A study on effects of *Acacia Nilotica* on inhibition of disaccharidase activity in Long Evans Rats

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

Submitted by

Tanjin Sultana

2013-3-70-057

Submitted to

Dr. JMA Hannan

Professor

Department of Pharmacy

East West University

July 16, 2017

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Place of study: Pharmacology laboratory, East West University

The Research Paper is dedicated to My Parents

Declaration by the Candidate

I am Tanjin Sultana hereby declare that the dissertation entitled **"**A study on effects of *Acacia Nilotica* on inhibition of disaccharidase activity in Long Evans Rats" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out during the period 2017 of our research in the Department of Pharmacy, East West University, under the supervision and guidance of Dr. JMA Hannan, Professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

Tanjin Sultana
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Certificate by the Supervisor

This is to certify that the thesis entitled **"**A study on effects of *Acacia Nilotica* on inhibition of disaccharidase activity in Long Evans Rats'' submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Summaya Islam Tania.

During the period 2016-2017 of their research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Dr. JMA Hannan
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Certificate by the Chairperson

This is to certify that the thesis entitled **''**Determination Anti diabetic Efficacy of leaves of *Asteracantha longifolia''* submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by –

Tanjin Sultana	**2013-3-70-057**

During the period 2016-2017 of their research in the Department of Pharmacy, East West University.

_____

Dr. Chowdhury Faiz Hossain
Chairperson
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Abstract

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

Keywords: Anti-Diabetic, *Acacia Nilotica* Hypoglycemic, Glucose, Sucrose.

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Diabetes, often referred to by doctors as **diabetes mellitus**, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). (Information et al., 2017)

1.2 Diabetes Mellitus

Diabetes (diabetes mellitus) is classed as a metabolic disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood - it is the principal source of fuel for our bodies.

When our food is digested, the glucose makes its way into our bloodstream. Our cells use the glucose for energy and growth. However, glucose cannot enter our cells without insulin being present - insulin makes it possible for our cells to take in the glucose.

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

A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because the body does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of

glucose, the cells are not getting it for their essential energy and growth requirements. (Information et al., 2017)

1.2.1 Types of diabetes

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

Type 1 diabetes

If you have type 1 diabetes, your body does not make insulin. Your immune system attacks and destroys the cells in your pancreas that make insulin. Type 1 diabetes is usually diagnosed in children and young adults, although it can appear at any age. People with type 1 diabetes need to take insulin every day to stay alive.

Type2 diabetes

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

Gestational diabetes

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

Other types of diabetes

Less common types include monogenic diabetes, which is an inherited form of diabetes, and cystic fibrosis-related diabetes

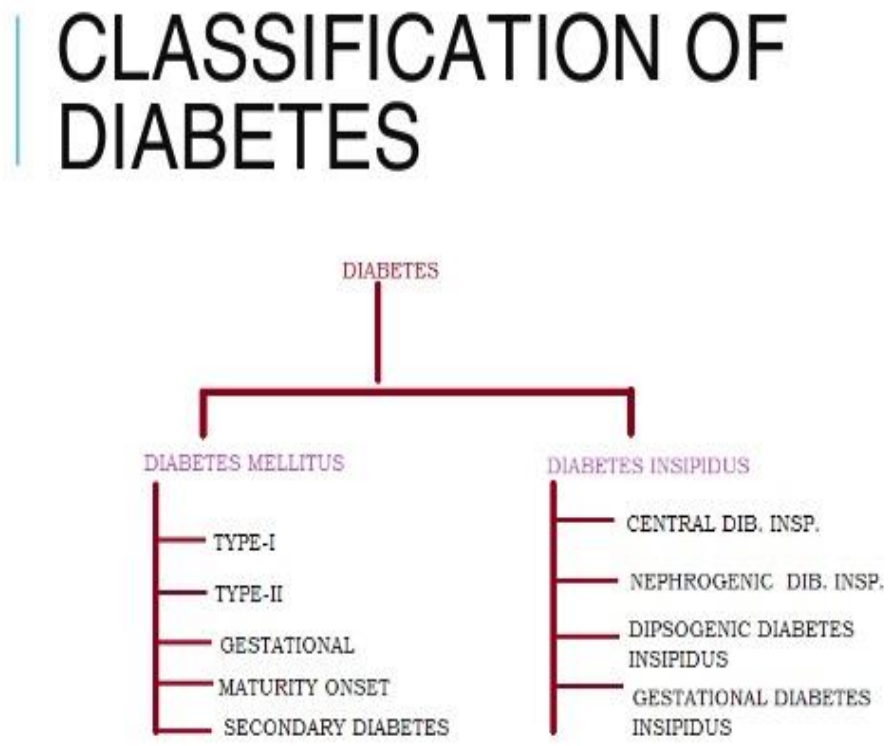

Figure 1: Classification of diabetes

1.2.2 Epidemiology

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 are 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 diabetes when you were pregnant. Learn more about risk factors for type 2 diabetes.

1.2.3 Health problems due to diabetes

Over time, high blood glucose leads to problems such as

- heart disease
- stroke
- kidney disease
- eye problems
- dental disease
- nerve damage

1.2.4 Difference between type 1 and type 2 diabetes

Table 1: Difference between type 1 and type 2 diabetes

Differences 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.

(Cold et al., 2017)

1.2.5 Facts on Diabetes

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

(Canadian Diabetes Association, 2017)

1.2.6 Symptoms of Diabetes

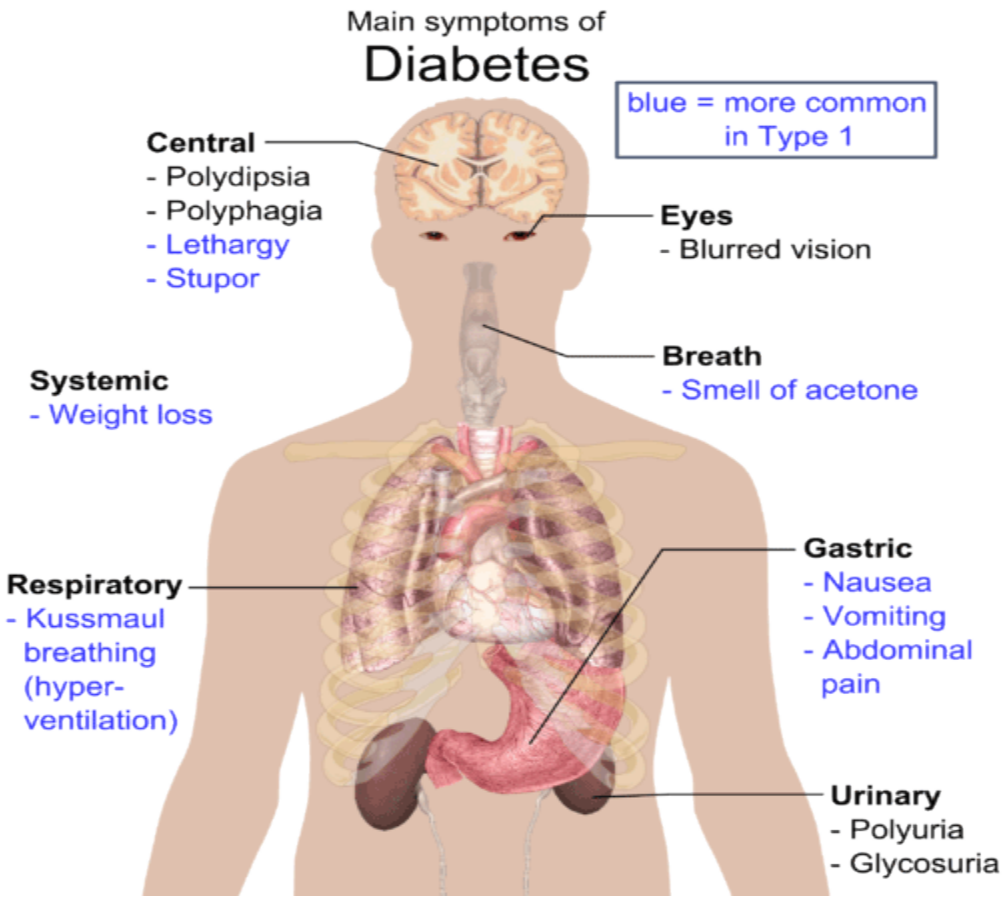

Figure 2: Symptoms of diabetes

1.2.7 Complications linked to badly controlled diabetes

- **Eye complications** - glaucoma, cataracts, diabetic retinopathy, and some others.
- **Foot complications** - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
- **Skin complications** - people with diabetes are more susceptible to skin infections and skin disorders
- **Heart problems** - such as ischemic heart disease, when the blood supply to the heart muscle is diminished

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

(Canadian Diabetes Association, 2017)

1.2.8 Diagnosis of Diabetes

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

- **The A1C test**
  - at least 6.5% means diabetes
  - between 5.7% and 5.99% means prediabetes
  - less than 5.7% means normal
- **The FPG (fasting plasma glucose) test**
  - at least 126 mg/dl means diabetes
  - between 100 mg/dl and 125.99 mg/dl means prediabetes
  - less than 100 mg/dl means normal

  An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG)
- **The OGTT (oral glucose tolerance test)**
  - at least 200 mg/dl means diabetes
  - between 140 and 199.9 mg/dl means prediabetes
  - less than 140 mg/dl means normal

  An abnormal reading following the OGTT means the patient has impaired glucose tolerance (IGT)

1.2.9 Insulin

Insulin is a small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains held together by disulfide bonds. The figure to the right shows a molecular model of bovine insulin, with the A chain colored blue and the larger B chain green.

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

1.2.9.1 Biosynthesis of Insulin

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

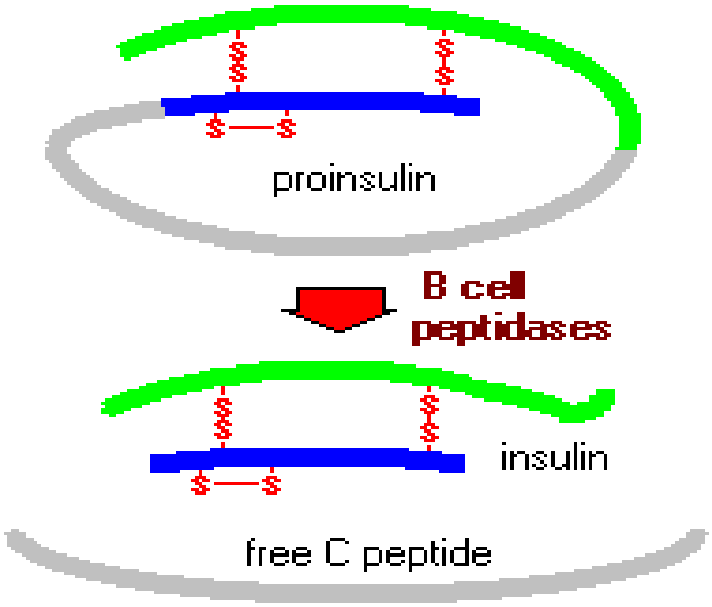

Figure 3: Biosynthesis of Insullin

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. (Vivo.colostate.edu, 2017)

1.2.9.2 Insulin secretion

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

1.2.9.3 Mechanism of Insulin secretion

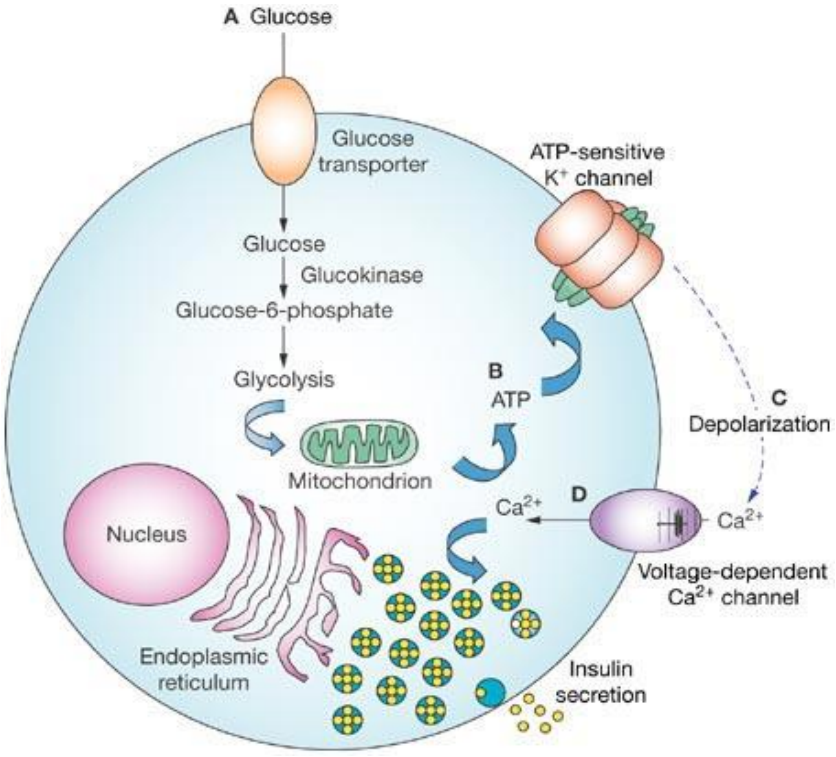

Figure 4: Mechanism of Insulin secretion

1.2.9.4 Insulin degradation

Insulin has a short half-life in the circulation following release, estimated at 4-6 minutes, allowing minute-to-minute regulation of metabolism. Circulating insulin is cleared by the liver as it passes through the portal circulation, which means that portal levels of insulin are higher than those in the systemic circulation. The kidney is largely responsible for insulin clearance in the systemic circulation, and delayed insulin clearance may cause problems with control in those with kidney disease. Some degradation occurs within the insulin granule, and insulin is degraded in other tissues after binding to the insulin receptor. In this receptor-mediated degradation, the insulin-insulin receptor complexes come together on the plasma membrane of the target cell, forming groups that are sequestered in so-called coated-pits. These invaginate to fuse with intracellular lysosomes, in which the insulin is enzymatically degraded. (Insulin synthesis, Valk and Collective, 2017)

1.2.9.5 Control of Insulin Secretion

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

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

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

The figure to the right depicts the effects on insulin secretion when enough glucose is infused to maintain blood levels two to three times the fasting level for an hour. Almost immediately after the infusion begins, plasma insulin levels increase dramatically. This initial increase is due to secretion of preformed insulin, which is soon significantly depleted. The secondary rise in insulin reflects the considerable amount of newly synthesized insulin that

is released immediately. Clearly, elevated glucose not only simulates insulin secretion, but also transcription of the insulin gene and translation of its mRNA. (Vivo.colostate.edu, 2017)

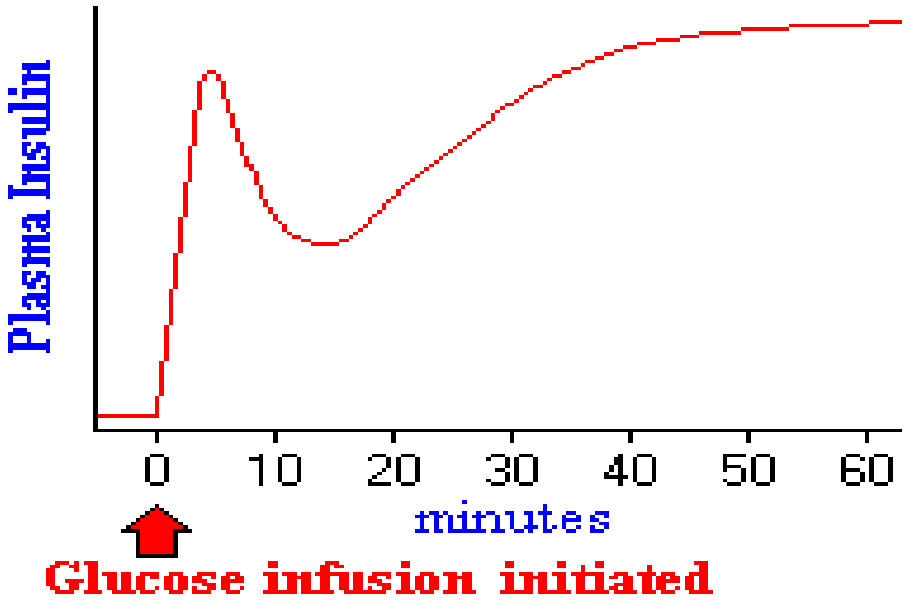

Figure 5: Control of Insulin Secretion

1.2.10 Drugs used as Antidiabetic agents

Table 2: Drugs used as Antidiabetic agents

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

Table 3: Oral Antidiabetic Drugs

Drug List – Oral Antidiabetics

Insulin secretagogues		Biguanides	Thiazolidinediones	α-glucosidase inhibitors
Sulfonylureas	Meglitinides			
Tolbutamide* Chloroprapamide* Glyburide** Glipizide** Glimepiride** (amaryl)	Repaglinide	Metformin	Rosiglitazone	Acarbose Miglitol
* 1st generation sulfonylureas, ** 2nd generation sulfonylureas				

(Drugs.com, 2017)

1.3 Medicinal Plants as Anti-diabetic

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

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

A large diversity of animal models has been developed to better understand the pathogenesis of diabetes mellitus and new drugs introduced in market to treat this disease. This review also studied the animal model used in testing of drug. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used

as herbal drugs. The active principal present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of insulin resistance. The ethanobotonical information reports about 800 plants that may possess antidiabetic potential and more than 1200 species of plants have been screened for activity on the basis of ethanopharmacology.

In India indigenous remedies have been used for treatment of diabetes since the time of charaka and sushruta. The World Health Organization has recommended the evaluation of traditional plant treatments for the diabetes. Diabetes mellitus can be induced by pharmacological, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. (Ayurhelp.com, 2017)

1.3.1Traditional herbal anti-diabetics

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

Several disadvantages associated with insulin and synthetic drugs and their failure to divert the course of diabetic complications have opened up tremendous horizons for searching possibilities in complementary and alternative medicine (CAM) for diabetes as well as many other chronic diseases. Plants, herbs and their derivatives owing to their wide spectrum of active principles representing numerous chemical compounds hold promising potentials for their consistent usages in the treatment of Diabetes. According to WHO, 21,000 plants around the globe have been reported for medicinal uses. India is posted to have an enormous medicinal flora of some 25,000 species, out of these 150 species are commercially exploited for medicinal extractions or drug formulation. There are about 800 plants species reported having the probability of possessing antidiabetic potentials in the ethnobotanical surveys. The antidiabetic effects of the plants are attributed to the wide

range of chemicals and secondary metabolites. Reports have essayed approximately 200 pure compounds from plant sources to show blood glucose lowering effect. These compounds range vividly in chemical nature like alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoid, triterpenoid, peptides and amino acids, lipids, phenolics, glycopeptides, and iridoids. (Ayurhelp.com, 2017)

1.3.2 Medicinal Plants with reported Antidiabetic Effect on experimental models

Table 4: Medicinal Plants used as Antidiabetics

Plant(Family)	Part of Plant Used	Material	Result
Annona Sqamosa (Annonaceae)	Fruit peel	Alcohol, ether, ethyl acetate	Significant increase body weight and diminished blood glucose level
Calamus erectus (Arecaceae)	fruit	Methanolic extract	Reduction of blood glucose level
Tamarandus indica Linn	Seeds	Aqueous extract	effective in type II diabetic rat model
Momordica Charantia (Cucurbitaceae)	Plant	Alcoholic extract	lower the blood sugar level
dactylifera linn (Arecaceae)	dried dates	Aqueous extract	reduction in blood glucose level
Zizyphus nummularia (Rhamnaceae)	Leaves	aqueous and 12% ethanolic extract	reduction in blood glucose level and body weight maintained
Swertia Chirata (Gentianaceae)	Whole plant	aqueous and 12% ethanolic extracts	Significant antidiabetic activity
Tamarandus indica Linn (Caesalpiniaceae)	Fruit pulp	ethanolic extracts	Antidiabetic effect
Parmelia Perlata. Ach (Permeliaceae)	Leaves	Aqueous extract	reduced the fasting blood glucose and HbA1C level
Psidium guvajava (Myrtaceae)	Leaves	Ethanolic extract	reduction in blood glucose level

(Ayurhelp.com, 2017)

1.4 Acacia nilotica

Babla or Babula is a small to medium-sized, almost evergreen tree with a short trunk. It has a spreading crown and feathery foliage. Leaves are bipinnate, flowers are golden-yellow in colour, fragrant, and are crowded in long-stalked globose heads. Fruits of this plant are stalked, constricted between the circular seeds that are densely and persistently grey downy. Flowering occurs generally during the rainy season, occasionally to December; fruiting usually from April to June. The botanical name of this plant is Acacia nilotica. Three varieties of Babla plant are recognized in India:

(1) Cupressiformis Stewart characterized by its broom-like ascending branches

(2) Vediana Cooke, a smaller variety with rough, fissured bark

(3) Indica, themost common variety found in natural and plantation forests.

Babla or Babula is also known as Indian gum Arabic tree in English, babul in Bangla, Urdu, Hindi and bamura in Gujrati. Though this plant is apparently native to Egypt, the Arabian Peninsula and India, Babla is cultivated and naturalized in many tropical and subtropical countries. It is a very common species in dry to moist inland habitats nearly throughout India from Punjab to West Bengal southwards, where it often forms pure stands or is dominant in mixed stands. It is usually confined to low elevation sites on flat or gently undulating terrain and ravines, though is occasionally found on sites up to 900 meter elevation. Its common associates include Acacia leucophloea, Prosopis cineraria, Azadirachta indica, Ziziphus jujube.

Babul or Babla is known for its medicinal usages. In Ayurveda the bark is considered astringent to the bowels, alexiphaxmic and anthelmintic; it is used to treat coughs, bronchitis, diarrhea, biliousness, leucoderma and urinary discharges, a decoction of the bark is used as a gargle to relieve sore throat and toothache. The leaves are considered useful for treating bronchitis, piles and eye diseases and to promote healing of bone fractures. In Unani medicine they are used as a liver and brain tonic, antipyretic, and for

treating leucoderma, gonorrhea, strangury and ophthalmia. The gum exuded from the cut bark (babul gum) is used as a substitute for true gum arabic as an astringent and styptic. It is used in Ayurveda practice to treat biliousness, leprosy, urinary, vaginal and uterine discharges, and in Unani medicine as an antipyretic, liver tonic and for treating sore throat, cough, piles, burns and colic. Among the Irulars of Tamil Nadu the powdered gum is mixed with egg-white and applied externally to relieve scalds and burns. A decoction of the pods is used in the treatment of urinogenital diseases. An infusion or the pulp of the tender leaves mixed with rice water is used as an astringent and remedy for diarrhea and dysentery. The twigs are used as toothbrushes in some locales. The tannin-rich bark is highly valued for tanning, particularly in northern India. A decoction of the bark is used as a substitute for soap, and the unripe pods are sometimes used to make ink.

Acacia nilotica Lam (Mimosaceae) indigenously known as ‘Babul’ or ‘Kikar’ is a proverbial, medium sized tree and is broadly scattered in tropical and subtropical countries. It has an inspiring range of medicinal uses with potential anti-oxidant activity. This plant contributes a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. A. nilotica is a medicinal plant acknowledged to be rich in phenolics, consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, (+) -catechin, (-) epi- gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate. Different parts of this plant such as the leaves, roots, seeds, bark, fruits, flowers, gum and immature pods act as anti-cancer, antimutagenic, spasmogenic, vasoconstrictor, anti-pyretic, anti-asthamatic, cytotoxic, anti-diabetic, anti-platelet agregatory, anti-plasmodial, molluscicidal, anti-fungal, inhibitory activity against Hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-I and antioxidant activities, antibacterial, anti-

Hypertensive and anti-spasmodic activities, and are also engaged for the treatment of different ailments in the indigenous system of medicine. This review spotlights on the detailed phytochemical composition, medicinal uses, along with pharmacological properties of different parts of this multipurpose plant.

Acacia nilotica (L.) Del. syn. Acacia arabica (Lam.) Willd. (Mimosaceae) is an imperative multipurpose plant (Kaur et al., 2005). A. nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black colored, grey-pinkish slash, fissured bark, exuding a reddish low quality gum. The plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2 to 1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly on peduncles 2 to 3 cm long located at the end of the branches. Pods are strongly constricted, white-grey, hairy and thick (baravker et al., 2008). A. nilotica is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. A.nilotica occurs naturally and is imperative in traditional rural and agro-pastoral systems (Shittu, 2010). A. nilotica is recognized by the following names: Acacia, Acacia Arabica, Babhul - Hindi and Napalese, Babla - Bengali, Babool - Unani, Babool Baum - German, Babhoola - Sanskrit, Babul, Babul Tree, Huanlong Kyain - Burmese, Kikar, Mughilan - Arabian Indogom - Japenese and Ummughiion – Persian (Steve, 2004). A. nilotica is an imperative multipurpose plant that has been used broadly for the treatment of various diseases (Singh et al., 2009b). Natural medicinal plants promote self-healing, good health and durability in ayurvedic medicine practices and have acknowledged that A. nilotica can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions). It also serves as a source of polyphenols (Singh et al., 2009a). The role of these polyphenols to the plant itself is not well implicit, but for the human kind they can be of prime strategies (Singh et al., 2009a). The phytochemicals contribute chemically to a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Banso, 2009). This plant contain a profile of a variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives (Singh et al., 2009a). It has been reported that different parts of the plant are

prosperous in tannins (ellagic acid, gallic acid and tannic acid), stearic acid, vitamin-C (ascorbic acid), carotene, crude protein, crude fiber, arabin, calcium, magnesium and selenium (Meena et al., 2006). A number of medicinal properties have been ascribed to various parts of this highly esteemed plant. Traditionally the bark, leaves, pods and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, tuberculosis and small pox, leprosy, bleeding piles, leucoderma and menstrual problems. They have spasmogenic, vasoconstrictor, anti/-hypertensive, -mutagenic, -carcinogenic, -spasmodic, -inflammatory, -oxidant and -platelet aggregatory properties (Singh et al., 2009b). A. nilotica has anti-plasmodial, molluscicidal, anti-fungal, anti-microbial activity, inhibitory activity against HCV and HIV-I (Sultana et al., 2007). The bark of the plant is used as astringent, acrid, cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic and nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, skin diseases and seminal weakness. Gum is used as astringent, emollient, liver tonic, antipyretic and antiasthmatic (baravkar et al., 2008). The bark is used extensively for colds, bronchitis, biliousness, diarrhoea, dysentery, bleeding piles and leucoderma (Del, 2009). It is used by traditional healers of different regions of Chattisgarh in treatment of various cancer types of mouth, bone and skin. In West Africa, the bark and gum are used against cancers and/or tumors (of ear, eye, or testicles) and indurations of liver and spleen, the root for tuberculosis, the wood for smallpox and the leaves for ulcers (Kalaivani and Methew, 2010a). Pods and tender leaves are given to treat diarrhoea and are also considered very useful in folk medicine to treat diabetes mellitus (Gilani et al., 1999). The tender twings are used as toothbrushes (Meena et al., 2006). So far no comprehensive review has been compiled encircling the efficacy of this plant in all proportions from the literature. Its stretchy utility as a medicine forced us to bridge the information gap in this area and to write a comprehensive review on the medicinal, phytochemical and pharmacological traits of this plant of high economic value. (Anon, 2017)

1.4.1 General Pharmacology

The saline extract of the pollen grains stimulated the ileum of guinea pig which was blocked by mepyramine and atropine; the pet. Ether extract stimulated the rat's uterus and the heart of pila which was blocked by 2- bromo LSD. The effect of acid treated acetone extract was blocked by mepyramine. A Quaternary base picrate, (mp 242-44^{0}C), isolated from 11 species including the stem bark of the plant was reported to be pharmacologically identical to choline. The 50 % ethanolic extract of the stem bark in a preliminary biological screening exhibited antiprotozoal activity against *Entamoebahistolytica*, CVS effect in dog/cat, antispasmodic activity in guinea pig ileum and CNS depressant activity as evidence by amphetamine hyperactivity test in mice. The extract was devoid of antibacterial, antifungal, antiviral, hypoglycemic and anticancer activities. The LD_{50} was found to be 500 mg/kg i.p. in mice. (Anon, 2017)

1.4.2 Pharmacological and Biological Studies

Wadood *et al*., demonstrated that *Acacia arabica seeds* contained a substance(s) which depressed the blood glucose level in normoglycemic but not in alloxan-diabetic rabbits, suggesting that the mechanism of action involved release of insulin from pancreatic beta-cells. The bark in the form of decoction (20 mg/kg) as well as the standard drug talbutamide produced a significant reduction in blood glucose levels in mild alloxonised diabetic rabbits fasted for 18 hr [38]. The *A. nilotica* ssp. Indica fed for one week were found to exhibit hypoglycemic effect (blood sugar lowered by 25.05%,) in normal rats, but did not show any significant hypoglycemic effect in alloxanised diabetic rats (blood sugar lowered by 2.14%). The hypoglycemic effect of the legumes was due to its direct or indirect stimulation of β–cells of islets of langerhans to secrete more insulin.

Figure 6: *Acacia Nilotica* (Babla)

1.4.3 Phytochemistry

Plant compounds have interest as a source of safer or more valuable substitutes than synthetically created antimicrobial agents. Phytochemical progress has been aided extremely by the development of rapid and accurate methods of screening plants for particular chemicals. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom. The phytochemicals are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Banso, 2009). Phytochemistry confirmed that all the tested extracts contain physterols, fixed oils, fats, phenolic compounds, flavanoids and saponins (Kalaivani et al., 2010b). The phytochemicals alkaloids and glycosides detected in the crude extracts of A. nilotica roots are indicated (Jigam et al., 2010) below. Phytochemical screening of the stem bark of A. nilotica exposed that the plant contain terpenoids, alkaloids, saponins and glycosides. Negative results were recorded for steroids and flavonoids which authenticate the absence of these phytochemicals (Banso, 2009). This plant recommends a variety of phytochemical such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-

diglucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6, 8-bis-Cglucopyranoside, m-catechol and their derivatives. A. nilotica contains gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, gallolyated flavan-3, 4-diol, robidandiol (7, 3, 4, 5-tetrahydroxyflavan-3-4-diol), androstene steroid, D-pinitol carbohydrate and catechin-5-galloyl ester (Singh et al., 2009a). The bark is prosperous in phenolics viz. condensed tannin and phlobatannin, gallic acid, protocatechuic acid pyrocatechol, (+)-catechin, (-) epigallocatechin-7-gallate, and (-) epigallocatechin-5, 7- digallate (Singh et al., 2009a). The bark is also reported to contain (-) epicatechin, (+) dicatechin, quercetin, gallic acid, (+) leucocyanidingallate, sucrose and (+) catechin- 5-gallate (Mitra and Sundaram, 2007). A.nilotica is a medicinal plant from which the polyphenolic compounds kaempferol has been reported for the first time]. Another compound umbelliferone has been reported from A. nilotica (Singh et al., 2010b).

Anti-diabetic activities Studies have confirmed anti-diabetic activities. However, pods and tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus (Gilani et al., 1999).

1.4.4 MEDICINAL USES AND PHARMACOLOGICAL EFFECTS:

A. nilotica also has numerous medicinal uses. The medicinal traits and pharmacological activities endorsed to various parts of A. nilotica are detailed as follows.

1.4.4.1 Anti-hypertensive and anti-spasmodic activities

A decrease in arterial blood pressure is reported by use of methanolic extract of A. nilotica pods and provides evidence of anti-hypertensive activities independent of muscarinic receptor stimulation. In the in vitro studies, A. nilotica has inhibitory effect on force and rate of spontaneous contractions in guinea-pig paired atria and rabbit jejunum. A. nilotica also inhibits K + induced contractions in rabbit jejunum advocating the antispasmodic action of A. nilotica which is mediated through calcium channel blockade and this may also be

responsible for the blood pressure lowering effect of A. nilotica, observed in the in vivo studies (Gilani et al., 1999). An aqueous extract of the seed of A. nilotica is also investigated on the isolated guinea-pig ileum which exposed the sustained dose-related contractile activity. A dose-related significant elevation of blood pressure is produced by intravenous administration of the extract (Amos et al., 1999). Antibacterial and antifungal activities The assays of the stem bark extracts confirms the antimicrobial activity against Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Shigellasonnei using the agar diffusion method. A. nilotica could be a potential source of antimicrobial agents (Banso, 2009). A. nilotica demonstrates highest activity against three bacterial (E. coli, S. aureus and Salmonella typhi) and two fungal strain (Candida albicans and Aspergillusniger) (Kalaivani and Methew, 2010a). Antiplasmodial activities. The ethyl acetate extract holds the highest activity on Plasmodium falciparum. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins (El-tahir et al., 1999). Crude methanolic root extracts of A. nilotica reveals significant activity against chloroquine sensitive strain of Plasmodium berghei in mice (Jigam, 2010). Antioxidant activity Water extract/fractions of A. nilotica (L.) in lipid peroxidation assay possess the peroxyl radical scavenging capacity and results prove the anti-oxidant activity of plant. The bark powder of the plant extracts with different solvents found the scavenging activity using maceration extraction (Del, 2009). Another study reveals that A. nilotica is easily accessible source of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc (Amos et al., 1999). Furthermore, the high scavenging property of A. nilotica may be due to hydroxyl groups existing in the phenolic compounds that can scavenge the free radicals (Kalaivani and Mathew, 2010).

Acetylcholinesterase inhibitory activities Acetylcholinesterase is a basic aim in the treatment of Alzheimer's disease. It has been found that A. nilotica has effect on central nervous system activities due to potent Acetylcholinesterase inhibitory activities. More investigations are required in the treatment of Alzheimier's (Crowch and Okello, 2009). Anti-diabetic activities Studies have confirmed anti-diabetic activities. However, pods and

tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus (Gilani et al., 1999). Chemopreventive, cytotoxic and anti-mutagenic activities It has been reported, that the antimutagenic and cytotoxic activities exhibited by acetone extract may be due to the presence of gallic acid and other polyphenols (Kaur et al., 2005). It is reported that the leaf extract of A. nilotica had significant chemopreventive and anti-mutagenic activity than the other parts (Kalaivani and Mathew, 2010a). The chemopreventive activity of A. nilotica gum, flower and leaf aqueous extracts, on 7,12– dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male swiss albino mice has been found. The chemopreventive and anti-mutagenic activity of the leaf extract of A. nilotica was the most significant, followed by the flower extract and then by gum (Meena et al., 2006). OTHER MULTIPLICITIES The extract of A.nilotica is found to stimulate the synthesis and release of prolactin in the female rate and may be give a better result for lactating women (Lompo et al., 2004). A. nilotica are used for tanning, dyeing of leather, for gastrointestinal disorders, syphilitic ulcers and toothache (Amos et al., 1999). A. nilotica pods have reported inhibited HIV-1 induced cythopathogenicity (Asres et al., 2005). Fresh roots extract used as narcotic, known as Desisharab (local bear), gum is used as aphrodisiac with water; branches are used for cleaning teeth (Badshah and Hussain, 2011). Methanolic bark extract of bark has significant inhibitory effects of Sudanese medicinal plant extracts on HCV protease (Hussein et al., 1999b). In the end, methanol extracts of bark and pods have considerable inhibitory effects against HIV-1 PR (protease) (Hussein et al., 2000a). Ali et al. 1495 FUTURE PROSPECTS based on the different studies on different parts of A.nilotica, there is a grim need to isolate and identify new compounds from different parts of the tree, which have possible antimutagenic and cytotoxic activities. Therefore, the spreadability of naturally occurring polyphenolic compounds having ability to provide protection against certain types of mutagens and carcinogens is of great importance. The A. nilotica extract was also studied for its possible interaction with serotonin (5-HT) receptors which is associated with hypertension. Furthermore, it contains additional serotonin blocking compounds, which may be further studied for detailed interaction with serotonin receptor subtypes (Gilani et al., 1999). The high scavenging property of A. nilotica exhibits high scavenging activity due

to presence of phenolic compounds. However, further research is required to identify individual components forming antioxidative system and develop their application for pharmaceutical and food industries (Kalaivani and Mathew, 2010a). Umbelliferone, a potent antioxidant isolated from A. nilotica plant and food derived antioxidants are implicated in the prevention of cancer and aging by destroying oxidative species that initiate carcinogenesis through oxidative damage of deoxyribonucleic acid (DNA) The supplementation of functional food with antioxidants, which inhibit the formation of free radicals, can lead to prevention of some diseases As most of the antimu- tagenic compounds act via scavenging of free radicals, There is intense need to investigate the antioxidant activity of the functional components present in the extract from A. nilotica (Singh et al., 2009b). Literature is however scarce in respect of the efficacy of gallotannins as antiplasmodial agents so more investigation is required (Jigam et al., 2010). Having potential uses of this plant, it is highly recommended to cultivate widely to get maximum production for welfare of mankind. (Ijpsr.com, 2017)

Table 5: Uses of different parts of *Acacia Nilotica*

Part used	**Uses**
Leaf	Chemo preventive, anitmutagenic, anti-bacterial, anticancer, astringent, anti-microbial activity Tender leaves are used to treat diarrhea, Aphrodisiac, Dressing of ulcers, anti-inflammatory and Alzheimer's diseases.
Gum	Astringent, emollient, liver tonic, antipyretic and antiasthmatic.
Stem bark	Anti-bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acridcooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small Pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma.
Seeds	Spasmogenic activity and antiplasmodial activity.
Pods	Anti-hypertensive and antispasmodic, anti-diarrhoerial, astringent,anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cythopathogenicity, antiplatelet aggregator activity and anti-oxidant.

1.4.4.2 Antibacterial and antifungal activities

The assays of the stem bark extracts confirms the antimicrobial activity against Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Shigella sonnei using the agar diffusion method. A. nilotica could be a potential source of antimicrobial agents (Banso, 2009). A. nilotica demonstrates highest activity against three bacterial (E. coli, S. aureus and Salmonella typhi) and two fungal strain (Candida albicans and Aspergillus niger) (Kalaivani and Methew, 2010).

1.4.4.3 Anti-hypertensive and anti-spasmodic activities

A decrease in arterial blood pressure is reported by use of methanolic extract of A. nilotica pods and provides evidence of anti-hypertensive activities independent of muscarinic receptor stimulation. In the in vitro studies, A. nilotica has inhibitory effect on force and rate of spontaneous contractions in guinea-pig paired atria and rabbit jejunum. A. nilotica also inhibits K+ induced contractions in rabbit jejunum advocating the antispasmodic action of A. nilotica which is mediated through calcium channel blockade and this may also be responsible for the blood pressure lowering effect of A. nilotica, observed in the in vivo studies (Gilani et al., 1999).

An aqueous extract of the seed of A. nilotica is also investigated on the isolated guinea-pig ileum which exposed the sustained dose-related contractile activity. A dose-related significant elevation of blood pressure is produced by intravenous administration of the extract (Amos et al., 1999).

1.4.4.4 Antiplasmodial activities

The ethyl acetate extract holds the highest activity on Plasmodium falciparum. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins (El-tahir et al., 1999). Crude methanolic root extracts of A. nilotica reveals significant activity against chloroquine sensitive strain of Plasmodium berghei in mice (Jigam, 2010).

1.4.4.5 Antioxidant activity

Water extract/fractions of A. nilotica (L.) in lipid peroxidation assay possess the peroxyl radical scavenging capacity and results prove the anti-oxidant activity of plant. The bark powder of the plant extracts with different solvents found the scavenging activity using maceration extraction (Del, 2009). Another study reveals that A. nilotica is easily accessible source of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc (Amos et al., 1999). Furthermore, the high scavenging property of A. nilotica may be due to hydroxyl groups existing in the phenolic compounds that can scavenge the free radicals (Kalaivani and Mathew, 2010).

1.4.4.6 Anti-diabetic activities

Studies have confirmed anti-diabetic activities. However, pods and tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus (Gilani et al., 1999). Chemopreventive, cytotoxic and anti-mutagenic activities It has been reported, that the antimutagenic and cytotoxic activities exhibited by acetone extract may be due to the presence of gallic acid and other polyphenols (Kaur et al., 2005). It is reported that the leaf extract of A. nilotica had significant chemopreventive and anti-mutagenic activity than the other parts (Kalaivani and Mathew, 2010a). The chemopreventive activity of A. nilotica gum, flower and leaf aqueous extracts, on 7,12– dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male swiss albino mice has been found. The chemopreventive and anti-mutagenic activity of the leaf extract of A. nilotica was the most significant, followed by the flower extract and then by gum (Meena et al., 2006).

CHAPTER 2: MATERIALS & METHOD

2.1 Plant Material

Plant sample of *Acacia Nilotica* were used for the experiment. They were processed in the laboratory.

2.1.1 Collection of plant

The Plant sample *Acacia Nilotica* was collected and washed with water several times.

2.1.2 Drying and grinding

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

2.1.3 Extraction (Ethanol extraction)

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

2.1.4 Extraction Procedure

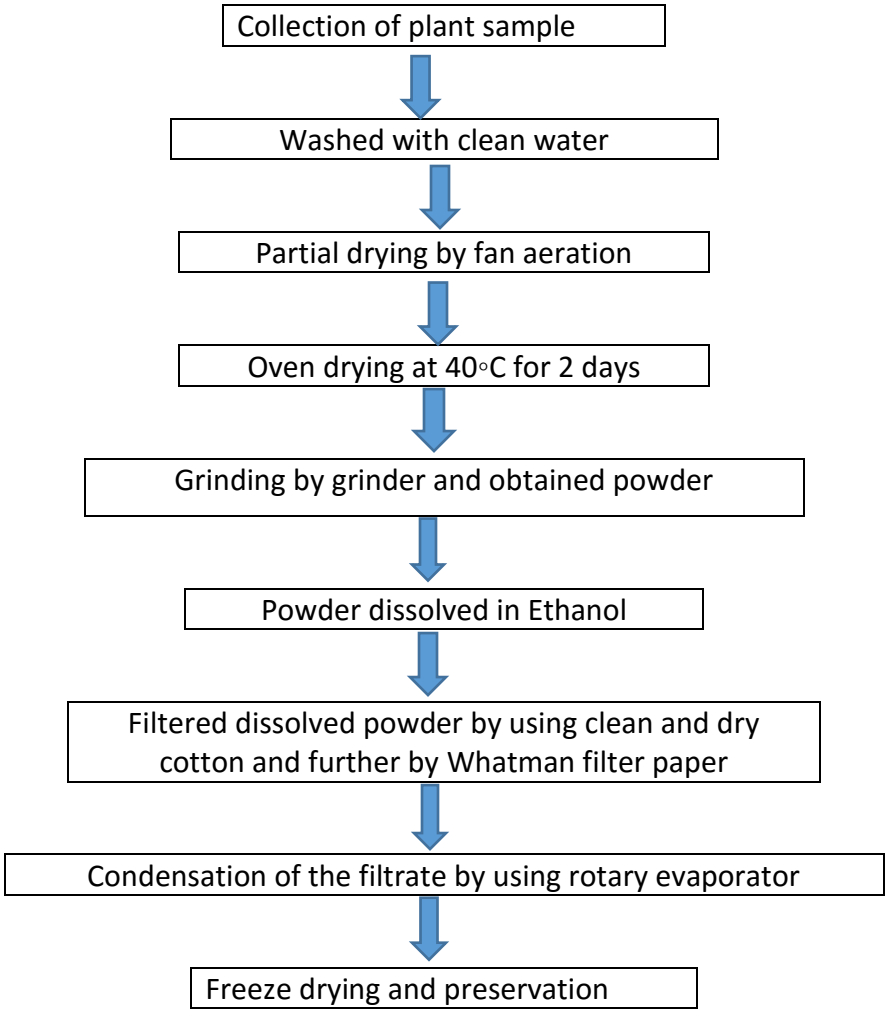

Figure 7: General Plant Extraction Procedure

2.2 Experimental animals

Long Evans rats (male and female), weighing 80-200g of either sex are bred in ICDDR, B and grown in the animal house of the Department of Pharmacy, East West University. All the animals acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2°C,

and 12 hours light dark cycle). The animals were fed with standard diet from ICDDR, B and had free access to filtered water (M.K. Sharif et al, 2011)

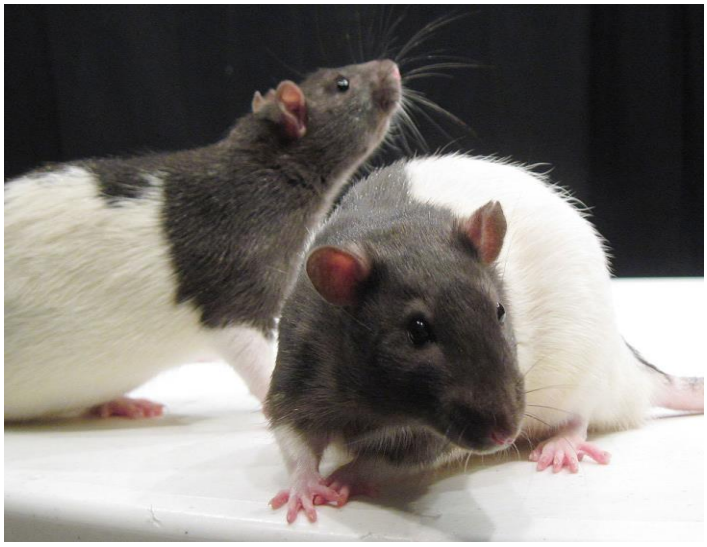

Figure 8: Long Evans Rat

2.2.2 Biomedical research

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

Figure 9: Rat used in research

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

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

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

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

2.3.1 Chemicals and reagents

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

Drug: 100mg/Kg body weight of rat

Kits:

Glucose kit was used for the determination of Glucose.

2.3.2 Procedure

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

2.3.3 Steps of the experiment

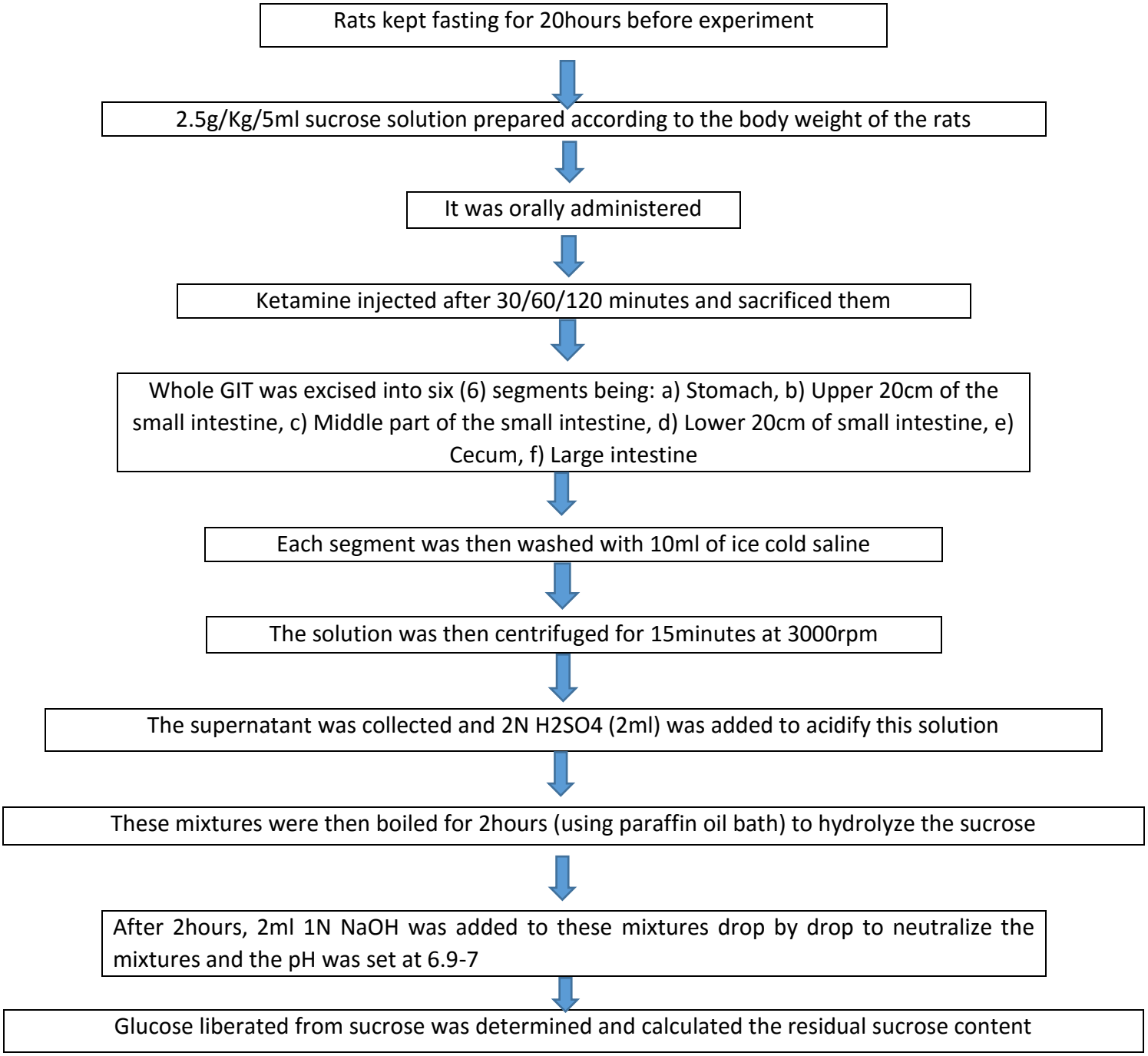

Figure 10: Steps of the experiment

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

2.4.1 Assessment of conditions

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

2.4.2 Mucosa/Tissue Collection

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

2.4.3 Enzyme activities

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

2.4.4 Steps of the experiment:

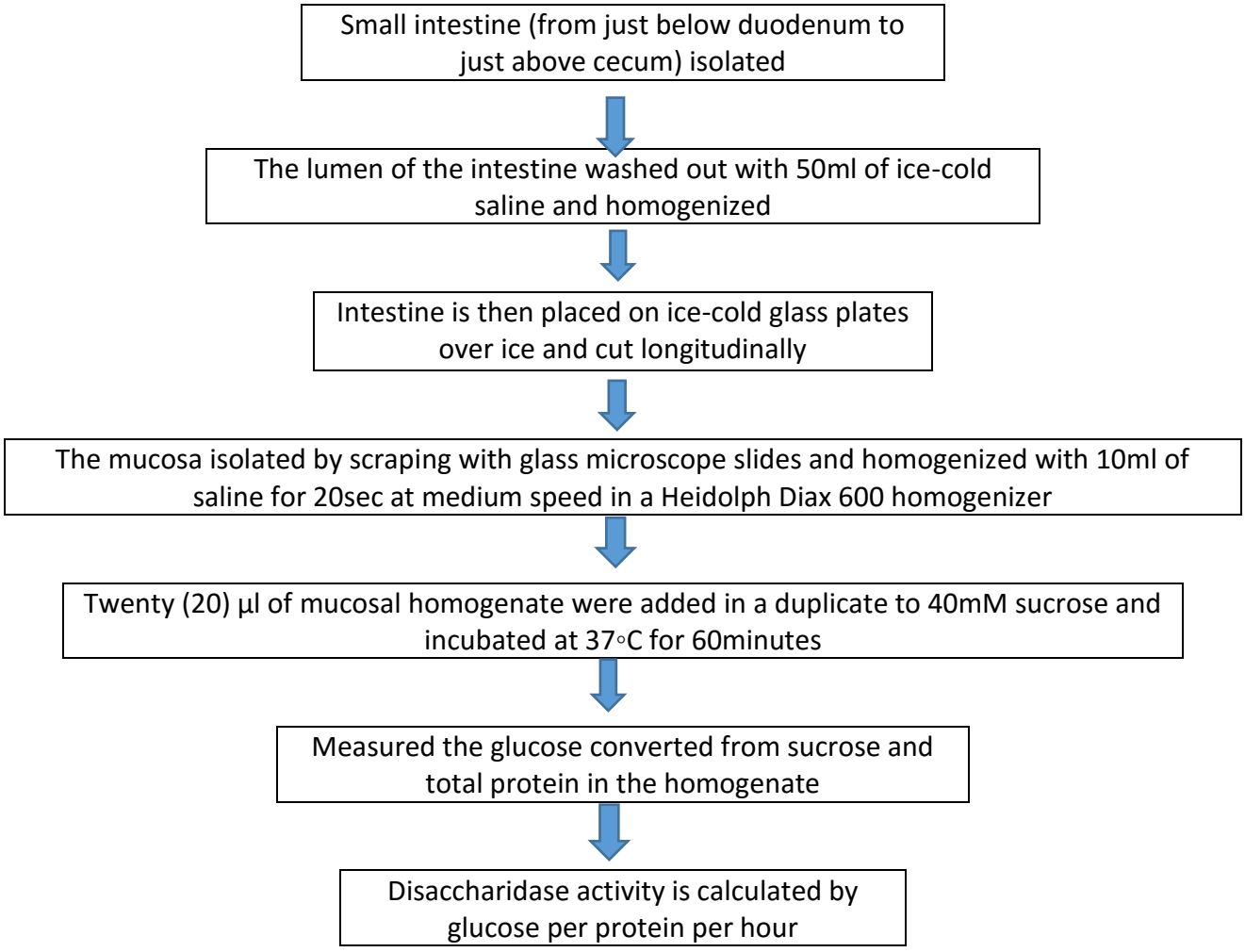

Figure 11: Steps of the experiment

CHAPTER 3: RESULT

3.1 Effect of *Acacia nilotica* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with ***A. nilotica*** (100mg/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1h. This amount of residual sucrose remained significant in caecum and large intestine till 4h ($p<0.05$; Table 1 - Table 6, Figure 1).

Table 6: Sucrose content in Stomach

Sucrose content in Stomach								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	52.3	8.5	31.8	5.9	7.2	1.4	1.1	0.3
Acacia nilotica	63.1	6.6	44.3	3.9	64.2	2.1	1.7	0.3

Table 7: Sucrose content in Upper 20cm of intestine

Sucrose content in Upper 20cm of intestine								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	13.3	2.3	10.2	1.8	4.9	1.1	1.7	0.4
Acacia nilotica	17.8	2.9	14.8	2.5	11.1	1.6	2.9	0.4

Table 8: Sucrose content in middle 20cm of intestine

Sucrose content in middle 20cm of intestine								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	20.0	4.3	16.0	4.2	7.5	1.8	1.3	0.3
Acacia nilotica	25.1	5.1	20.9	3.6	12.2	2.1	2.4	0.8

Table 9: Sucrose content in Lower 20cm of intestine

Sucrose content in Lower 20cm of intestine								
Groups	**30 min**		**60 min**		**120 min**		**240 min**	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	2.0	0.5	4.5	1.1	1.7	0.4	1.0	0.3
Acacia nilotica	2.9	0.5	5.3	0.3	2.4	0.4	1.7	0.2

Table 10: Sucrose content in Caecum

Sucrose content in Caecum								
Groups	**30 min**		**60 min**		**120 min**		**240 min**	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	1.6	0.5	1.7	0.3	1.6	0.4	1.8	0.5
Acacia nilotica	2.3	0.2	2.0	0.4	1.9	0.2	2.0	0.3

Table 11: Sucrose content in Large intestine

Sucrose content in Large intestine								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	1.0	0.3	0.9	0.3	1.2	0.2	1.1	0.2
Acacia nilotica	1.2	0.2	1.4	0.2	2.1	0.2	1.6	0.3

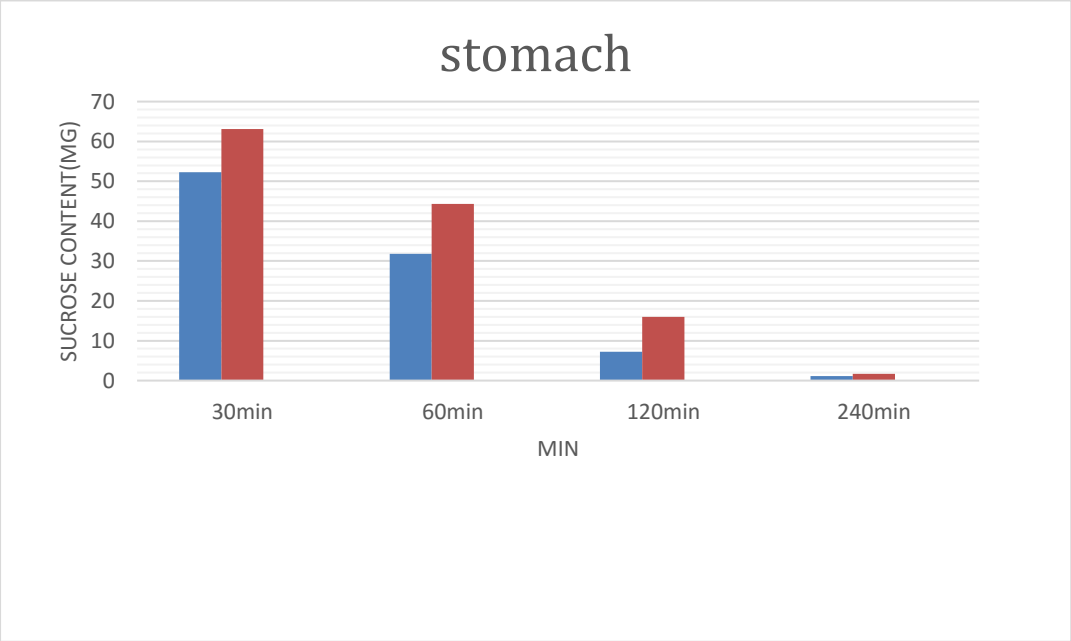

Figure 12: Sucrose content in Stomach

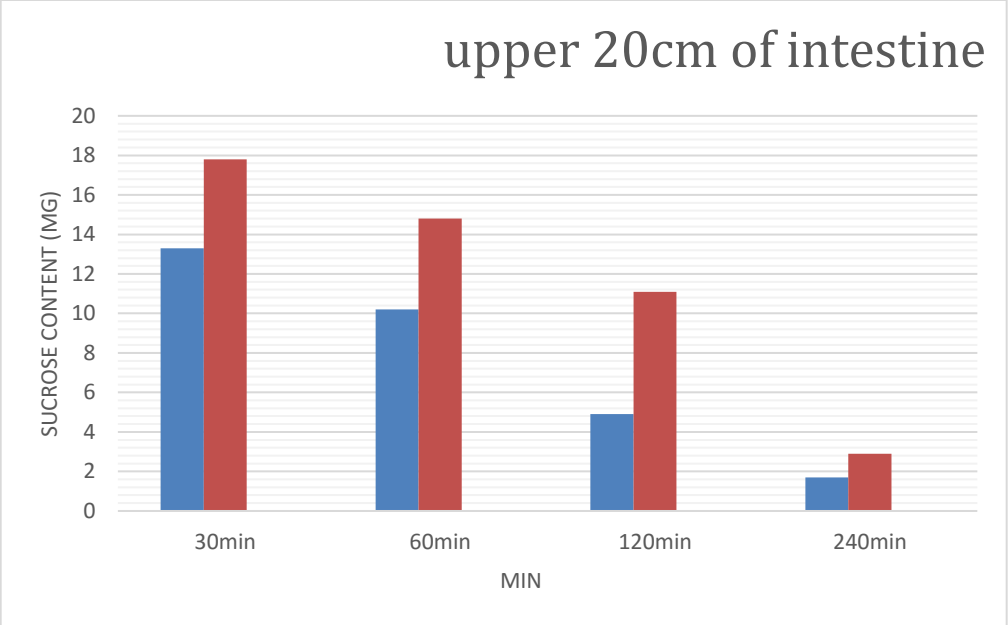

Figure 13: Sucrose content in Upper 20cm of intestine

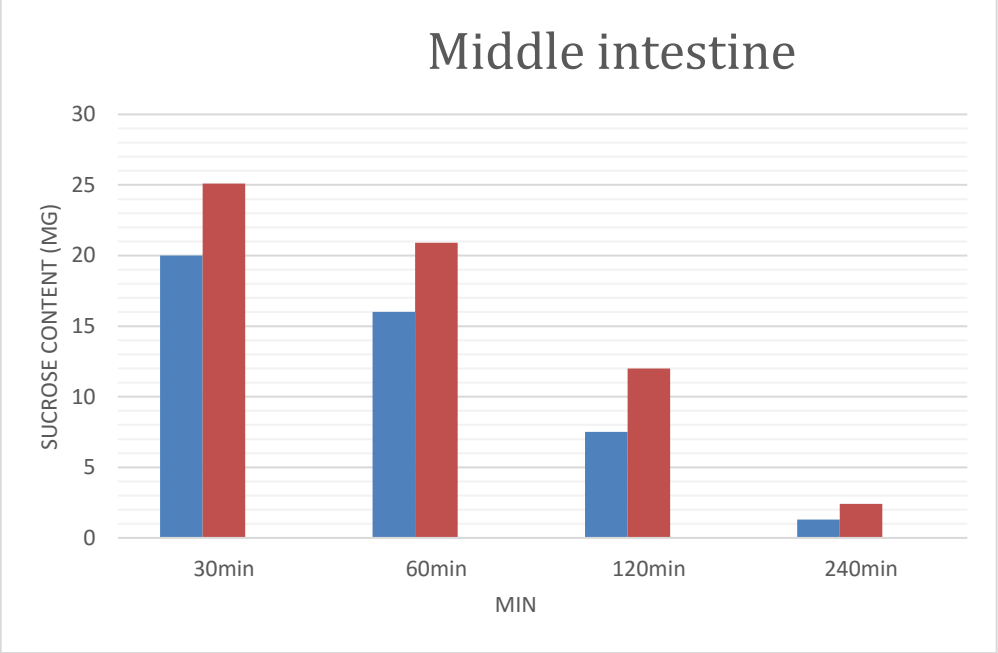

Figure 14: Sucrose content in middle 20cm of intestine

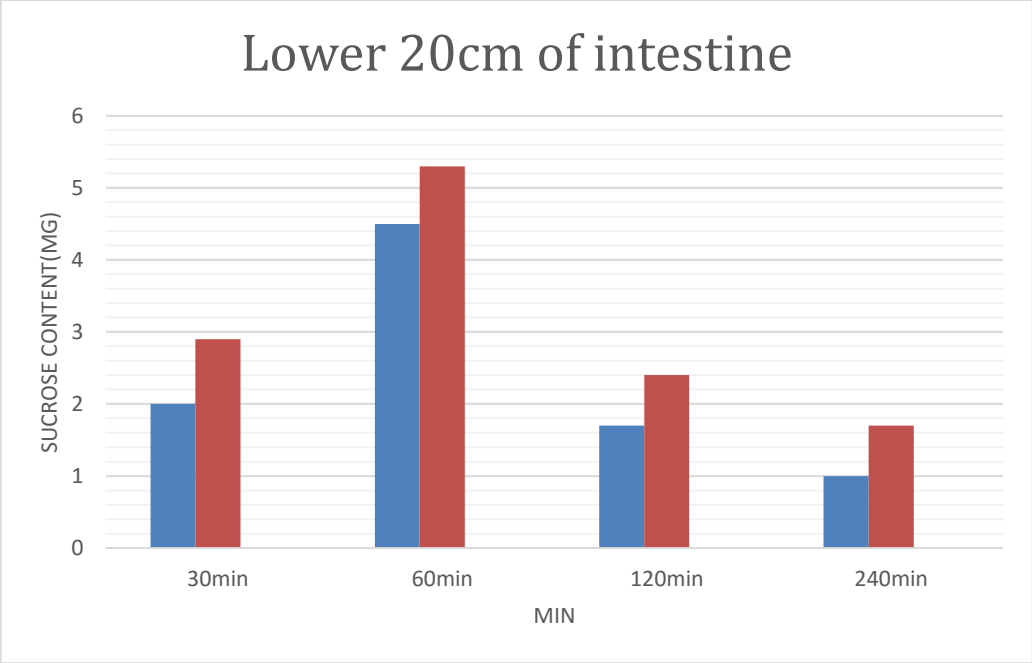

Figure 15: Sucrose content in Lower 20cm of intestine

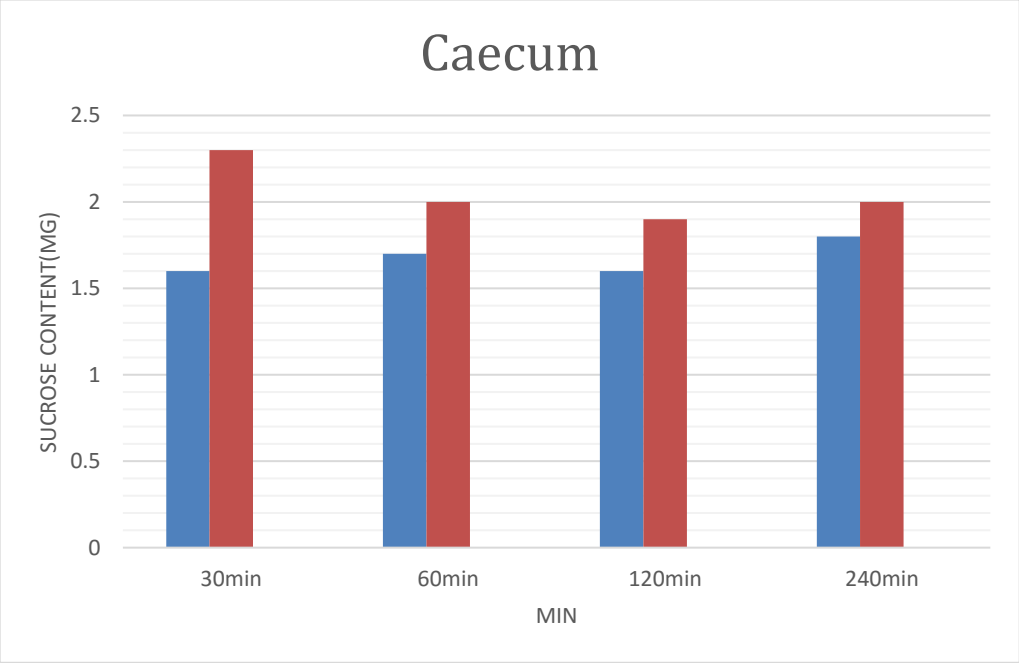

Figure 16: Sucrose content in Caecum

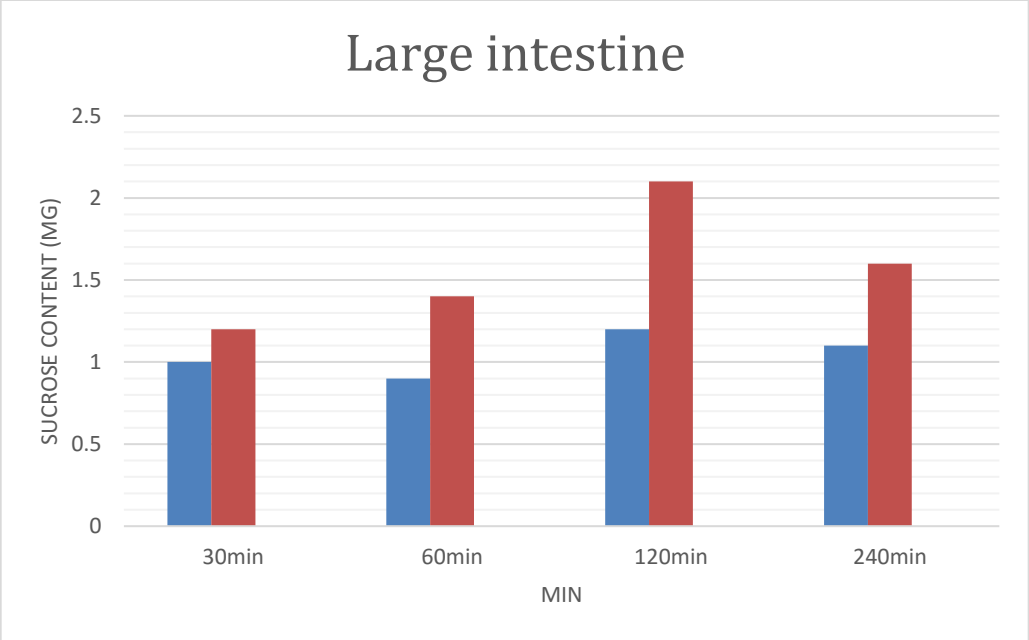

Figure 17: Sucrose content in Large intestine

Figure: Effects of ethanol extract of *A. nilotica* on gastrointestinal sucrose content after oral sucrose loading in normal rats: Rats were fasted for 20 h before the oral administration of a sucrose solution (2·5 g/kg body weight) with (treated group) or without (control group) ethanol extract of *Acacia nilotica* (100mg/kg body weight). Values are means and standard deviations represented by vertical bars. This is derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

Effect of *Acacia nilotica* on Intestinal Disaccharidase Enzyme Activity

Acacia nilotica extract showed significant ($p<0.05$) inhibition of disaccharidase enzyme activity.

Figure 18: Effect of Acacia nilotica on Intestinal Disaccharidase Enzyme Activity

Groups	Disaccharidase activity (µmol/mg/h)	SEM
Control	1.6	0.2
Acacia nilotica	1.03	0.1
Acarbose	1.07	0.17

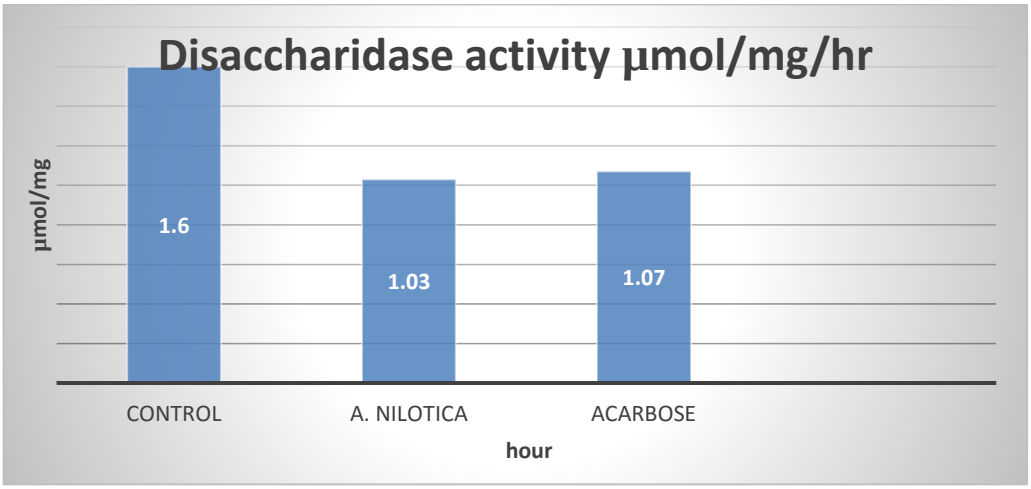

Figure 19: Effect of Acacia nilotica on Intestinal Disaccharidase Enzyme Activity

Figure 19: Effects of ethanol extract of *A. nilotica* on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of *A. nilotica* (100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 mg/Kg) was used as reference control for disaccharidase activity test. Values are means and standard deviations represented by vertical bars (n=12). It significantly decreased ($p<0.05$) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

CHAPTER 4: DISCUSSION & CONCLUSION

4.1 Discussion

Diabetes and its complications is becoming the third leading cause of death after cancer and cardiovascular diseases. Many serious side effects of insulin therapy and oral hypoglycaemic drugs necessitate the search for newer effective and safer class of compounds to overcome diabetic problems. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines. It has been estimated that more than 1000 plant species are used as folk medicine for treating diabetes though most lack scientific evidence. Our study is directed to evaluate the anti-diabetic property of aethanolic extract of stalks of ***Acacia nilotica*** on normal rats. Additionally, unpublished, preliminary screening data, of this plant, showed highly promising hypoglycemic activity. Oral treatment with the defatted ethanolic leaf extract showed hypoglycemic activity in normal rats. However, the tissue level mechanism of action of ***Acacia nilotica*** antidiabetic property is yet to be investigated. According to established studies, the initiator of diabetic tissue damage is the hyperglycaemic states. The cells which are damaged by hyperglycemia cannot maintain a constant internal level of glucose which ultimately results in altered cellular mechanism and long-term changes in cellular macromolecular content. Postprandial glucose spike causes perturbation in endothelial cell function, and increased blood coagulation. An increase in the products of glycosylation is another result of hyperglycaemic states, which significantly influences the development of diabetic induced vascular disease. Thus, management of hyperglycaemic states in diabetes patients is the most important method of diabetes control. Commonly used diabetic drugs follow the basic mechanism of enhancing insulin secretion or enhancing sensitivity to insulin, improving peripheral glucose utilization, inhibiting glucose absorption and intestinal disacharidase enzymes. Through our studies on ***Acacia nilotica***, after using several techniques, we are trying to prove any of the above mentioned mechanism that this plant follows.

Six Segment test showed significantly higher amount of sucrose in stomach, upper, middle and lower intestine in ***Acacia nilotica*** administered groups. The latter three part of GI are most important for absorption of nutrients including sugar. Disaccharides in its own form

does not get absorbed due to lack to sucrose carriers, as carriers monosaccharaides only are present in the GI tract. Therefore, it is imperative that disaccharides get converted to monosaccharaides first for absorption. Higher sucrose content in the GI Tract clearly reflects a reduced sucrose digestion throughout the GI Tract. This in turn, is shown by a significantly higher concentration of sucrose reaching the large intestine and caecum, which eventually remains unabsorbed and egested with faeces.

In the intestinal disaccharidase activity assay, ***Acacia nilotica*** was shown to have reduced the catabolism of sucrose and starch respectively. Since complex carbohydrates and disaccharides have first to be broken down into simpler monosaccharaides, it follows that any inhibition of this catabolic process would retard sugar absorption, which would in turn, be shown as a lower glycemic peak.

Dietary fibers of plant ingredients or powders can often provide a barrier to diffusion caused due to its high viscosity and ability to bind to glucose. Because, dietary fibers are capable of significantly reducing the transit time in GI Tract of ingested food. Reduced transit time is responsible for lesser time available for di-and polysaccharides in the meal to be digested and absorbed.

So, our results can be fully attributed to the significant increase amount of unabsorbed sucrose was remained in 6 different parts of intestine and decrease in disaccharide enzyme activity which validates anti-hyperglycemic activity of ***Acacia nilotica***.

Further research is underway, in our labs, for identifying the active molecules responsible for inhibiting α-amylase and disaccharidase enzyme activity. We also intend to study if there is any significant lipid lowering or obesity controlling ability of ***Acacia nilotica*** in diabetic models.

4.2 Conclusions

Our studies confirm the previous findings showing anti-hyperglycemic action of ***Acacia nilotica.*** Additionally, we have elucidated that ***Acacia nilotica*** has significant capabilities of inhibiting absorption of glucose by inhibition of intestinal disaccharidase enzyme. Therefore, its traditional use, as mentioned above is justified and calls for further research, to optimize its anti-diabetic activity.

CHAPTER 5: REFERENCES

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