# Studies on the Mechanism of Anti-Hyperglycemic Effects of *Acacia nilotica*

***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.***


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## Declaration by the Candidate

I, Marzia Binta Alam hereby declare that the dissertation entitled **"Studies on the Mechanism of Anti-Hyperglycemic Effects of Acacia nilotica"** 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 by us 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.

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## Certificate by the Supervisor

This is to certify that the thesis entitled **"Studies on the Mechanism of Anti-Hyperglycemic Effects of Acacia nilotica"** 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 Marzia Binta Alam (student ID: 2013-3-70-076). 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.

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## Certificate by the Chairperson

This is to certify that the thesis entitled **"Studies on the Mechanism of Anti-Hyperglycemic Effects of Acacia nilotica"** 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 Marzia Binta Alam (student ID 2013-3-70-076) During the period 2016-2017 of their research in the Department of Pharmacy, East West University.

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# DEDICATION

This research paper is dedicated to my beloved parents for their unconditional support.

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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 Dissacharide 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.*

# CHAPTER 1

# INTRODUCTION

## 1.1 Diabetes Mellitus

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2001).

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 (WHO, 2002).

## 1.2 Epidemiology of Diabetes Mellitus

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 (WHO 2002). 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).

Bangladesh has a disproportionately high diabetes population with more than 7.1 million, 8.4% or 10 million according to research published in WHO bulletin in 2013, of the adult population affected by the disease. The number will be 13.6 million in 2040 (WHO, 2013).

## 1.3 Classification of Diabetes Mellitus

The World Health Organization (WHO) classifies diabetes into main groups: type 1 diabetes also called insulin-dependent diabetes mellitus or IDDM and type 2 diabetes also called non-insulin dependent diabetes mellitus or NIDDM. Maturity-Onset Diabetes of the Young (MODY) and gestational diabetes are less frequently occurring forms of diabetes (WHO, 2002).

### 1.3.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (DM) is a multisystem disease with both biochemical and anatomic consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin, which results from the marked and progressive inability of the pancreas to secrete insulin because of autoimmune destruction of the beta cell. Type 1 DM can occur at any age. It is most common in juveniles but can also develop in adults, especially in those in their late 30s and early 40s (Dansinger, 2014).

Common symptoms of type 1 diabetes include:

- Excessive thirst
- Increased urination (sometimes as often as every hour)
- Unusual weight loss
- Fatigue or tiredness
- Nausea, perhaps vomiting
- Blurred vision
- In women, frequent vaginal infections
- In men and women, yeast infections (thrush)
- Dry mouth
- Slow-healing sores or cuts
- Itching skin, especially in the groin or vaginal area.

Symptoms of type 1 diabetes can develop quickly, over weeks or sometimes days (Dansinger, 2014).

#### 1.3.1.1 Pathophysiology of type 1 diabetes

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in IDDM patients. Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with IDDM, glucagons secretion is not suppressed by

hyperglycemia. The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. There are multiple biochemical mechanisms that account for impairment of tissue's response to insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju, 2010).

### 1.3.2 Type 2 Diabetes Mellitus

Diabetes mellitus type 2 (formerly noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin. (Kumaret al, 2005).

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity. As of 2010 there were approximately 285 million people diagnosed with the disease compared to around 30 million in 1985. (Smyth & Heron, 2006) Long-term complications from high blood sugar can include heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor blood flow in the limbs leading to amputations. The acute complication of ketoacidosis, a feature of type 1 diabetes, is uncommon, (Fasanmade, Odeniyi &Ogbera, 2008).

Common symptoms of type 2 diabetes include:

- Increased thirst
- Increased hunger (especially after eating)
- Dry mouth
- Frequent urination
- Unexplained weight loss (even though you are eating and feel hungry)
- Fatigue (weak, tired feeling)
- Blurred vision

- Headaches
- Loss of consciousness (rare)

Other symptoms of type 2 diabetes may include:

- Slow-healing sores or cuts
- Itching of the skin (usually around the vaginal or groin area)
- Frequent yeast infections
- Recent weight gain or unexplained weight loss
- Velvety dark skin changes of the neck, armpit, and groin, called acanthosis nigricans
- Numbness and tingling of the hands and feet
- Decreased vision
- Impotency (Dansinger, 2014).

### 1.3.2.1 Pathophysiology of Type 2 Diabetes

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups:

i) Those with normal glucose tolerance.

ii) Chemical diabetes (called impaired glucose tolerance).

iii) Diabetes with minimal fasting hyperglycemia (fasting plasma glucose less than

140mg/dl).

iv) Diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose greater than140mg/dl).The individuals with impaired glucose tolerance have hyperglycemia in spite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in the average NIDDM patients (Holt, 2004).

### 1.3.2.2 The risk factors for type 2

- Age and ethnicity: The older people are at higher risk, especially over 40 (for white people), and over 25 (for black, South Asian and some minority groups). It has been found in the UK that black people and people of South Asian origin have five times the risk of developing Type 2 compared to white people.
- Diabetes in the family: If a relative has/had diabetes risk might be greater. The risk increases if the relative is a close one.
- Bodyweight (and inactivity combined with bodyweight): Four-fifths of people who have Type 2 became so because they were overweight. The more overweight a person is the higher his/her risk will be. The highest risk is for a person who is overweight and physically inactive.
- Cardiovascular problems and stroke: A person who has had a stroke runs a higher risk of developing Type 2. This is also the case for people who suffer from hypertension (high blood pressure), or have had a heart attack. Any diagnosis of a problem with circulation indicates a higher risk of developing Type 2.
- Gestational Diabetes: A woman who became temporarily diabetic during pregnancy - gestational diabetes - runs a higher risk of developing Type 2 later on. Women who give birth to a higher weight baby may run a higher risk, too.
- Impaired fasting glycaemia (IFG) - Impaired glucose tolerance (IGT): A person who has been diagnosed as having impaired fasting glycaemia or impaired glucose tolerance and does not have diabetes runs a significantly higher risk of eventually developing Type 2. People with IFG or IGT have higher than normal levels of glucose in their blood.
- Severe mental health problems: It has been found that people with severe mental health problems are more likely to develop Type 2.

## 1.4 Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy. GDM is detected through the screening of pregnant women

for clinical risk factors and testing for abnormal glucose tolerance that is usually, but not invariably, mild and asymptomatic. GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. Indeed, women with GDM are at high risk for having or developing diabetes when they are not pregnant. Thus, GDM provides a unique opportunity to study the early pathogenesis of diabetes and to develop interventions to prevent the disease (Thomas, 2005).

### 1.4.1 Causes

Body digests the food which is eaten to produce sugar (glucose) that enters your bloodstream. In response to pancreas (large gland behind stomach) produces insulin. Insulin is a hormone that helps glucose move from your bloodstream into body's cells, where it's used as energy. During pregnancy, the placenta, which connects baby to blood supply, produces high levels of various other hormones. Almost all of them impair the action of insulin in cells, raising blood sugar. Modest elevation of blood sugar after meals is normal during pregnancy. As baby grows, the placenta produces more and more insulin-blocking hormones. In gestational diabetes, the placental hormones provoke a rise in blood sugar to a level that can affect the growth and welfare of baby. Gestational diabetes usually develops during the last half of pregnancy sometimes as early as the 20th week, but generally not until later (Mayo Clinic Staff, 2013).

## 1.5. Biochemical abnormalities involved in the pathogenesis of diabetes

Diabetes mellitus is a heterogeneous group of metabolic disorders. The major metabolic lesions associated with diabetes mellitus include defective insulin secretion and insulin sensitivity by peripheral tissue targets.

### 1.5.1 Defective insulin secretion in diabetes mellitus

Defective insulin secretion is a feature of type 2 diabetes that results from inadequate compensatory increase of β cell mass and impaired glucose-dependent insulin release (Rutter 2001, Kahn and Porte 1990, Leahy 1990, Flatt et al 1992). The ability of pancreatic β cells to synthesize, store, and release insulin in response to variations in circulating metabolite levels and intracellular glucose metabolism is regulated by changes in ATP/ADP ratios resulting in $Ca^{2+}$ mobilization (Matschinsky et al 1996). Alterations of this sensing loop occur early in the pathogenesis of type 2 diabetes, but are initially compensated by an increase of β cell mass (Bonner-Weir 1994). In this respect, pancreatic β cells appear to differ from other terminally differentiated cell types by retaining their ability to proliferate, as demonstrated in both physiological conditions (growth, gestation) and disease states (obesity, insulin resistance) (Bonner-Weir, 2000). In addition to presumptive proliferation of existing β cells, there is evidence for β cell neogenesis from undifferentiated progenitors, apparently arising from the epithelial lining of pancreatic ducts (Bendayan 1987, Bertelli et al 2001, Bonner-Weir 2000, Bouwens & Pipeleers 1998).

The factors inducing β cell proliferation under normal or pathological conditions are largely unknown, although some evidence exists about the involvement of fibroblast growth factors (FGFs) (Hart et al 2000), hematopoietic growth factors (HGFs) (Garcia-Ocana et al 2000), and placental lactogen (Vasavada 2000). Moreover, signaling by receptor tyrosine kinases has been implicated as a regulatory mechanism in both β cell proliferation (Rhodes 2000, Withers 1999, Hugl et al 1998) and insulin release (Hart et al 2000, Khan et al 2001, Leibiger et al 1998). In particular, insulin/insulin-like growth factor (IGF) signaling through insulin receptor substrate (IRS) and phosphoinositide 3-kinase (PI 3-kinase) appears to regulate several aspects of β cell function. Thus, ablation of the insulin/IGF receptor substrate IRS-2 impairs β cell proliferation (Withers 1998, Kubota 2000), whereas ablation of $p70^{s6k1}$, an Akt substrate, is associated with a decrease in β cell size (Pende 2000).

The mutations of insulin receptor (IR) (Kulkarni 1999a) or IRS-1 (Kulkarni 1999b) impair insulin synthesis and secretion mediated by PI 3-kinase–dependent pathways (Aspinwall 2000, Kulkarni 1999b). The signals regulating β cell proliferation and insulin secretion diverge

downstream of PI 3-kinase and this strongly suggests that Akt is not the sole effector of PI 3-kinase. Nevertheless, the role of growth factor signaling through PI 3-kinase as related to insulin secretion remains poorly understood. It has been demonstrated that mice lacking IRS-1 develop defective insulin secretion, whereas mice lacking IRS-2 develop impaired β cell proliferation (Accili 2001). Recently it has been shown that β cells lacking IGF1R exhibit a profound decrease of insulin secretion in response to both glucose and arginine (Xuan et al 2002).

### 1.5.2 Defective insulin action in diabetes mellitus

Insulin-mediated glucose utilization and metabolism is the final result of the activation of a complex cascade of events involved in the insulin signaling process (Khan 1993). Alteration of one or more of these events can result in impaired insulin action. Three main steps are involved in the generation of insulin resistance: 1) insulin binding to the cell membrane receptor, 2) insulin receptor phosphorylation, and 3) intracellular insulin signaling.

The insulin receptor is consisting of two subunits in the extracellular domain and two subunits with main intracellular domain. Upon insulin binding of the subunits, the intrinsic kinase activity in the □-subunits is activated leading to phosphorylation of the adjacent subunit. The autophosphorylation of the insulin receptor allows the activation of insulin receptor substrate (IRS-1, -2, -3, -4) protein family. These proteins exert an important regulatory action on other mediators like phospho-inositol-3-kinase (PI3-kinase). The contribution of IRS-1 and IRS–2 to insulin resistance has been recently demonstrated with knock-out genetic experiments. These studies proved that IRS-2 can play a vicariate role in absence of IRS-1, while IRS-2 knock-out results in impaired insulin action (Mauvais-Jarvis et al 2002).

Activation of PI3-kinase catalyses the formation of PI-3, 4,5-phosphate allowing the activation of PKB/AKT and phosphatidylinositol-3,4,5-phosphate kinase-1 (PDK-1). The phosphorylation of PKB/AKT regulates the kinase cascade involved in the insulin signal transduction responsible for GLUT-4 translocation from the intracellular membrane compartment to the cell membrane allowing active transmembrane glucose transport and

phosphorylation, activation of the glycolytic flux, as well as glycogen and protein synthesis (Khan & Pessin 2002).

Several of the steps of insulin signaling cascade involved in the generation and propagation of the insulin signal can contribute to the molecular defect of insulin action. A reduced expression and a phosphorylation of the elements involved in the first steps of insulin signaling (IRS, PI3-kinase, PKB) have been found in tissue of type 2 diabetic patients. The role of specific defects of these proteins has been established by knock-out animal models (Mauvais-Jarvis et al 2002). For instance, IRS-1, IRS-2 and GLUT-4 knock-out mice have been shown to develop insulin resistance and glucose intolerance. □-cell insulin receptor knock-out (□IRKO) mice lose acute insulin response to glucose and develop glucose intolerance. Human pancreatic islets carrying the $Gly^{972}\rightarrow$Arg IRS-1 polymorphism have impaired insulin action (Marchetti et al 2002).

The principle defect in type 2 is the loss of insulin sensitivity in peripheral tissue such as muscle and liver resulting in impairment of glucose uptake and utilization by these tissues. Together with excessive glucose production by the liver, these defects lead to widespread disruption of nutrient homeostasis (DeFronzo 1988). Insulin resistance appears to be the primary metabolic defect with relative (but not absolute) insulin deficiency being the factor determining conversion to diabetes (Stern 1988). Once diabetes is established, the abnormalities of insulin secretion and insulin resistance worsens, hand in hand, in direct relationship to the degree of fasting hyperglycemia (Zimmet et al 1978, Kolterman et al 1981). In type 2 diabetes, gross insulin resistance combined with hyperinsulinaemia results in a state of relative (not absolute) insulin deficiency (Campbell et al 1988). Postreceptor defects are currently believed to be primarily related to insulin resistance in human diabetes (Olefsky & Kolterman 1981, Becker & Roth 1990, Kahn & Folli 1993, Kahn 1994).

## 1.6 Signs and Symptoms

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.

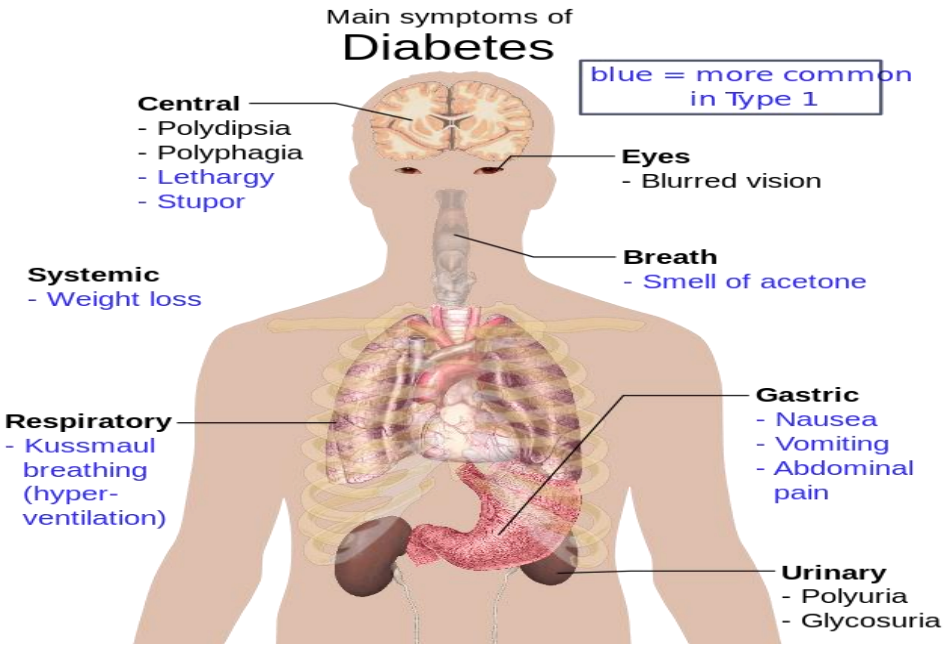



Figure 1.1: Overview of the most Significant Symptoms of Diabetes Mellitus (Jackson et al, 1991)

## 1.7 Diabetic Emergencies

Low blood sugar is common in persons with type 1 and type 2-diabetes. Most cases are mild and are not considered medical emergencies. Effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior, seizures, unconsciousness, and (rarely) permanent brain

damage or death in severe cases. Mild cases are self-treated by eating or drinking something high in sugar. Severe cases can lead to unconsciousness and must be treated with intravenous glucose or injections with glucagon.

People (usually with type 1 diabetes) may also experience episodes of diabetic ketoacidosis, a metabolic disturbance characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness.A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration.

## 1.8 Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves.Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant.Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes.The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5 fold greater rate of decline in cognitive function

## 1.9 Prevention

There is no known preventive measure for type 1 diabetes. Type 2 diabetes can often be prevented by a person being a normal body weight, physical exercise, and following a healthful diet.[2] Dietary changes known to be effective in helping to prevent diabetes include a diet rich in whole grains and fiber, and choosing good fats, such as polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help in the prevention of diabetes. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well. Although the genes you inherit may influence the development of type 2 diabetes, they take a back seat to behavioral and lifestyle factors. Data from the Nurses' Health Study suggest that 90 percent of type 2 diabetes in women can be attributed to five such factors: excess weight, lack of exercise, a less-than-healthy diet, smoking, and abstaining from alcohol.

### 1.9(a) Simple Steps to Lowering the Risk

Making a few lifestyle changes can dramatically lower the chances of developing type 2diabetes. The same changes can also lower the chances of developing heart disease and some cancers.

### 1.9 (b) Control Your Weight

Excess weight is the single most important cause of type 2 diabetes. Being overweight increases the chances of developing type 2 diabetes seven fold. Being obese makes you 20 to 40 times more likely to develop diabetes than someone with a healthy weight.

### 1.9 (c) Dietary Changes

Four dietary changes can have a big impact on the risk of type 2 diabetes-

- Choose whole grains and whole grain products over highly processed carbohydrates. Whole grains don't contain a magical nutrient that fights diabetes and improves health.

It's the entire package—elements intact and working together—that's important. The bran and fiber in whole grains make it more difficult for digestive enzymes to break down the starches into glucose. This leads to lower, slower increases in blood sugar and insulin, and a lower glycemic index. As a result, they stress the body's insulin-making machinery less, and so may help prevent type 2-diabetes.

- Skip the sugary drinks, and choose water, coffee, or tea instead. Several studies show that children and adults who drink soda or other sugar-sweetened beverages are more likely to gain weight than those who don't, and that switching from these to water or unsweetened beverages can reduce weight. Even so, however, weight gain caused by sugary drinks may not completely explain the increased diabetes risk. There is mounting evidence that sugary drinks contribute to chronic inflammation, high triglycerides, decreased good (HDL) cholesterol, and increased insulin resistance, all of which are risk factors for diabetes. Water is an excellent choice. Coffee and tea are also good calorie-free substitutes for sugared beverages (as long as you don't load them up with sugar and cream). And there's convincing evidence that coffee may help protect against diabetes.
- Choose good fats instead of bad fats. The types of fats in your diet can also affect the development of diabetes. Good fats, such as the polyunsaturated fats found in liquid vegetable oils, nuts, and seeds can help ward off type 2 diabetes.
- Eating polyunsaturated fats from fish—also known as ―long chain omega or marine omega fats does not protect against diabetes, even though there is much evidence that these marine omega fats help prevent heart disease. If you already have diabetes, eating fish can help protect you against a heart attack or dying from heart disease.
- Limit red meat and avoid processed meat; choose nuts, whole grains, poultry, or fish instead. It may be that the high iron content of red meat diminishes insulin's effectiveness or damages the cells that produce insulin; the high levels of sodium and nitrites (preservatives) in processed red meats may also be to blame. Red and processed meats are a hallmark of the unhealthful Western dietary pattern, which seems to trigger diabetes in people who are already at genetic risk.

### 1.9 (d) Quit Smoking

Add type 2 diabetes to the long list of health problems linked with smoking. Smokers are roughly 50 percent more likely to develop diabetes than nonsmokers, and heavy smokers have an even higher risk.

### 1.9 (e) Alcohol Now and Then May Help

A growing body of evidence links moderate alcohol consumption with reduced risk of heart disease. The same may be true for type 2 diabetes. Moderate amounts of alcohol—up to a drink a day for women, up to two drinks a day for men—increases the efficiency of insulin at getting glucose inside cells. And some studies indicate that moderate alcohol consumption decreases the risk of type 2 diabetes. If you already drink alcohol, the key is to keep your consumption in the moderate range, as higher amounts of alcohol could increase diabetes risk.

## 1.10 Management

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing low blood sugar. This can usually be accomplished with a healthful diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). Learning about the disease and actively participating in the treatment is vital for people with diabetes, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels.The goal of treatment is an HbA1C level of 6.5%, but should not be lower than that, and may be set higher.

Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however.

### 1.10.1 Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

### 1.10.2 Insulin

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  cells (Eisenbarth 1986, Rossini et al 1993), 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 (Schiffrin & Belmonte 1982). 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 (Dorzbach and Muller 1971), 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 (Galloway & Chance 1994, Skyler 1998). 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.

Examples of rapid acting insulins include

- Regular insulin (Humulin R, Novolin R)
- Insulin lispro (Humalog)
- Insulin aspart (Novolog)
- Insulin glulisine (Apidra)
- Prompt insulin zinc (Semilente, Slightly slower acting)

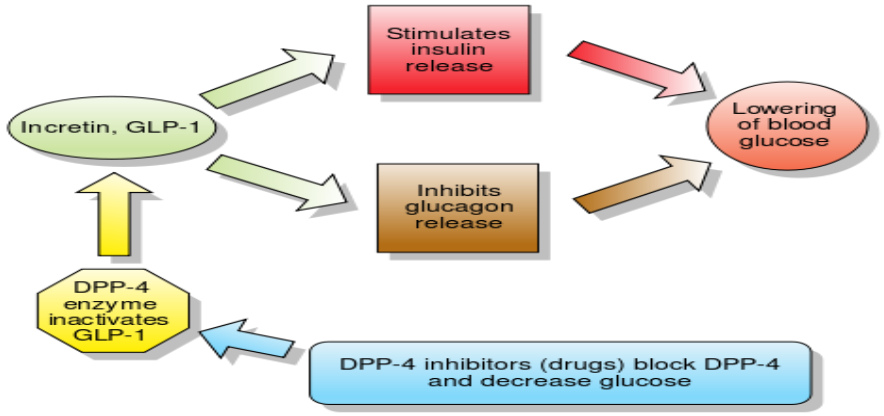



Fig 1.2: Overview of Insulin Secretion.

Examples of intermediate acting insulins include

- Isophane insulin, neutral protamine Hagedorn (NPH) (Humulin N, Novolin N) • Insulin zinc (Lente)

Examples of long acting insulins include

- Extended insulin zinc insulin (Ultralente)
- Insulin glargine (Lantus)
- Insulin detemir (Levemir)

### 1.10.3 Anti-Diabetes Medications

Many anti-diabetes drugs are available as generics. These include:

- Sulfonylureas - glimepiride, glipizide, glyburide
- Biguanides - metformin
- Thiazolidinediones (Tzd) - pioglitazone, Actos generic
- Alpha-glucosidase inhibitors - Acarbose
- Meglitinides - nateglinide
- Combination of sulfonylureas plus metformin - known by generic names of the two drugs.

Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality.It works by decreasing production of glucose by the liver.Several other groups of drugs, mostly given by mouth, may also decrease blood surgar in type II DM. These include agents that increase insulin release, agents that decrease absorption of sugar from the intestines, and agents that make the body more sensitive to insulin.When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications.Doses of insulin are then increased to effect.

Since cardiovascular disease is a serious complication associated with diabetes, some recommend blood pressure levels below 120/80 mmHg;however, evidence only supports less than or equal to somewhere between 140/90 mmHg to 160/100 mmHg.Amongst medications that lower blood pressure, angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications angiotensin receptor blockers (ARBs) do not.Aspirin is also recommended for patient with cardiovascular problems, however routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes.

## 1.11 Medicinal Plants

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical semi-synthesis. When a plant is designated as ‘medicinal’, it is implied that the said plant is useful

as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes (Ghani, 1998).

## 1.12 History of the Use of Medicinal Plants

History of medicine practically dates back to existence of human civilization. Fossil records date back the use of plants, at least, to the middle Paleolithic age some 60,000 years ago.

The basis of development of modern medicine is rooted in traditional medicine and therapies. The Greek physician Galen (AD 129-200) devised a pharmacopoeia describing the appearance, properties and use of many plants of his time. Natural products chemistry actually began with the work of Seturner, who first isolated morphine from opium in this, in turn, was obtained from opium poppy (Papaver somniferum L.) by process that have been used for over 5000 years.

Use of plants as medicines is a very older tradition of Chinese, Egyptians, Babylonians, Indians and Native Americans. The oldest list of those medicinal plants was found in Shennong Ben Cao Jing (c. 3000 B.C.) by Shen Nung.

Materia Medica, compiled by Dioscorides (c. 40-c. 90) and Galen (131-200 A.D.) is another evidence of the use of medicinal plants in Greek and Rome. The book is almost 1500 years old. It also gives a proof that the knowledge of medicinal plants and its use were also spread in Spain, France, Germany and England.

The knowledge of the use of medicinal plants, however, those were preserved in the monasteries of Britain and mainland Europe, in the middle ages. At that time they served as the medical school.

In the seventh and eighth centuries the herbal medical texts of Greek and Roman were acquired by the Arabic scholars. Iranian physician Ibn Sina, (980-1037 A.D.) is known for his contribution as he combined the work of both Galen and Dioscorides. The work is combined

in 'The Canon of Medicine'. Within the eleventh and twelfth century this work spread in many countries of Europe.

In the mid-fifteenth century, mass production of plant extract followed the guidance from the work of Galen, Dioscorides and Ibn Sina and those extracts were available outside monastery and medical schools. General people just had to collect the medicines and use the, as per the prescribed manner and dosage.

Though the uses of medicinal plants have always been proved to be very effective but inappropriate use of them may also cause health issues. This fact was emphasized by Paracelcus (1493-1541). According to him each herb has a characteristic 'sign' which could be observed by its color, scent and the environment where it grows and thus lead to the direction of its proper use. Marigold and dandelion and some other plants with yellow flowers were used for jaundice and flowers with heart shaped petals and white pansies ere used for heart diseases (University of Virginia, N.D).

## 1.13 Use of Medicinal Plants to Treat Diabetes

Popularity of herbal medicine in Bangladesh is high. There are many people, who have chosen to take alternative medicine over allopathic medicine as ailment. There are lots of people who still believes in natural treatment. That is why, the market share by herbal medicine is high. The market value for Bangladeshi medicinal plants are approximately Tk. 3,300 million at trade prices. The yearly turnover for the Ayurvedic sector is around Tk. 1,000 million, Unani around Tk. 1,800 million and homeopathy around Tk. 500 million (Hosseinzadeh *et al*., 2015).

Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years, and continue to play a major role in the primary health care of about 80% of the world's inhabitants (Farnsworth et al 1985). It is estimated that 66-80% of medicines used in developing countries are based on plants (Farnsworth 1983). Many of the currently available drugs have been derived directly or indirectly from plants. Within developed countries 25% of medicinal therapies contain active priciples derived from plants (Day & Bailey 1988). Besides providing active raw materials, plants can offer molecules that serve as templates for the development of new drugs.

World ethnobotanical information about medicinal plants reports that almost 800 plants are used in the control of diabetes mellitus (Ajgaonkar 1979, Alarcon-Aguilara et al 1998). Over the last two decades, several comprehensive reviews (Oliver-Bever & Zahnd 1979, Bailey & Day 1989, Ivorra et al 1989, Marles & Farnsworth 1995) have been written on the evidence that higher plants are of use in the treatment of diabetes, providing discussions of the botany, phytochemistry, pharmacology, and in some cases, toxicology, of the botanical agents. Literally hundreds of extracts of higher plants used in folk medicine for diabetes (or active principles derived from these plants) have been screened for their biologic activity in both in vitro and in vivo assays. The most extensive review (Marles & Farnsworth 1995) evaluated available data on more than 1000 species of plants reported to have been used to treat diabetes and/or been investigated for antidiabetic activity, and indicated that approximate 80% of the traditional plants used for the treatment of diabetes demonstrated some antidiabetic activity. In many instances the chemical constituent in the plant responsible for the biological activity has been isolated and identified, and information is also available concerning the mechanism of action. Galega officinalis (goat's rue), used in Europe as a treatment for diabetes since medieval times, yields a hypoglycemic principle rich in guanidine (Bailey 1985). Further derivatives of this principle have given rise to biguanides and the present anti-diabetic agent metformin (Sterne 1969).

Prior to the discovery of insulin in 1992 and the later development of oral hypoglycemic agents, the major form of treatment of diabetes mellitus involved dietary manipulation and the use of plant therapies. The recommended use of plants dates back to the Ebers papyrus of around 1550 BC. More than 400 plants world-wide have been documented for the treatment of diabetes and the majority await proper scientific and medical evaluation (Day & Bailey 1988). Most of these traditional medicines are prepared from herbs, spices and plants, which do not form part of the normal diet (Day & Bailey 1988, Bailey & Day 1989). However, several common components of the diet are traditionally recommended for regular consumption, and some are additionally taken as infusions, decoctions or alcoholic extracts. The World Health Organization has recommended accordingly that traditional plant treatments for diabetes warrant further evaluation (WHO 1980).

With few exceptions, traditional plant treatments for diabetes have not claimed to be alternatives to insulin therapy in type 1. Isolated reports have described plant-derived materials that exert an insulin-like effect in type 1 diabetes (Chandola & Tripathi 1981, Khanna et al 1981). However these reports have not been independently evaluated, and there is no evidence that they could provide a long-term botanical substitute for insulin. A small number of review articles exist which document hypoglycemic agents derived from plant treatments for diabetes (e.g. Oliver-Bever & Zahnd 1979, Duke 1985, Day 1990). Many traditional plant treatments owe their folklore reputation, at least in part, to the presence of polysaccharides, which achieve beneficial effects through reduction of gastrointestinal processing and post-prandial hyperglycemia. However for the majority of traditional plant treatments the active principles present together with their mode of action have yet to be realized (Ajgaonkar 1979, Day & Bailey 1988). Hypoglycemic compounds from plants that help directly combat insulin resistance and/or promote endogenous insulin release are realistic possibilities.

## 1.14 Traditional Herbal Anti-Diabetics

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

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

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

## 1.15 Medicinal Plants with reported Antidiabetic Effect on experimental models

| Plant(Family) | Part of Plant Used | Material | Result |
|---|---|---|---|
| Annona Sqamosa (Annonaceae) | Fruit peel | Alcohol, ether, ethyl acetate | Significant increase body weight and diminished blood glucose level |
| Piper longum (Piperaceae) | Root | Aqueous and ethanolic extract | Streptozotocin HbAlc level were well regulated near to normal |
| 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 |
| Gomphrena gobosa (Amaranthaceae) | whole plant | Methanolic n-Hexane, chloroform, Carbon tetrachloride and aqueous extracts | Lower the blood glucose level |
| Psidium guvajava (Myrtaceae) | Leaves | Ethanolic extract | reduction in blood glucose level |

Table 1.1: Medicinal Plants with reported Antidiabetic Effect ( Ghani, 2002)

## 1.16 *Acacia nilotica* (Babla)

Babul or Babla is known for its medicinal usages. In Ayurveda the bark is considered astringent to the bowels, alexipharmic and anthelmintic; it is used to treat coughs, bronchitis, diarrhea, biliousness, leukoderma 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 leukoderma, gonorrhea, and strangury and ophthalmic. 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.

### 1.16.1 General Description

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 bi pinnate, flowers are golden-yellow in color, 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*.

Fig 1.3: Babla Tree with Flower

## 1.16.2 Chemical Constituents

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.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).

Acacia nilotica (L.) Del. syn. Acacia arabica (Lam.) Willd.(Mimosaceae) is an imperative multipurpose plant. 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).

## 1.16.3 Classification of Acacia

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Mimosoideae

Genus: Acacia

Species: Acacia nilotica (Linn.). Bel.

Fig1.3: Leaf of *Acacia nilotica*

## 1.16.4 Medicinal Uses and Pharmacological Effects

- **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 spreadilbility 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)

| Part used | Uses |
|---|---|
| Leaf | Chemopreventive, 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. |

Table 1.2: parts used of Babla (Ghani, 2002)

- **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).

- **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).

- **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 METHODS

## 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

Extraction process can be described in flow charts

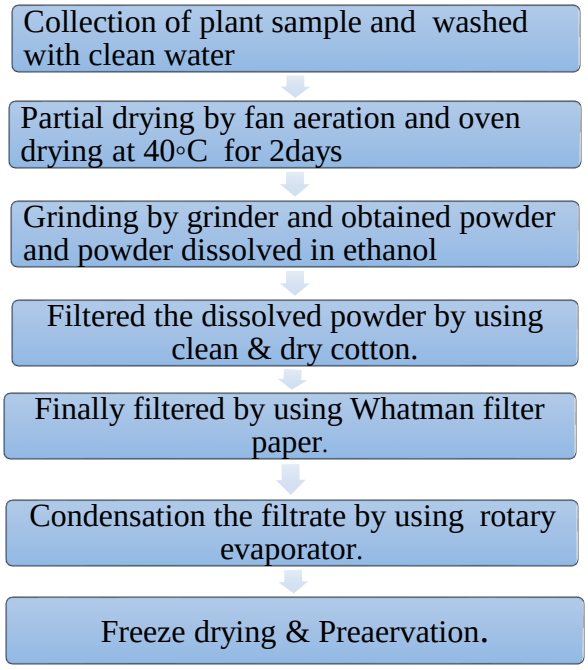



Fig 2.1: General Plant Extraction Procedure

## 2.2 Experimental Animals

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

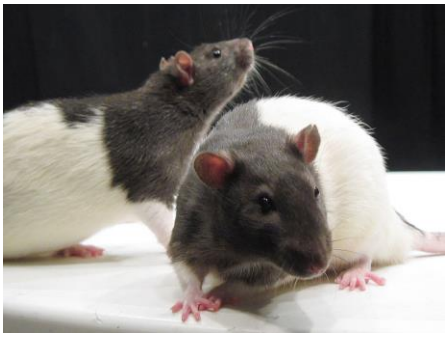


Fig 2.2: Long Evans rats

### 2.2.1 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.

Fig 2.3: 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.

The gastrointestinal tract was excised and divided into 6 segments. They are –

**1.** Stomach,

**2.** Upper 20 cm of small intestine,

**3.** Middle part of small intestine,

**4.** Lower 20 cm of small intestine,

**5.** Cecum and

**6**. Large intestine.

### 2.3.3 Steps of the experiment

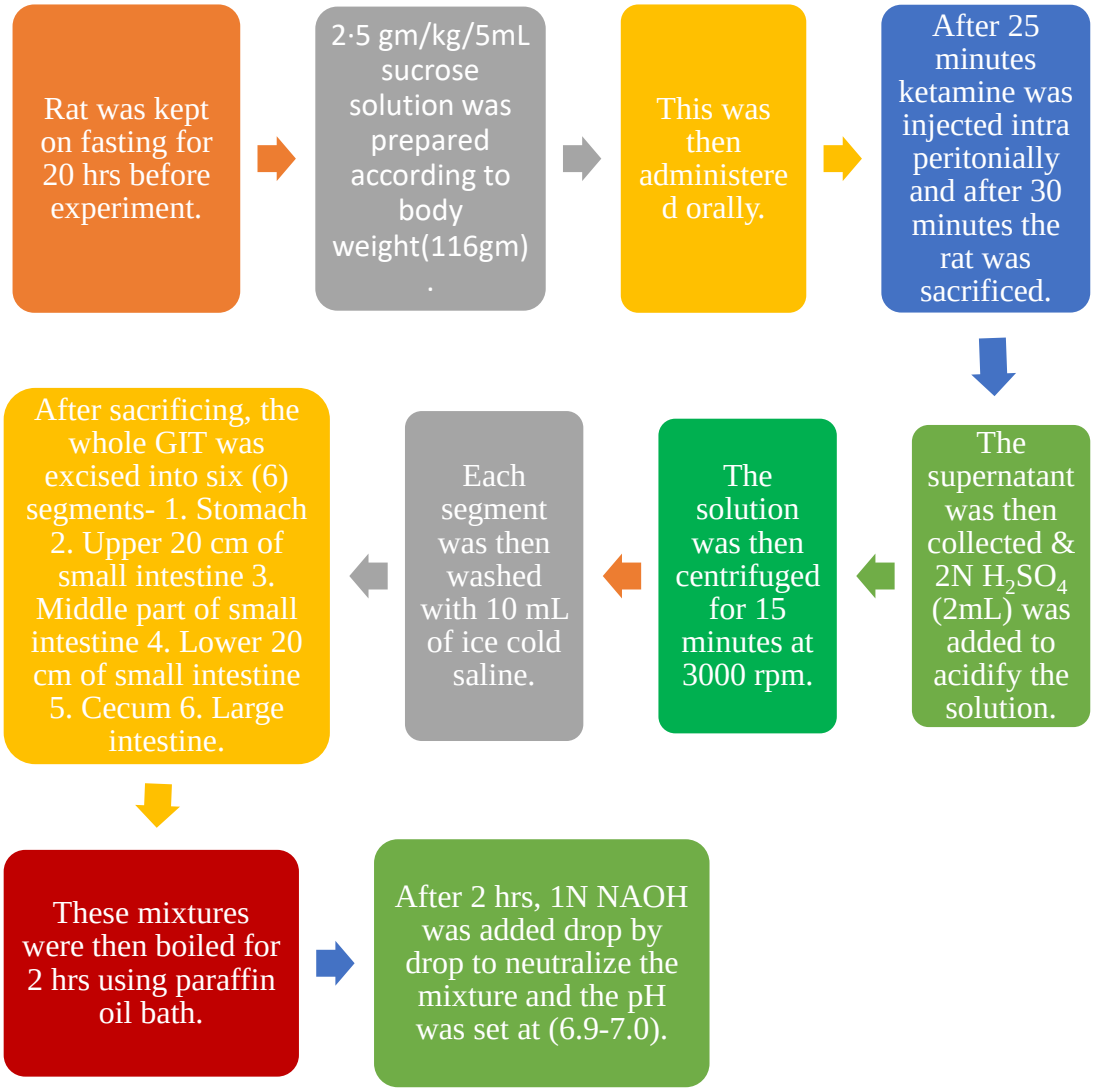



Figure-2.4: Flowchart of the experiment

## 2.4 Assessment of the Effect of Plant Materials on Intestinal Disaccharidase Activity

### 2.4.1 Assessment of Conditions

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

### 2.4.2 Mucosa/Tissue Collection

After one hour 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:

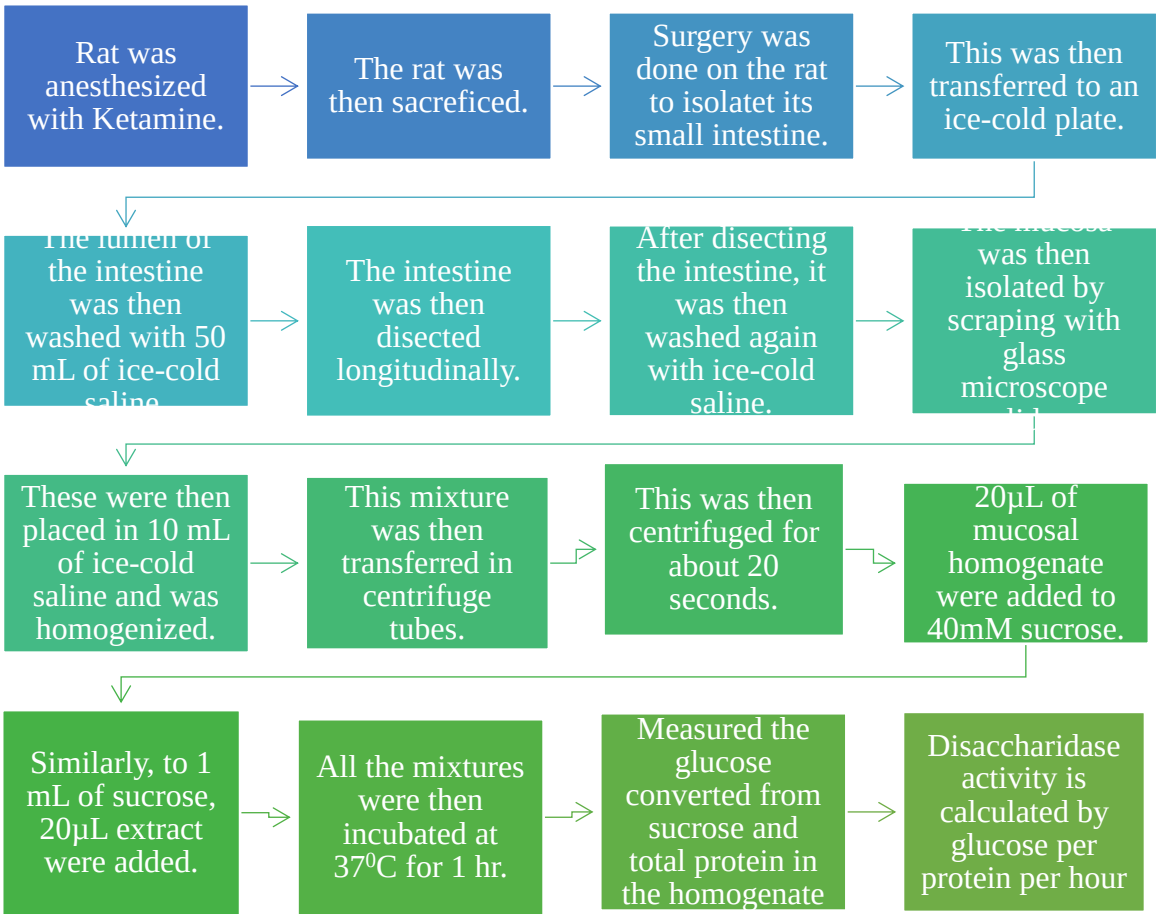



Fig 2.5: Flowchart of the Experiment

# CHAPTER 3

# RESULT

## 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: 3.1 (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: 3.2 (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: 3.3 (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: 3.4 (sucrose content in Lower 20cm of intestine)**

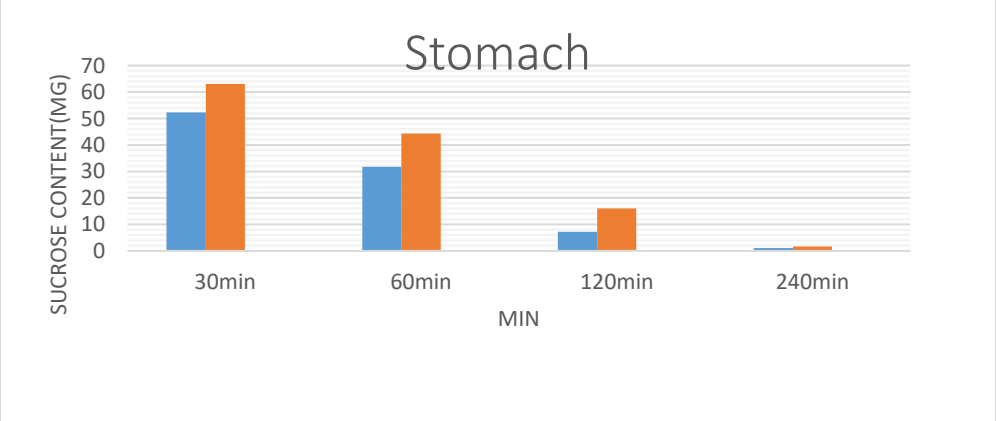
| **Groups** | **30 min** | | **60 min** | | **120 min** | | **240 min** | |
|---|---|---|---|---|---|---|---|---|
| | **Sucrose(mg)** | **SD** | **Sucrose(mg)** | **SD** | **Sucrose(mg)** | **SD** | **Sucrose(mg)** | **SD** |
| **Control** | **2.0** | **0.5** | **4.5** | **1.1** | **1.7** | **0.4** | **1.0** | **0.3** |
| ***Acacia nilotica*** | **2.9** | **0.5** | **5.3** | **0.3** | **2.4** | **0.4** | **1.7** | **0.2** |

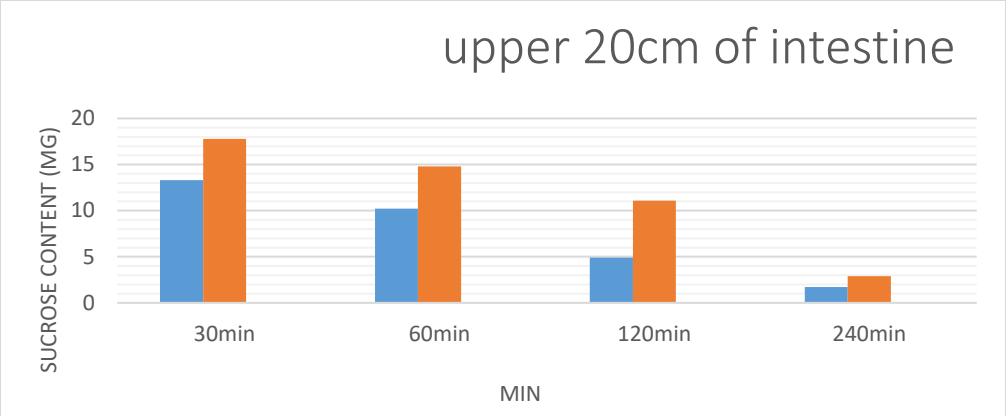
**Table: 3.5 (sucrose content in Caecum)**

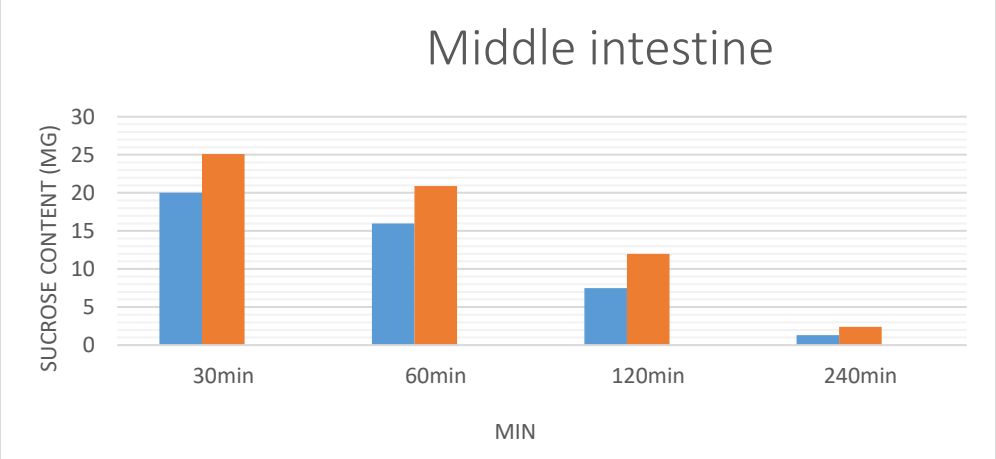
| Groups | 30 min | | 60 min | | 120 min | | 240 min | |
|---|---|---|---|---|---|---|---|---|
| | Sucrose(mg) | SD | Sucrose(mg) | SD | Sucrose(mg) | SD | Sucrose(mg) | SD |
| **Control** | **1.6** | **0.5** | **1.7** | **0.3** | **1.6** | **0.4** | **1.8** | **0.5** |
| ***Acacia nilotica*** | **2.3**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

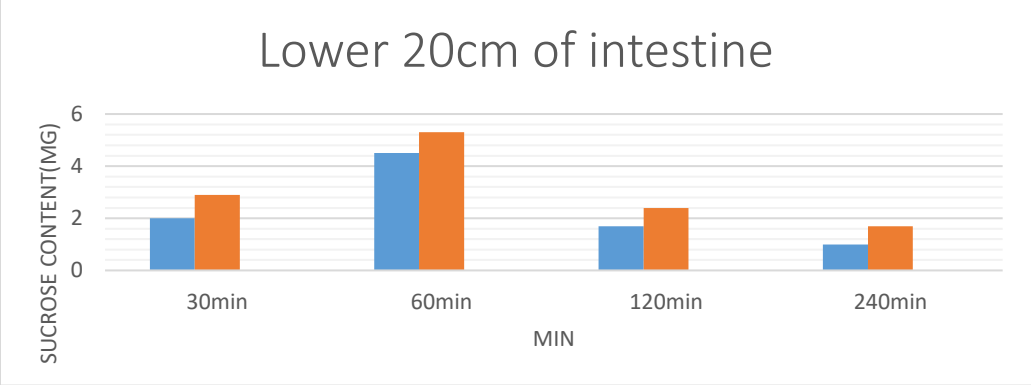
**Table:3.6 (sucrose content in Large intestine)**

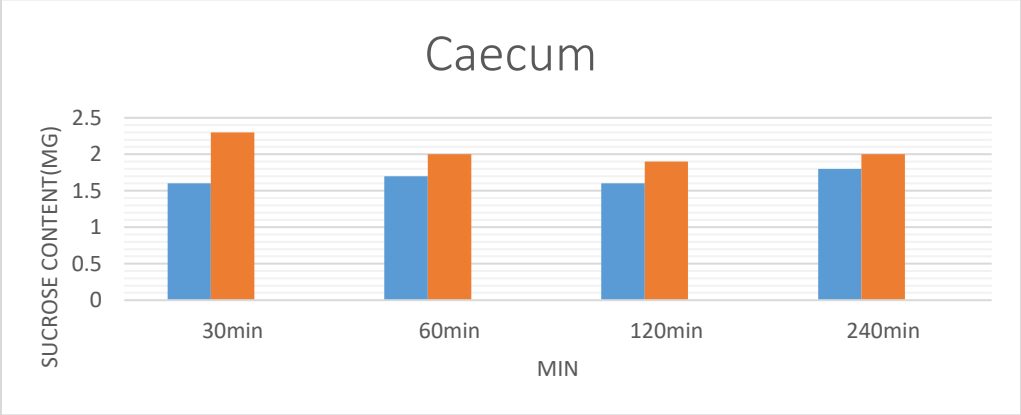
| Groups | 30 min | | 60 min | | 120 min | | 240 min | |
|---|---|---|---|---|---|---|---|---|
| | **Sucrose (mg)** | **SD** | **Sucrose(mg)** | **SD** | **Sucrose(mg)** | **SD** | **Sucrose(mg)** | **SD** |
| Control | 1.0 | 0.3 | 0.9 | 0.3 | 1.2 | 0.2 | 1.1 | 0.2 |
| ***Acacia nilotica*** | 1.2 | 0.2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




upper 20cm of intestine
SUCROSE CONTENT (MG)
20
15
10
5
0
30min
60min
120min
240min
MIN


Middle intestine
SUCROSE CONTENT (MG)
30
25
20
15
10
5
0
30min
60min
120min
240min
MIN


Lower 20cm of intestine
SUCROSE CONTENT(MG)
6
4
2
0
30min
60min
120min
240min
MIN



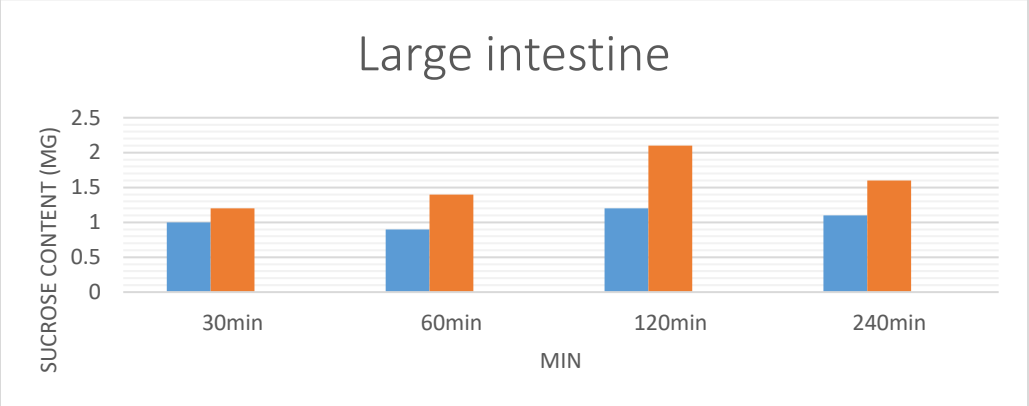




Figure 3.1: Effects of ethanol extract of *A. nilotica* on gastrointestinal sucrose content after oral sucrose loading in normal rats: Rats were fasted for 20 h before the oral administration of a sucrose solution (2·5 g/kg body weight) with (treated group) or without (control group) ethanol extract 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.

| **Table: 3.7** | | |
|---|---|---|
| Groups | **Disaccharidase activity (µmol/mg/h)** | **SEM** |
| Control | 1.6 | 0.2 |
| ***Acacia nilotica*** | 1.03 | 0.1 |
| Acarbose | 1.07 | 0.17 |

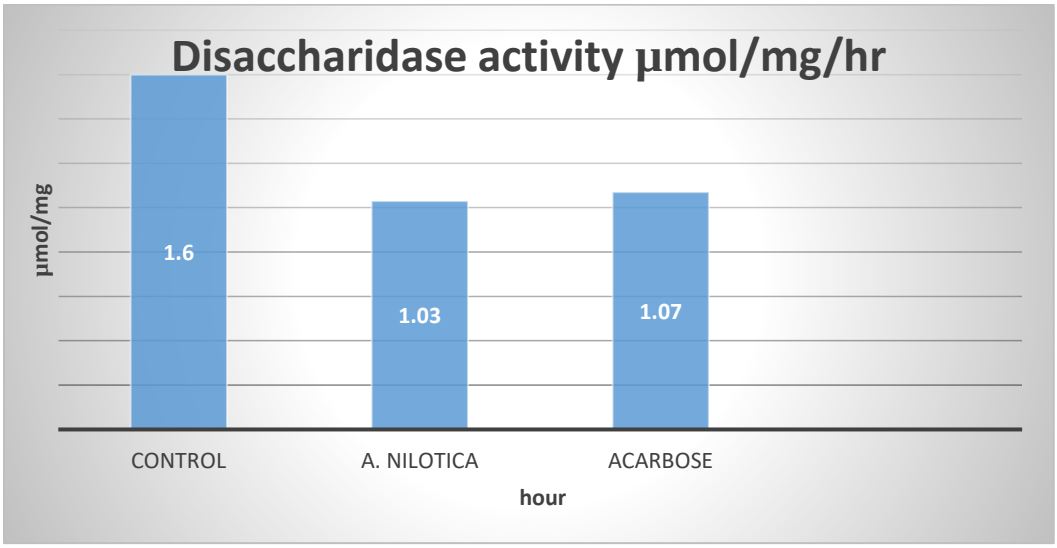



Figure 3.2: Effects of ethanol extract of *A. nilotica* on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of *A. nilotica* (100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 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

## 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.

## Conclusion

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

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