‘’Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Acacia nilotica* in Long Evans Rats’’

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

Submitted by

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Submitted To

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Declaration by the Candidate

I, Ilma Ahmed (ID: 2013-3-70-017), hereby declare that the dissertation entitled ‘’Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Acacia nilotica* 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 by us during the period 2016-2017 of my 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 ‘’Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Acacia nilotica* 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 Ilma Ahmed (ID: 2013-3-70-017) during the period 2016-2017 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Chairperson

This is to certify that the thesis entitled'' Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Acacia nilotica* 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 Ilma Ahmed (ID: 2013-3-70-017) during the period 2016-2017 of her research in the Department of Pharmacy, East West University

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Dedication

To

My Beloved
Parents & My Research
Supervisor

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Abstract

Diabetes is increasingly affecting a growing number of patients and seriously reducing their quality of life. Use of conventional drugs in diabetes management is expensive, thus, unaffordable to most patients. Furthermore most of these conventional drugs are associated with undesirable side effects. Incorporation of herbal medicine into conventional healthcare system may significantly improve the overall healthcare system. Evaluation of efficacy and safety by scientific method is necessary to validate herbal medicine utilization, in most cases even where efficacy of the plants has been established the standard dosage required to bring about healing is not clear. The intraperitoneal route of herbal extract administration was found to be more effective than the oral route. Our present studies were focused on the probable anti-hyperglycemic effect of ethanolic extract of *Acacia nilotica* in long-evans rats and the statistical significance of such effect. The leaf extract was subjected to anti-diabetic study through Inhibition of Carbohydrate Absorption (six segment method) and Intenstinal Disaccharide activity method. In six segment, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 100mg/kg extract at 30 minutes,1 h, 2h & 4 h. In Disaccharide 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 decreased ($p<0.05$) in dose dependent inhibition of glucose absorption and showed Anti-hyperglycemic effects in long-evans rats weighing from 150-200 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 leaves of *Acacia nilotica* claimed in Ayurveda medicine.

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

Chapter: 01

Introduction and Literature Review

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 affects on both the macrovascular and microvascular system. Diabetic complications associated with macrovascular diseases are atherosclerotic macrovascular disease and ischemic coronary heart disease. Diabetic complications related to microvascular disease include retinopathy, nephropathy, neuropathy, and peripheral vascular diseases (Perring et al 1985, Clements & Bill 1986, WHO 2002).

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).

1.1.1 Types of diabetes

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

1.1.1.1 Type 1 diabetes

Type 1 diabetes, defined by an absolute requirement for administration of exogenous insulin, results from the autoimmune destruction of the insulin-secreting pancreatic β cells. Type 1 diabetes is a severe form associated with ketosis in the untreated state. It arises most commonly in juveniles but occasionally in non-obese adults and elderly. It is a catabolic disorder in which circulating insulin is virtually absent with elevated level of plasma glucagon. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis and reduce the elevated blood glucose level. It is thought to result from an infectious or toxic environmental-induced autoimmune disorder (Karam 1998). Autoimmunity has been proposed to be the main reason for β cell destruction associated with type 1 diabetes (Eisenbarth 1986, Rossini et al 1993).

1.1.1.2 Type 2 diabetes

Type 2 or non-insulin-dependent diabetes mellitus is characterized (American Diabetes Association, 2001) by a relative insulin deficiency due to predominantly an insulin secretory defect with insulin resistance.Type 2 diabetes represents a heterogeneous group of disorders comprising milder forms of diabetes that occur predominantly in adults but occasionally in adolescents. Circulating exogenous insulin is sufficient to prevent ketoacidosis but is often either subnormal or relatively inadequate because of tissue insensitivity (Rodger 1991). Obesity, which generally results in an impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type 2 are obese. Genetic factors also underlie the disease (Karam 1998).

1.1.1.3 Difference between type 1 and type 2 diabetes

Table: 1.1 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.

(Leonid Barski, 2013)(Cold et al., 2017)

1.1.1.4 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.(American Diabetes Association 2001).

However, women who have had gestational diabetes are at greater risk of developing type 2 diabetes at a later stage in their lives (Landon & Gabbe 1988).

1.1.1.5 Other types of diabetes

Diabetes caused by other identifiable etiologies such as:

- Genetic defects of beta cell function (eg MODY 1, 2, 3)
- Genetic defects in insulin action
- Diseases of the exocrine pancreas (eg cancer of the pancreas, cystic fibrosis, pancreatitis)
- Endocrinopathies (eg Cushing's)
- Drug or chemical induced (eg steroids)
- Infection (eg rubella, Coxsackie, CMV)
- Uncommon forms of immune-related diabetes
- Other genetic syndromes.

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

1.1.2 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 is overweight. Physical inactivity, race, and certain health problems such as high blood pressure also affect your chance of developing type 2 diabetes. You are also more likely to develop type 2 diabetes if you have prediabetes or had gestational you were pregnant. Learn more about risk factors for type 2 diabetes (Nicholas J. Wareham 2014).

1.1.3 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 (Leonid ,2013) .

1.1.4 Symptoms of diabetes

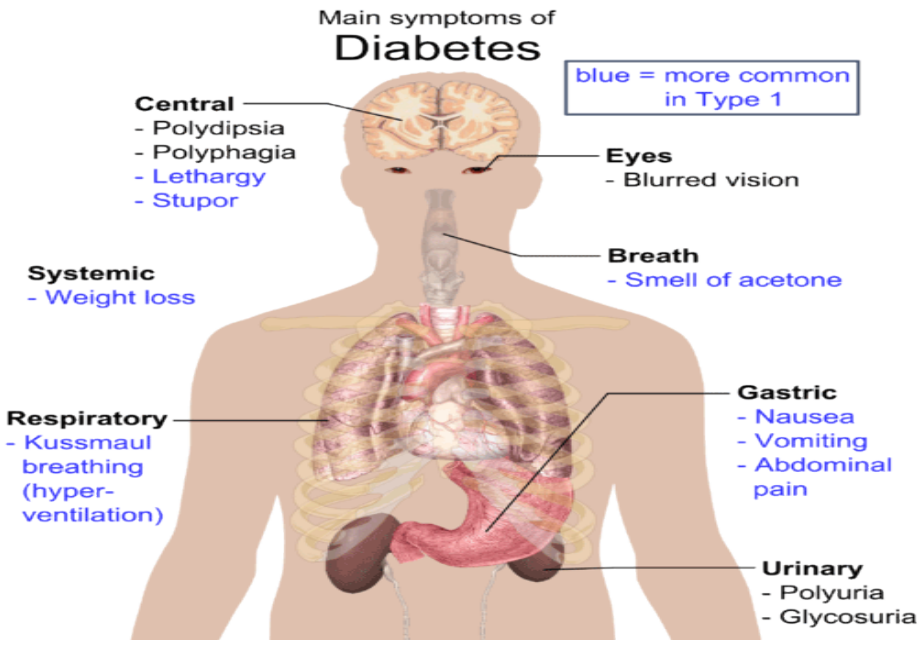

Figure: 1.1 Symptoms of diabetes - by Mikael Häggström

1.1.5 Complications linked to badly controlled diabetes

Diabetes is a complex heterogeneous disease where multiple levels of abnormalities are present in various tissues. Defects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The major long-term complications of diabetes mellitus are **macrovascular** diseases such as coronary and peripheral vascular diseases & **microvascular** diseases such as nephropathy, retinopathy and neuropathy (Donnelly et al 2000).

- **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. Stroke can occur.
- **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
- **Erectile dysfunction** - male impotence.
- **Infections** - people with badly controlled diabetes are much more susceptible to infections
- **Healing of wounds** - cuts and lesions take much longer to heal (Donnelly et al 2000) (Pessin & Saltiel 2000).

1.1.6 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 (Donnelly et al 2000).

1.1.7 Physiology of insulin secretion and action

Insulin is the most potent anabolic hormone promoting the synthesis and storage of carbohydrates, lipids and proteins, and inhibiting their degradation and release back into the circulation. Insulin regulates glucose homeostasis by inhibiting gluconeogenesis and the breakdown of glycogen in the liver and by stimulating glucose uptake, utilization and storage in insulin-sensitive tissues, such as adipose tissue, skeletal muscle and cardiac muscle. In muscle and liver, insulin increases glycogen synthesis (Pessin & Saltiel 2000).

1.1.7.1 Mechanism of insulin secretion

Insulin secretion occurs by the process of exocytosis in which the granule membrane fuses with the cell membrane, the membranes are disrupted at the point of fusion, and insulin crystals are discharged to the extracellular space. The process of exocytosis is the rate-limiting step for the physiologic insulin secretion. In this mechanism, cytoplasmic free calcium concentration and two second messenger systems, the cyclic-AMP and phosphoinositide systems are critically important for controlling the secretory steps and for setting the sensitivity of the release sites to the prevailing free calcium level (Daniel & Gerald 1997). The levels of the second messengers are tightly regulated by various secretagogues, such as glucose, other nutrients, hormones, and neurotrasmitters (McClenaghan & Flatt 1999b, Rutter 2001). Such stimulators can be further divided into two categories including initiators and potentiators. The fuel hypothesis has been proposed and is the generally accepted model of glucose induced insulin secretion (Trus et al 1981, Ashcroft & Ashcroft 1992). It is based on the following observations. Firstly, glucose induced insulin secretion is tightly related to glucose utilization and oxidation and blocking glucose phosphorylation or glycolysis abolishes insulin secretion (Sweet et al 1996).

In addition, non-metabolizable sugars, such as 3-O-methylglucose, galactose, and fructose characteristically do not induce insulin secretion whereas metabolizable nutrients such as the amino acid, leucine are potent stimulators of insulin secretion (McClenaghan et al 1996b, McClenaghan et al 1996c, Lindskog et al 1998). As such, fuel metabolism plays a fundamental role in the initiation of insulin secretion. In contrast, the potent insulinotropic actions of other agents, including incretin hormones, require the presence of fuel secretagogues to mediate their actions and are referred to as potentiators of insulin secretion. The potentiation of insulin secretion by these agents is usually mediated by second messengers, such as cAMP, via binding and regulation of specific G protein-coupled receptor pathways.

1.1.7.1.1 ATP-sensitive K+ channels (K_{ATP} channels) – membrane depolarization – voltage dependent calcium channel (VDCC) pathway

Glucose is the main stimulator of insulin secretion and utilizes this pathway. Glucose (>5 mM) is transported into pancreatic □ cells (via GLUT2) and metabolized through glycolysis and Krebs cycle inside the mitochondria (Katagiri et al 1994). This process leads to the

elevation of the intracellular ATP. The increase of intracellular ATP, results in the increase of ATP/ADP ratio, causes closure of K_{ATP} channels and inhibits the efflux of potassium ions (Deeney et al 2000). Under basal glucose levels (0 – 3 mM), the membrane potential of pancreatic cells is about –60 to –70 mV (Ashcroft et al 1992). However, with membrane depolarization via the closure of K_{ATP} channels, the resting cell membrane will be depolarized (raising to 0 mV from –70 mV) and results in the opening of the voltage-dependent calcium channels (VDCC). The intracellular Ca^{2+} concentration is increased by the influx of calcium via VDCC. Finally, the mobilization of secretory granules will be triggered and insulin will be discharged by exocytosis (Rotig et al 1996, Rutter 2001).

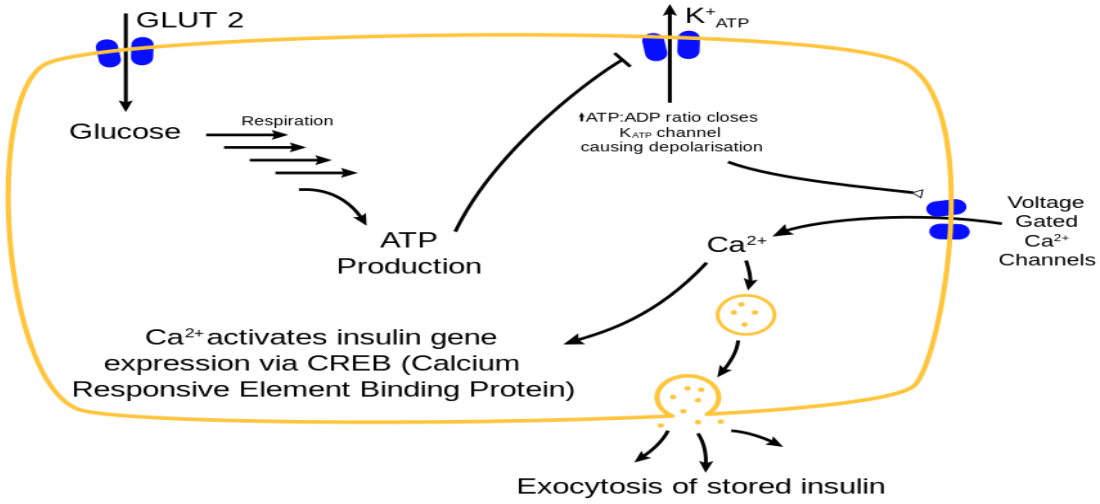

Figure 1.2: Mechanism of Insulin Secretion

Activation of certain key components of this pathway can trigger secretion. Firstly, amino acids, such as leucine, and keto acids, can generate intracellular ATP via metabolism resulting in a rise of the ATP/ADP ratio (Meglasson et al 1986). In this way these agents stimulate insulin secretion utilizing essentially the same pathway as glucose. In addition, the oral hypoglycemic agents, such as the sulphonylureas, tolbutamide and glibenclamide, can trigger insulin secretion by closure of K_{ATP} channels as a consequence of binding to the sulphonylurea binding subunit (SUR1) (Ashcroft et al 1992). Moreover, membrane depolarization agents, such as KCl and arginine, have been shown to increase intracellular calcium via opening VDCCs (Herchulz et al 1984, Hermans et al 1987). On the other hand, alanine depolarizes the cell membrane by co-transportation with Na^+ which depolarizes the cells and thereby increases intracellular calcium via activation of VDCCs (Yada 1994).

1.1.7.1.2 K_{ATP} channel independent pathway (amplification pathway)

Glucose can stimulate insulin secretion in pancreatic cells under conditions where K_{ATP} channels are fully opened by KCl and diazoxide (Henquin 2000). Interestingly, a significantly reduced first phase but maintained second phase of glucose induced insulin secretion was observed in SUR knockout mice .These observations suggest that glucose stimulated insulin secretion is not only via K_{ATP} channel–VDCC pathway but also by K_{ATP} channel-independent pathways (Seghers et al 2000).

1.1.7.1.3 Potentiation of insulin secretion via regulation of second messengers

(i) cAMP – Protein kinase A pathway:

Cyclic AMP augments glucose-induced insulin secretion through a number of mechanisms including increased opening of voltage-sensitive Ca^{2+} channels (Kanno et al 1998), calcium-induced Ca^{2+}-release (Kang & Holz 2003), activation of ryanodine receptors in the ER (Islam et al 1998, Holz et al 19990, stimulation of cell lipolysis (Yaney et al 2001) and direct effects on exocytosis (Harndahl et al 2002, Hedskov 1980, Wollheim & Sharp 1981, Weidenkeller & Sharp 1983, Supattapone et al 1988, Sculptoreanu et al 1993). Most actions of cyclic AMP in the cell seem to be mediated through protein kinase A (PKA)-catalysed phosphorylation events but direct effects of the cyclic nucleotide on exocytosis are partly PKA-independent (Renstrom et al 1997). PKA-independent effects on exocytosis can be mediated by the cyclic AMP-binding protein cAMP-GEFII, interacting with Rim2, a target of the small G-protein Rab3 (Kashima et al 2001).

Furthermore, incretin hormones, such as glucagons-like peptide 1 (GLP-1) and gastric–inhibitory-polypeptide (GIP), can enhance glucose-induced insulin secretion by binding to their own specific stimulatory G protein coupled receptors, thereby increasing intracellular cAMP by activation of adenylate cyclase (Hedeskov 1980, Wolheim & Sharp 1981). An increase in intracellular cAMP by activation of adenylate cyclase with forskolin has been shown to enhance glucose induced biphasic insulin secretion . Although it has been accepted that cAMP regulates insulin exocytosis due to protein phosphorylation; nonetheless, cAMP-dependent pathways still remained to be fully characterized (Weidenkeller et al 1983).

Cyclic AMP is hydrolysed to its biologically inactive 5′ derivative by cyclic nucleotide phosphodiesterases (PDE1-PDE11) enzymes. Selective inhibition of phosphodiesterases (PDEs) augments insulin secretion by increasing cyclic AMP. Thus PDEs offer a target for developing drugs for the treatment of type 2 diabetes mellitus (Pyne & Furman 2003). IBMX, an inhibitor of cyclic AMP phosphodiesterase, has been shown to augment glucose-induced insulin secretion via increased levels of intracellular cAMP (Sharp 1979). Several selective PDE3 inhibitors (Org 9935, siguazodan, SK&F 94120, ICI118233) augmented glucose-induced insulin secretion from rat and human islets (Shafiee-Nick et al 1995). Org 9935 and siguazodan augmented insulin secretion in the insulin-secreting cell line BRIN-BD11 (Ahmad et al 2000). A novel piperazine hypoglycaemic agent was shown to inhibit PDE3 and PDE4 in islets and augmented insulin secretion (Leibowitz et al 1995).

(ii) Phospholipase C- protein kinase C pathway:

Phospholipase C (PLC) is a key component of activation of the calcium-calmodulin and protein kinase C system (Niwa et al 1998). This activation is via hydrolysis of $PtdInsP_2$ into $InsP_3$ and diacylglycerol (DAG). As a result, IP_3 increases intracellular calcium via mobilazation of intracellular calcium stores in ER or microsomes (McClenaghan & Flatt 1999b). Elevation of intracellular calcium is directly associated with insulin exocytosis and along with DAG activates PKC which has been suggested to contribute in K_{ATP} channel independent pathways for insulin release (McClenaghan & Flatt 1999b). Neurotransmitters, such as acetylcholine, and the gastrointestinal hormone, cholecystokinin-8 (CCK-8), enhance glucose induced insulin secretion by activation of the PLC-PKC pathway following binding to specific muscarinic and CCK-8 receptors, respectively (Karlsson & Ahren 1991, Tang et al 1995). Direct activation of PKC with the phorbol ester, phorbol 12-myristate 13 acetate (PMA) stimulates insulin secretion (Wolf et al 1989). However, down-regulation of PKC activity by chronic culture with phorbol esters has little effect on glucose-stimulated insulin secretion (Hii et al 1988).

1.1.7.2 Mechanism of insulin action

Insulin binds to specific, high-affinity receptors in the cell membrane of most tissues, including liver, muscle, and adipose. This is the first step in a cascade of reactions ultimately leading to a diverse array of biologic actions **(**Champe & Harvey 1994**)**.

1.1.7.2.1 Insulin receptor

The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into α and β subunits, which are then assembled into a tetramer linked by disulfide bonds. A hydrophobic domain in each β subunit spans the plasma membrane. The extracellular α subunit contains the insulin-binding site. The cytosolic domain of the β subunit is a tyrosine kinase, which is activated by insulin (Champe & Harvey 1994).

1.1.7.2.2 Insulin receptor substrates

The insulin receptor belongs to a subfamily of tyrosine kinases that includes the insulin-like growth factor (IGF)-I receptor and the insulin receptor-related receptor (IRR). These receptors are tetrameric proteins consisting of two α- and two β-glycoprotein subunits (Saltiel & Kahn 2001). Primary substrates of the insulin receptor include the four proteins, insulin receptor substrate (IRS)-1, -2, -3 and -4. The participation of IRS proteins in mediating intracellular signals from the insulin receptor is well documented (Cheatham 2000).

1.1.7.2.3 Signal transduction

The binding of insulin to the α subunits of the insulin receptor induces conformational changes that are transduced to the β subunits, promoting a rapid autophosphorylation of specific tyrosine residue of each β subunit (Champe & Harvey 1994).

The signaling mechanism involved in the various biologic responses to insulin remains somewhat elusive, but recent progress has shed light on a few pathways that are critical for its regulation of glucose and lipid metabolism (Pessin & Saltiel 2000). The action of insulin is characterized by a diverse variety of effects, including changes in vesicle trafficking, stimulation of protein kinases and phosphatases, promotion of cellular growth and differentiation, and activation, or in some cases, repression of transcription. The diverse mechanisms involve multiple signaling pathways that diverge at or near the receptor (Christian et al 2001). It has also been documented that both phosphoinositide (PI) 3-kinase-independent and -dependent signaling pathways are a necessary component of insulin-stimulated GLUT4 translocation (Christian et al 2001). Insulin-stimulated activation of PI 3-kinase is a crucial step linking signaling of GLUT4 translocation (Cheatham & Kahn 1995).

1.1.8 Effects of insulin on glucose uptake

Insulin stimulates glucose uptake in muscle and adipose tissue by translocating intracellular glucose transporter protein-4 (GLUT4) units to the plasma membrane. Basal glucose uptake is mediated primarily by GLUT1 and GLUT3. Any increase in the plasma glucose levels will enhance glucose uptake into peripheral tissues by these transporters (Kruszynska 2003).

1.1.9 Glucose transport and GLUT4

Glucose, being hydrophilic, cannot diffuse across the cell membrane. Entry of glucose into tissues from the bloodstream is by a family of facilitative GLUTs, which catalyze (in an energy-independent process) the transport of glucose down its concentration gradient. Seven functional GLUT isoforms (GLUT14 and GLUT8-10) have so far been identified; GLUT5 is a fructose transporter (Kruszynska 2003). However GLUT4 is the only major insulin regulator glucose transporter and its expression is limited to insulin-responsive tissues, namely adipose tissue, skeletal muscle and cardiac muscle. Unlike most of the other GLUTs, which are primarily localized to the cell surface membrane, GLUT4 sequestered in specialized vesicles are predominantly located in the cytosol under basal conditions.

Insulin stimulates glucose transport in muscle and adipocytes primarily by causing the translocation of vesicles containing GLUT4 to the plasma membrane. They function as pores allowing glucose entry (Kruszynska 2003). This process is reversible when circulating insulin levels fall, GLUT4 proteins are removed from the plasma membrane by endocytosis and are recycled back to their vesicular storage compartment. In the long-term, insulin plays a role in maintaining normal levels of the GLUT4 protein in muscle and fat (Kruszynska 2003). However, the exact mechanisms of these processes are unknown. The docking and fusion of the GLUT4 vesicle at the plasma membrane may be subjected to regulation by insulin (Saltiel & Kahn 2001). Furthermore, the GLUT4 compartment is enriched in v-SNARE protein VAMP2 (Christian et al 2001). Again the plasma membrane target for the GLUT4 vesicle is the t-SNARE, syntaxin 4 (Syn4) (Christian et al 2001). The v-SNARE protein VAMP2 physically interacts with its t-SNARE counterpart in the plasma membrane during GLUT4 vesicles docking and fusion (Saltiel & Kahn 2001). Several lines of evidence have suggested that insulin specifically stimulates the translocation of the GLUT4 from VAMP2-containing compartments (Pessin & Saltiel 2000).

The intravenous administration of insulin thus causes an immediate decrease in the concentration of blood glucose (Champe & Harvey 1994). The β-cells specialization for regulating blood glucose levels in the normal range (roughly 90 mg/dl or 5 mM).

1.1.10 Current therapies for diabetes mellitus

Since diabetes conditions encompass a multiplicity of endocrine and metabolic disturbance, it is necessary to consider a wide range of pharmacological approaches to manage these. These may be required individually or in combinations to treat different features of the disease process. Ideal treatments will target the fundamental causes of insulin resistance, defective beta cell function, and loss of cell mass, and reinstate near-normal glucose homeostasis (Bailey & Flatt 1995). Currently glycaemic control is achieved by dietary manipulation, oral hypoglycamics agents (for example sulphonylurase or biguanides) or insulin injections. Approximately 75% of diabetic patients in UK achieve glycaemic control without exogenous insulin treatment (Campbell 1990).

1.1.10.1 Diet

The regulation of food intake is central to the treatment of diabetes mellitus and various dietary regimes have been considered to assist in the control of hyperglycemia. The control of diet should be the first treatment offered to type 2 patients before drugs are considered. The main goal of nutritional management is to correct obesity as weight loss will improve glucose control (Savage et al 1979, Knowler et al 1991, Ohneda et al 1995), lower blood pressure and lipid concentration, all of which may help in preventing or diminishing long term complications (Henry & Griver 1998). Various dietary regimes have been considered to assist in the control of hyperglycemia. However, in most cases the dietary recommendations for type 2 diabetic patients are identical to those for the general population (British Diabetic Association 1981). Calorie restriction in the overweight and obese, with the emphasis on low-fat, high-carbohydrate and high-fibre is recommended (Simpson et al 1979b).

1.1.10.2 Insulin as drug

The discovery of insulin by Banting, Best and co-workers in 1922 dramatically improved the prospects of individuals with diabetes mellitus. As type 1 is characterized by insulin insufficiency caused by partial or total destruction of insulin releasing pancreatic beta cells (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. 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 (Galloway & Chance 1994, Skyler 1998).

1.1.10.3 Ant-diabetic drugs

Those patients who fail to achieve glycemic control through dietary intervention measures require oral hypoglycemic agents. Approximately 50% of type 2 patients in the UK are treated with oral hypoglycemic agents (Campbell 1990). Although there are new oral hypoglycemic agents on the horizon, the choice at the present is primarily between sulphonylureas and biguanide (metformin).

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Sulphonylureas, developed after initial observations of sulphonamide in patients with typhoid fever (Janbon et al 1942), have been the foundation of antidiabetic therapy for many years. The various sulphonylureas differ in potency, pharmacokinetic properties and side effects (Ferner & Chaplin 1987, Lebovitz 1990). The sulphonyurea drugs have direct and immediate stimulating effects on the □ cell (Pfiefer et al 1984, Gorus et al 1988, Panten 1989, Henquin

1990) mediated via the inhibition of K_{ATP} channels in the cell (Henquin 1988, Henquin 1990). The potentiation of the stimulatory effect of the amino acids alanine and leucine by sulphonylureas through enhanced cell recognition has been documented (Fajans 1967). Some authors claim an extrapancreatic action for sulphonylureas on the insulin receptor (Beck-Neilson et al 1984) and at the post-receptor level (Mandarino & Gerich 1984) which require the presence of endogenous insulin. Recently promotion of insulin exocytosis was demonstrated and was shown to be partly independent of K_{ATP} channels and dependent on protein kinase C (Eliasson 1996).

Repaglinide has recently been introduced in the US. The reports of trials in patients with type 2 diabetes have demonstrated that it promptly increase insulin concentrations and reduce postprandial hyperglycemia without causing interprandial glucose concentration to fall bellow the normal range (Graul & Castener 1996).

Metformin, the major biguanide in clinical use, was used before the characteristic insulin resistance was discovered. In contrast to sulphonylurea drug, metformin enhances the extrapancreatic actions of insulin in insulin resistance and hyperglycemic status but has no effect on glycemia of type 1 diabetic individuals. Metformin does not change insulin-receptor binding (Bailey 1988) or alter phosphorylation and kinase activity of insulin receptors after insulin-mediated glucose uptake *in vitro* with metformin indicating a post-receptor site of action (Jacobs et al 1986). In addition to insulin-mediated glucose disposal, metformin and related biguanides decrease hepatic glucose output and increase glucose utilization by the small intestine. Some of these effects are independent of insulin but in patients devoid of insulin these drugs are ineffective. The glucose-lowering efficacy of sulphonylureas and metformin in type 2 diabetes are reviewed elsewhere (Bailey & Nattrass 1988, Bailey & Day 1989, Henquin 1990, Lebovitz 1990, Bailey 1991).

Troglitazone, rosiglitazone and pioglitazone (thiazolidinediones derivative), are more recently discovered antidiabetic drugs that improve action of insulin through different cellular mechanisms (Cusi & DeFronzo 1998, Saleh et al 1999).

Acarbose is a glucosidase enzyme inhibitor, is a new class of antidiabetic drug that reduces postprandial peak of glucose level, by inhibiting the breakdown of oligosaccharides and disaccharides in the proximal half of the small intestine so that they must be digested throughout the length of the small intestine (Puls 1996, Puls 1980, Caspary 1978).

There are also many other promising agents, such as gluconeogenesis inhibitors, amylin, glucagon-like-peptide 1 (GLP-1) and analogues (Druker 2001), gastric inhibitory polypeptide (GIP) and analogues (Gault et al 2003, Meier et al 2002), DPP IV inhibitors (Scharpe & De-Meester 2001), and insulin mimic agents (Bailey & Flatt 1995), considered as potential drugs for the future treatment of diabetes.

1.1.10.4 The need for new treatments for diabetes mellitus

The management of diabetes mellitus is on the threshold of a revolution. Approach as to the control of blood glucose and prevention of hyperglycemia are central to the treatment of diabetes mellitus .At present none of these therapies either alone or in combination can reinstate normal blood glucose homeostasis or eliminate long-term complications and many limitation exist in the use of antidiabetic drugs. In type 1 diabetes a more physiological means of insulin delivery is required. Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration, and in the event of excess dosage – fatal hypoglycemia – limits its usage (Rang et al 1991). Currently available sulfonylureas, the most commonly used pharmacologic agents in treatment of type 2 diabetes; have gradually increasing secondary failure rates reaching 50% at the end of 5 y of disease, though the initial response is good in 70-75% of patients. The biguanides are mainly used as adjuvants to sulfonylureas. The gastrointestinal intolerance limits their use in many patients. Thus, large number of patients with type 2 diabetes fails to achieve persistent good metabolic control (American Diabetes Association 1995). New therapies are needed which reinstate a normal metabolic environment and prevent long-term complications. The development of new antidiabetic drugs, which address the underlying metabolic lesions in type 2 diabetes, ideally requires new pharmacological treatments, which stimulate both the secretion and action of insulin (Bailey & Flatt 1995).Drug research conducted over the past three decades shows that natural products are a potential source of novel molecules for drug development (Farnsworth 1990, Farnsworth 1994). Much evidence has been published indicating the potential use of plants in the treatment of type 2 diabetes (Oliver-Bever & Zahnd 1979, Bailey & Day 1989).

1.2 Traditional plants for diabetes treatment

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. 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.

1.2.1 Medicinal Plants with reported Anti diabetic Effect

Table 1.2 Medicinal Plants with reported Anti diabetic Effect

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
Dactyl lifera 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

(Gilani et al., 1999; Asres et al., 2005; Shah et al., 1997; Singh et al., 2009)

1.3 Acacia nilotica

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, anti-bacterial, antihypertensive 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 (Steve, 2004) (Shittu, 2010).

It is also known as *Vachellia nilotica*. Common name is Babla or Babula which 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.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 (Singh et al., 2009b) (Kaur et al., 2005).

1.3.1 Plant Morphological Description

Acacia nilotica 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). *Acacia 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).

Figure 1.3: ***Acacia nilotica***

1.3.2 Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Mimosoideae

Genus: Acacia

Species: *Acacia nilotica*

1.3.3 Some common medicinal uses of different parts of *Acacia nilotica.*

Table 1.3 Medicinal uses of different parts of *Acacia nilotica*

Parts Used	**Uses**
Root	The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen.
Leaf	Chemoprventive, 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, acrid cooling, 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.
Pods	Anti hypertensive and antispasmodic, anti-diarrhoerial, astringent,anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cythopathogenicity, antiplatelet aggregatory activity and anti oxidant.

(Kalaivani and Mathew, 2010) (Baravkar et al., 2008)

1.3.4 Ethno medicinal uses of *Acacia nilotica*

Acacia nilotica is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder. Babul plant is therapeutic used as anti-cancer, anti tumours, antiscorbutic, astringent, antioxidant, natriuretic, antispasmodial, diuretic, intestinal pains and diarrhea, nerve stimulant, cold, congestion, coughs, dysenter, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis. A. nilotica is the most important tree and almost all its parts are used in medicine, including leaves, bark, root, flower, pods, gum, etc. (Kalaivani and Mathew, 2010).

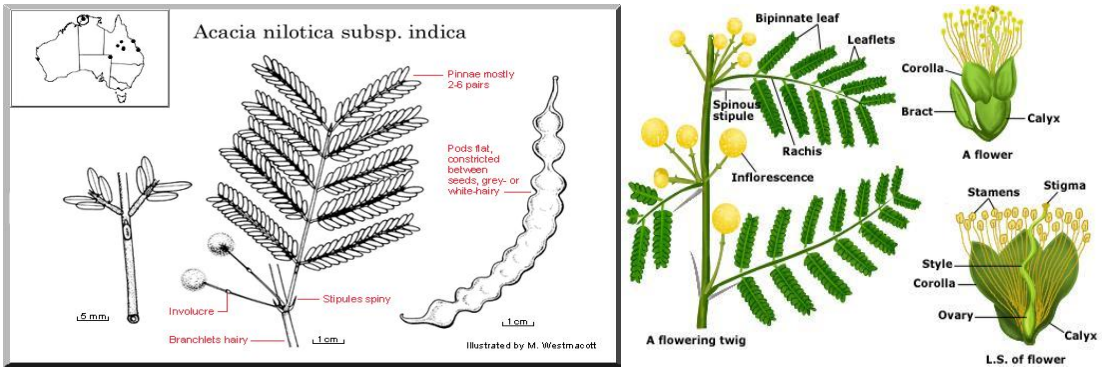

Fig 1.4: S**ome parts of *Acacia nilotica***

1.3.5 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 (Del, 2009) (Singh et al., 2008a).

1.3.6 Pharmacological and biological studies

Wadood *et al*. demonstrated that *Acacia nilotica 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 (Agrawal et al., 2010).

1.3.7 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)

1.3.8 Medicinal uses & pharmacological effects

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 (Steve, 2004).

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 (Kalaivani and Mathew, 2010a).

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

1.3.8.1 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)

1.3.8.2 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).

1.3.8.3 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).

1.3.8.4 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).

1.3.8.5 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).

1.3.8.6 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).

1.3.8.7 Antidiarrhoeal activity

This study was carried out on perfused isolated rabbit jejunum and castor oil-induced diarrhoea in mice. The aqueous methanol extracts (0.5, 1.0, 2.0 and 3.0 mg/ml) were generally found to cause a dose-dependent response in the isolated rabbit jejunum, though this was not uniform in all the plants. *Gmelina arborea* and *Vitex doniana* showed concentration dependent relaxation at low doses (0.5, 1.0 mg/ml), but showed no significant relaxation at higher doses (2.0, 3.0 mg/ml). Other extracts showed biphasic effects. For example, *Acacia nilotica* at 3.0 mg/ml caused initial relaxation quickly followed by contraction. In the castor oil-induced diarrhoeal, 100% protections were shown by extracts of *Acacia nilotica* and *Parkia biglobosa* (100, 200 mg/kg) while *Vitex doniana* showed a dose-dependent effect. (Kalaivani and Mathew, 2010a).

1.3.8.8 Chemo preventive, 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).

1.3.8.9 Antiviral activity

The crude extract of the leaves of the plant showed *in vitro* antiviral activity against the *Turnip mosaic* virus. There was a decrease in lesions numbers on the hosts *Chenopodium amaranticolor* (93.77 %) and *C. album* (80.2 %). There was also decrease in lesions when the extract was on the host leaves. The bark extract inhibited the potato virus (Jigam, 2010).

1.3.8.10 Nematicidal activity

The aqueous leaf extract of the plant as also of *Acacia nilotica* showed nematicidal activity against *Meloidogyne incogenita* as it inhibited its hatching.

1.3.8.11 Abortifacient activity

Aqueous or 90 % ethanol extracts of the plants of interest were studied in rats orally dosed for 10 days after insemination with special reference to effects on foetal development. Leaf extracts of *Moringa oleifern* and *Adhatoda vasica* were 100% abortive at doses equivalent to 175 mg/kg of starting dry material. Only the flowers of *Acacia arabica* and *Hibiscus rosa-sinensis* appeared to lack teratologic potential at the doses tested (Kalaivani and Mathew, 2010).

1.3.9 Other multiplicities

The extract of *Acacia 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).

Acacia nilotica are used for tanning, dyeing of leather, for gastrointestinal disorders, syphilitic ulcers and toothache (Amos et al., 1999).

Acacia nilotica pods have reported inhibited HIV-1 induced cythopathogenicity (Asres et al., 2005).

resh roots extract used as narcotic, known as Desi sharab (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).

1.3.10 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 *Acacia nilotica* (Singh et al., 2009b).

Chapter: 02

Objective of the Study

2.1 Research Objective:

The objective of this research work was therefore focused on the following point:

- To evaluate the anti-hyperglycemic effect of the ethanolic extract of the leaves of plant *Acacia nilotica* in long evans rats.

- To determine the anti diabetic efficacy of the plant *Acacia nilotica*.

Chapter: 03

Methods and Materials

3.1 Extraction techniques of medicinal plants

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum.

The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modern drugs. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

3.1.1 Plant material collection

Plant leaf sample of **Acacia *nilotica*** was used for the experiment which was processed in the laboratory. The Plant leaves were collected and washed with water several times.

3.1.2 Drying and grinding

The collected plant leaves 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.

3.1.3 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.

3.1.4 Extraction procedure

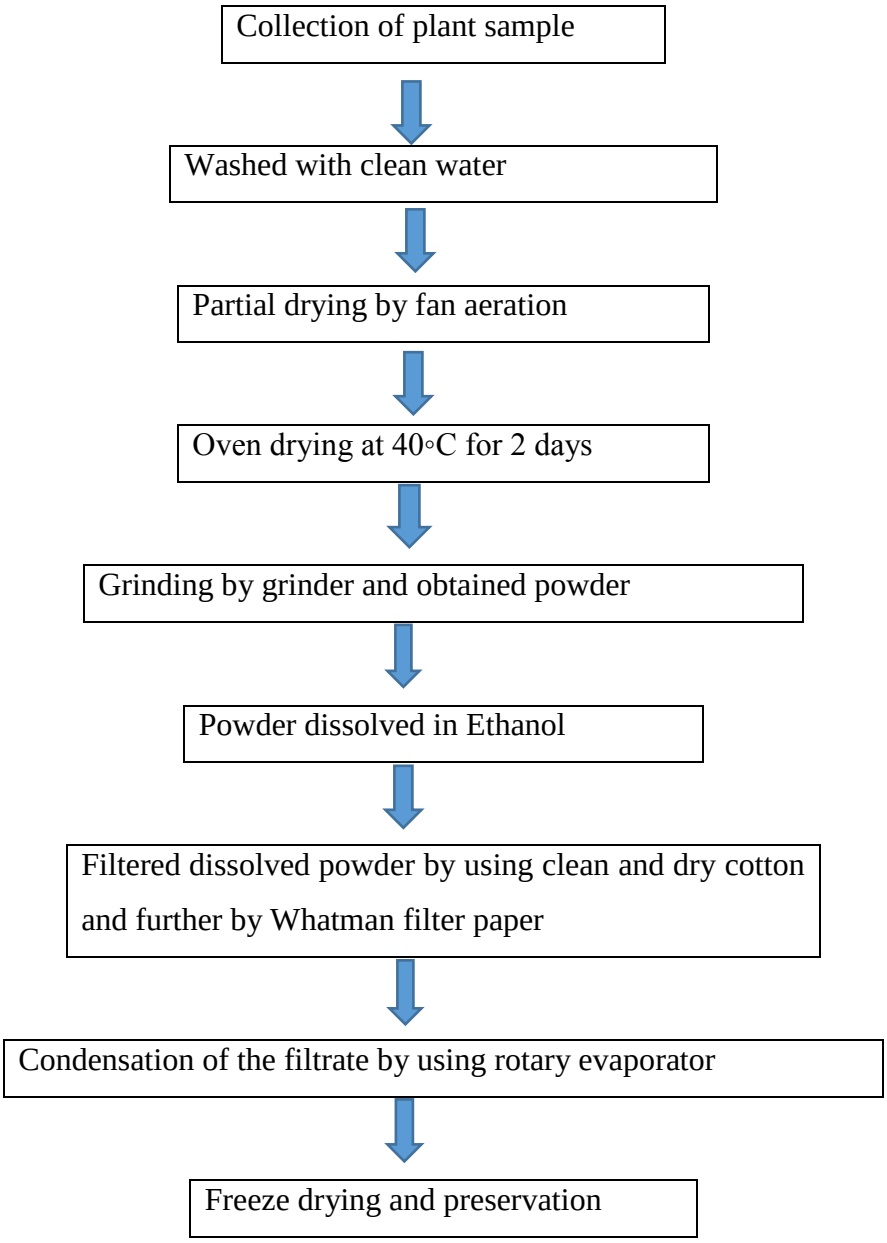

Figure 3.1: General Plant Extraction Procedure

3.2 Experimental animals

Long Evans rats (male and female), weighing 150-200g of either sex are bred in ICDDRB 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 ICDDRB and had free access to filtered water .The overall nutrient composition of the diet was 36.2% carbohydrate, 20.9% protein, 4.4% fat and 38.5% fiber with metabolisable energy content of 1.18 MJ/100 gm (282Kcal/100 gm). The animals were maintained in the laboratory and the treatment was scheduled.

Animal described as fasted were deprived of food for at least 12hr but allowed free access to drinking water.

Figure 3.2: Long Evans Rats

3.2.1 Description of our model

This model was developed by Dr Long and Dr Evans in 1915.The long evans rat is the result of a cross between a female albino from the WISTAR Institute and a wild male (*Rattus norvegicus*) captured near Berkeley and offspring selection.

The long evans rat is small and resistant to oncogenesis. This strain is widely used in behavioral, learning, ageing (visual acuity less affected than that of albino strains), addiction – especially to alcohol – studies (M. D. Mordechai Hallak ,2002).

3.2.2 Biomedical research

Rats have 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 3.3: Rat handling

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. (Thomas H. J. Burne, 2014

3.3 Screening for the Possible Inhibition of Carbohydrate Cbsorption by Plant Material

3.3.1 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.

3.3.2 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.

3.3.3 Steps in six segments

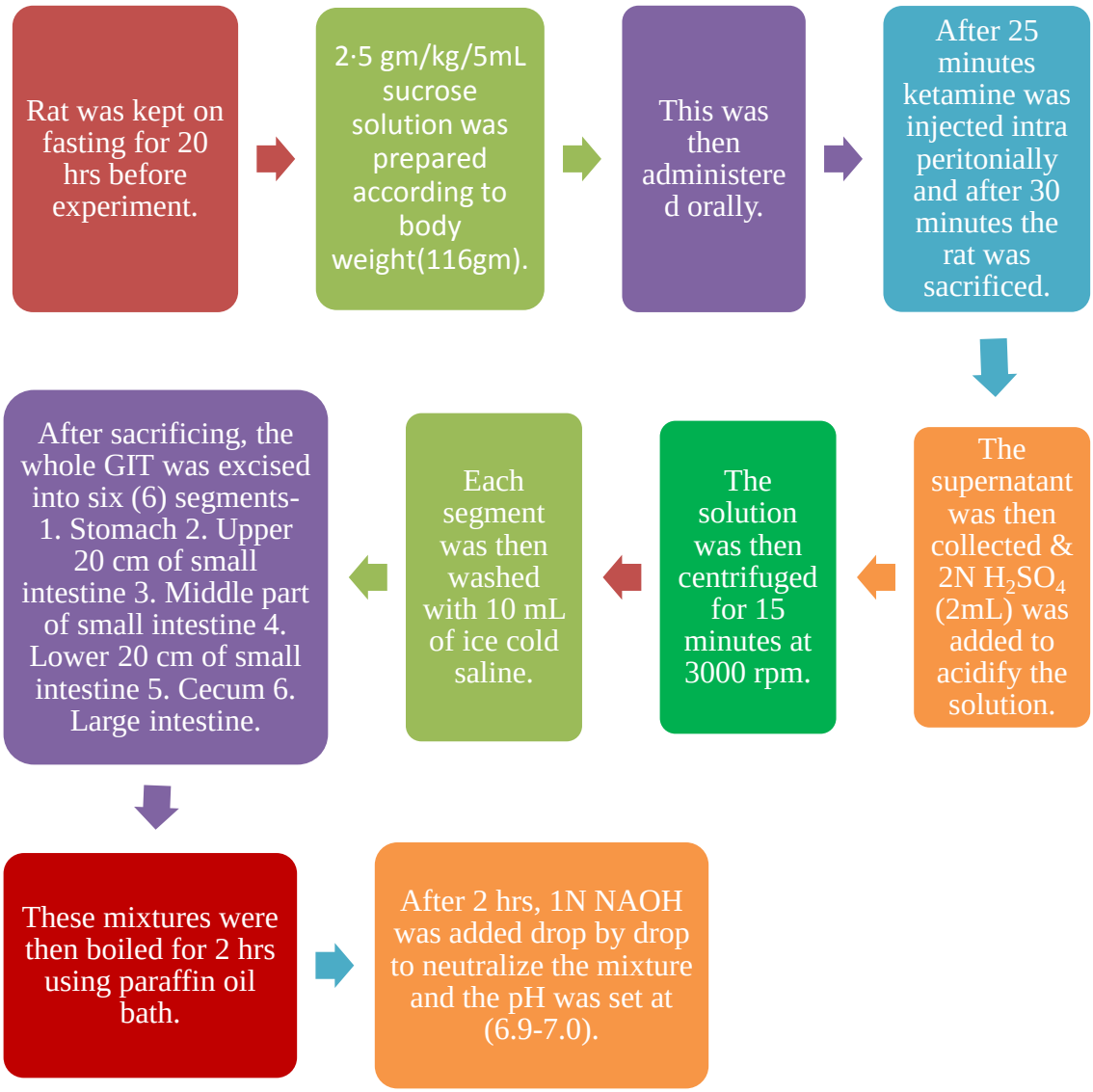

Figure 3.4: Steps in Six Segments

3.4 Assessment of the Effect of Plant Materials on Intestinal Disaccharidase Activity (Enzyme activity).

3.4.1 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.

3.4.2 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.

3.4.3 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.

3.4.4 Steps in Enzyme Activity

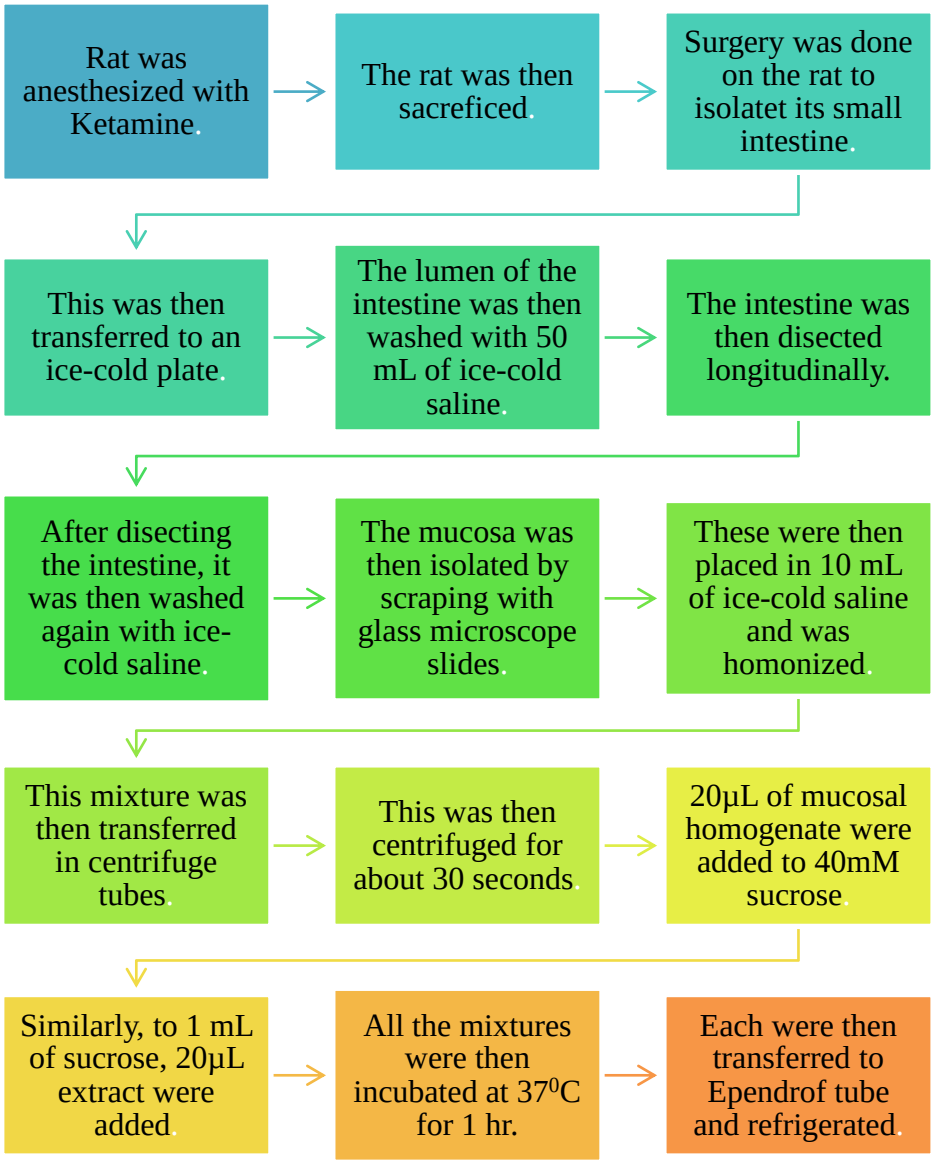

Figure 3.5: Steps in Enzyme Activity

Chapter: 04

Result

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

Upon oral administration of sucrose along with ***Acacia 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 4.1. - Table 4.6, Figure 4.1-4.6)

In Figures 4.1 – 4.6, blue color graph indicates control groups & red color graph indicates *Acacia nilotica* (drug) group.

Table:4.1 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

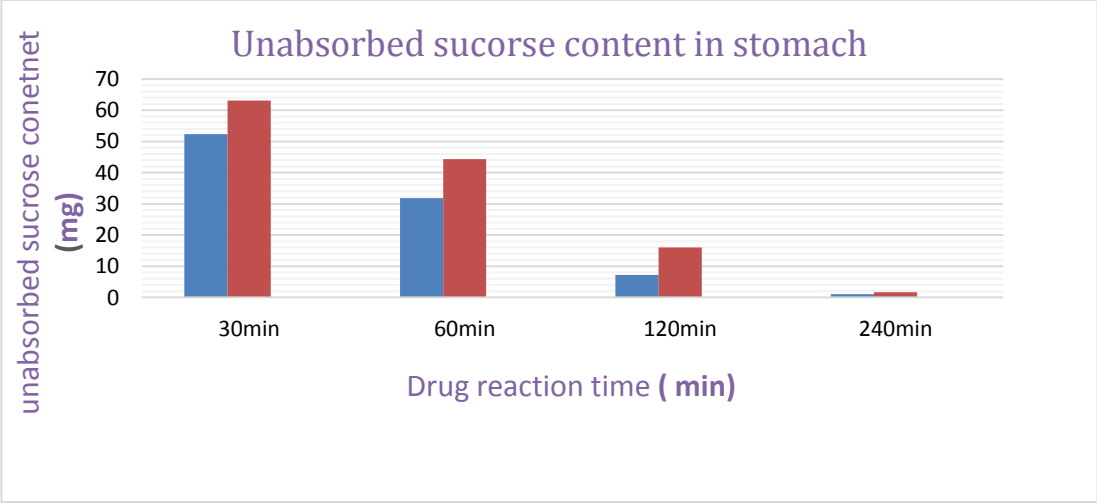

Figure 4.1: Anti Hyperglycemic Effect of *Acacia nilotica* in Stomach

Table: 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

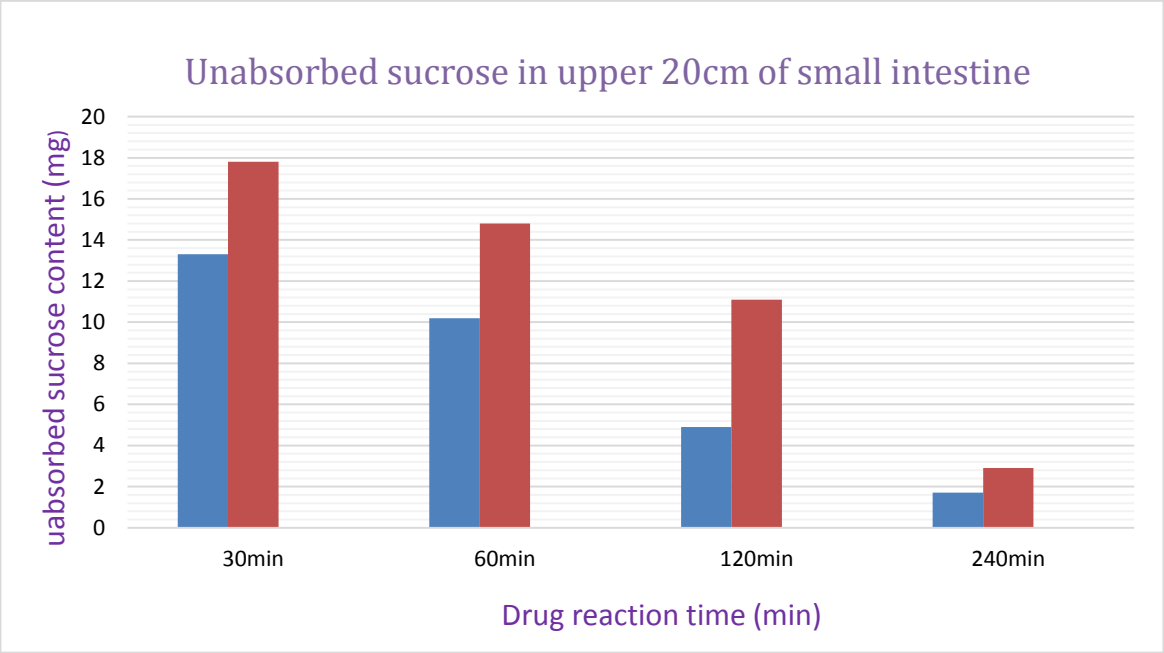

Figure 4.2: Anti Hyperglycemic Effect of *Acacia nilotica* in upper 20 cm of small intestine.

Table: 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

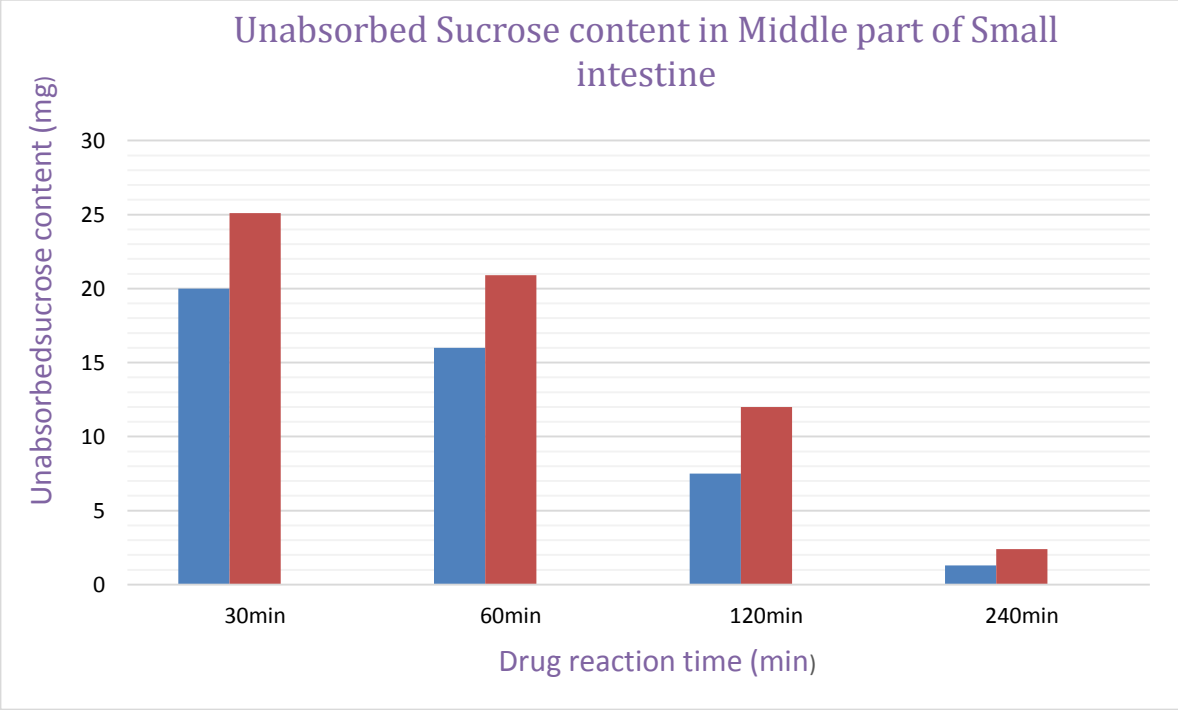

Figure 4.3: Anti Hyperglycemic Effect of *Acacia nilotica* in middle part of small intestine.

Table: 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**

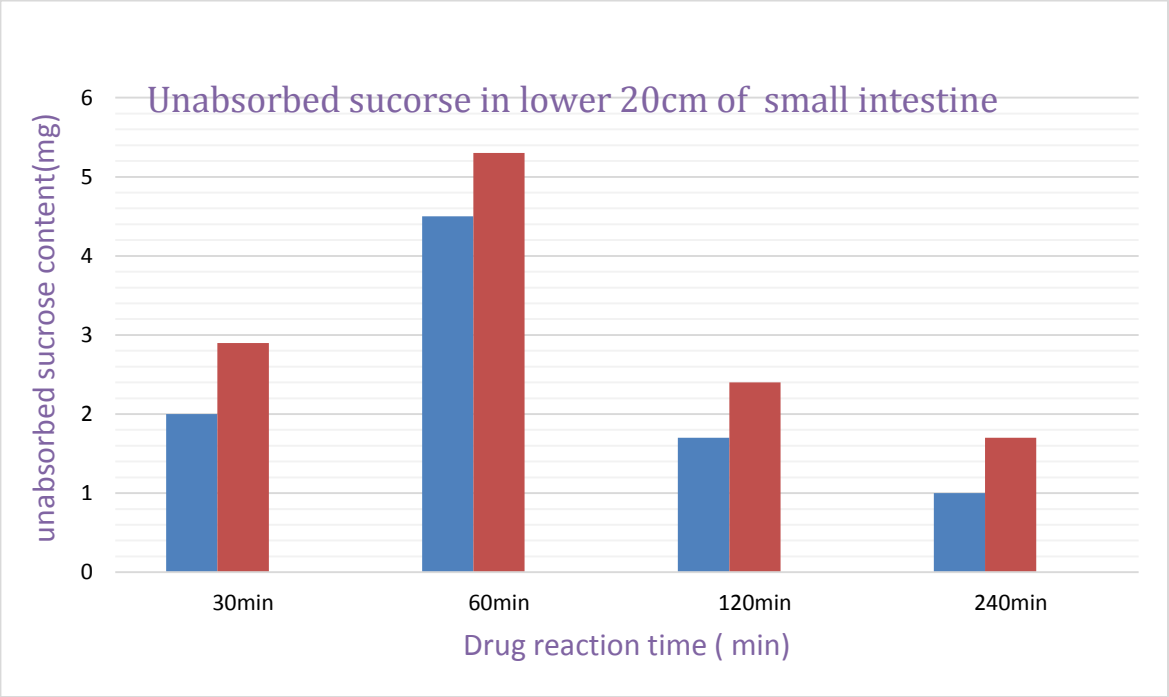

Figure 4.4: Anti Hyperglycemic Effect of *Acacia nilotica* in lower 20 cm of small intestine

Table: 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**

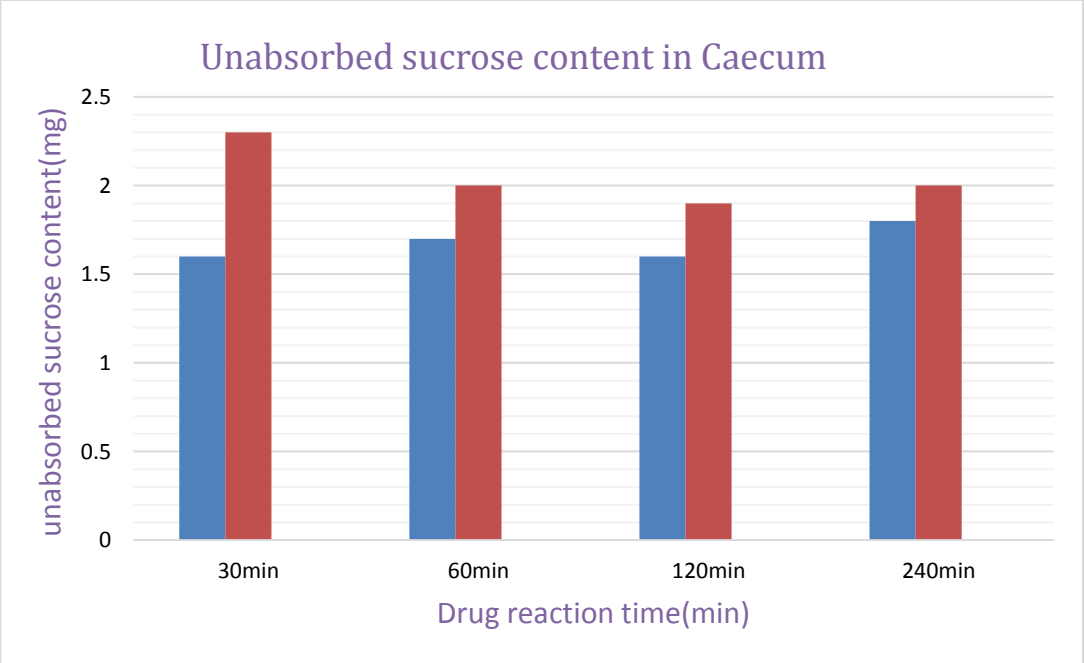

Figure 4.5: Anti Hyperglycemic Effect of *Acacia nilotica* in cecum

Table: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

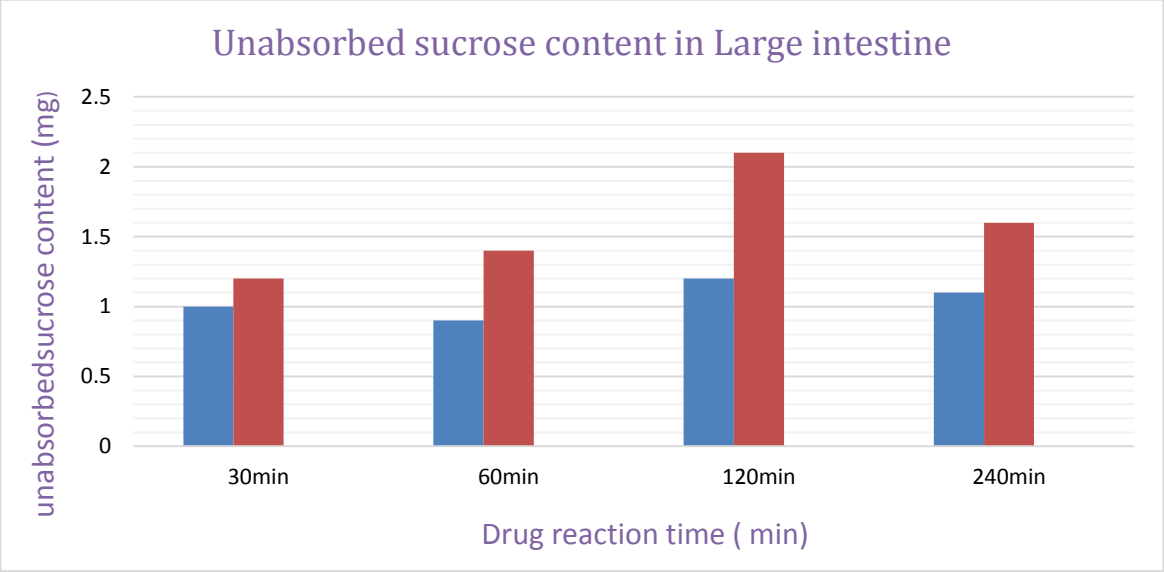

Figure 4.6: Anti Hyperglycemic Effect of *Acacia nilotica* in cecum

Figure 4.1-4.6 represents Effects of ethanol extract of *Acacia 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.

4.2 Effect of *Acacia nilotica* on Intestinal Disaccharidase Enzyme Activity

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

Table 4.2: 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

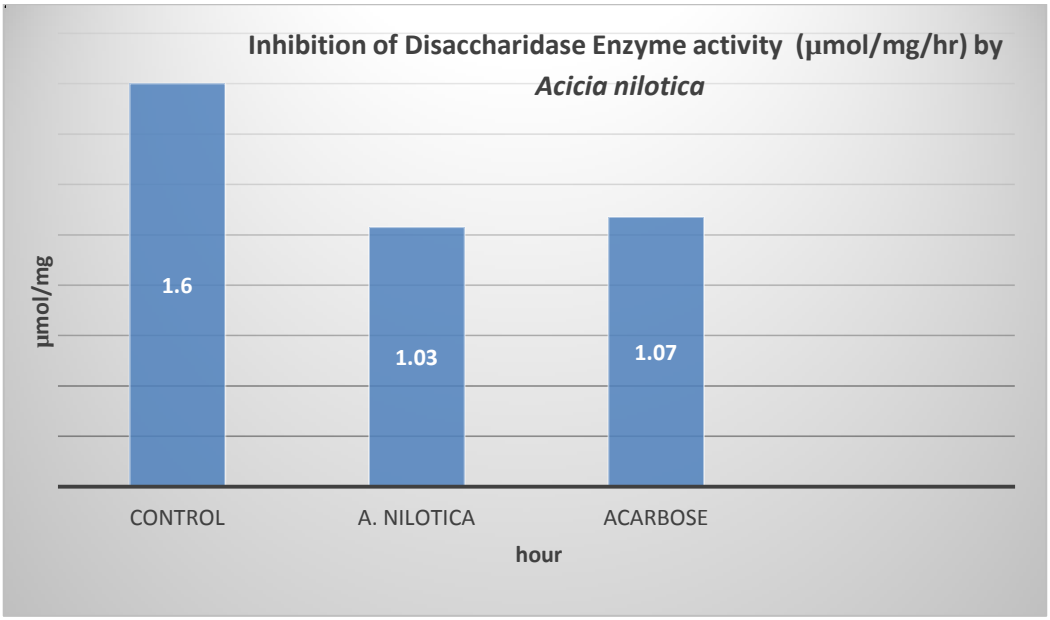

Figure 4.7: Effects of ethanol extract of *A. nilotica* on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of *A. nilotica* (100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 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: 05

Discussion & Conclusion

5.1 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

5.2 Conclusions

Acacia nilotica plant is rich in phytochemicals and has been in use since ancient times to treat a wide range of diseases in traditional system medicine. The present study would be helpful to create awareness among people for taking control measures based on, herbal plants against infectious diseases. Further more detail clinical researches are needed to explore its medicinal value in order to establish it as a standard drug. 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: 06

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