activity on disaccharidase enzyme which also suggest the anti-hyperglycemic effect of both root and bark extract.

Chapter 7

Conclusion

The prevalence of diabetes is rising relentlessly around the world. Current estimates suggest that, globally, the number of persons with diabetes will rise from 151 million in the year 2008, to 221 million by the year 2010, and to 300 million by 2025 (Rahim et al 2007). This rise is predicted to occur in virtually every nation, with the greatest increases expected in developing countries. Nature has been a source of medicinal treatments for thousands of years, and plants-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries. The rapidly increasing prevalence of diabetes mellitus throughout the world will continue to challenge the existing therapies and encourage new approaches to counter DM.

The present study has evaluated potential antidiabetic activity of *Heritiera fomes plants,* can be a new era in the treatment of DM. Because, the anti-hyperglycemic effect has been proved in most two important section , disaccharidase inhibition and carbohydrate absorption. And finally by giving good laxative effect, the experiment carried out showed positive hypoglycemic effects of the plant without constipation as a side effect.

Hopefully this will provide as a lead to carry out further investigation to assess whether or not *Heritiera fomes* extracts may be used commercially.

Chapter 8

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