“Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of Aegle marmelos 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 Master’s of Pharmacy

Submitted To

Dr. JMA Hannan

Professor

Submitted by

Md. Arif ullah siddique

ID: 2016-1-79-003

EAST WEST UNIVERSITY

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Declaration by the Candidate

I, Md. Arif ullah siddique (ID: 2016-1-79-003), hereby declare that the dissertation entitled "Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Aegle marmelos* in Long Evans Rats" submitted by me to the Department of Pharmacy, East West University and in the partial fulfilment of the requirement for the award of the degree Master's 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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Md. Arif ullah siddique

ID: 2016-1-79-003

Department of Pharmacy

East West University, Dhaka

Certificate by the Supervisor

This is to certify that the thesis entitled "Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Aegle marmelos* in Long Evans Rats" submitted to the Department of Pharmacy, East West University for the partial fulfilment of the requirement for the award of the degree Master's of Pharmacy, was carried out by Md. Arif ullah siddique (ID: 2016-1-79-003) during the period 2016-2017 of his 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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Dr. JMA Hannan

Professor

Department of Pharmacy

East West University, Dhaka.

Certificate by the Chairperson

This is to certify that the thesis entitled "Evaluation of Anti-hyperglycemic Effect of Ethanolic Extract of *Aegle marmelos* in Long Evans Rats" submitted to the Department of Pharmacy, East West University for the partial fulfilment of the requirement for the award of the degree Master's of Pharmacy, was carried out by Md. Arif ullah siddique (ID: 2016-1-79-003) during the period 2016-2017 of his research in the Department of Pharmacy, East West University.

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Dr. Chowdhury Faiz Hossain

Chairperson & Professor

Department of Pharmacy

East West University, Dhaka

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Dedication

To

My Beloved Parents & My Research Supervisor

Abstract

Bael (***Aegle marmelos*** (Linn), family Rutacae, is also known as Bale fruit tree, is a moderate sized, slender, aromatic tree. The different parts of Bael are used for various therapeutic purposes, such as for treatment of Diabetes, Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea, Healthy Mind and Brain, Typhoid, Troubles during Pregnancy. ***Aegle marmelos*** has been used as an herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India, Bangladesh and Srilanka. The main usage of the parts of this tree is for medicinal purposes. The unripe dried fruit is astringent, digestive, stomachic and used to cure diarrhea and dysentery. 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 ***Aegle marmelos*** 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) method. In six segments, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 100mg/kg extract at different time frame. However, in this test, the amount of unabsorbed sucrose remaining in the gastrointestinal tract was found significantly higher in the extract treated rat group than the control. The Long Evans rats weighing from 150-200 gm were taken for this test. In conclusion, the results of all these studies suggested that the extract possess significant anti-diabetic activity.

Keywords: Anti-Diabeti*c, Aegle marmelos,* Anti-hyperglycemic, Glucose, Sucrose.

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Introduction and Literature Review

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).

Types of diabetes

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

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).

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).

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.

Table: 1.1 Difference between type 1 and type 2 diabetes

Gestational diabetes

Gestational diabetes develops in some women when they are pregnant. Most of the time, this type of diabetes goes away after the baby is born.(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).

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).

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).

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)

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Symptoms of diabetes

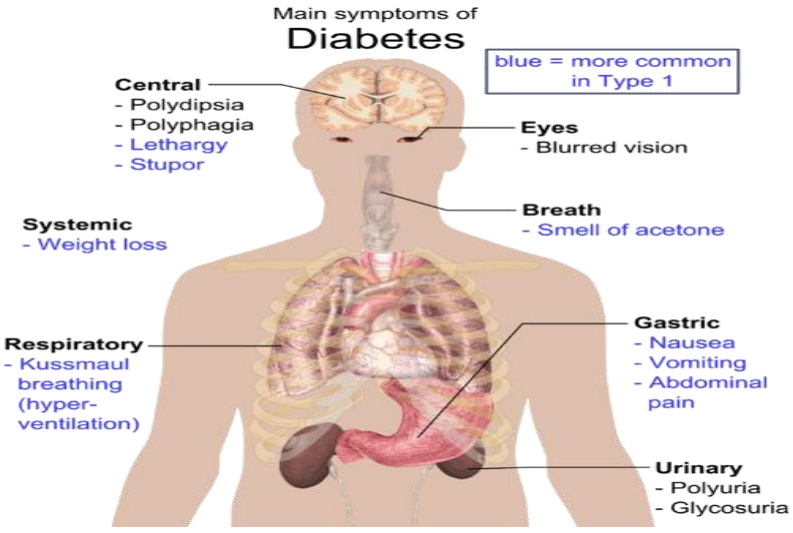

Figure: 1.2 Symptoms of diabetes - by Mikael Häggström

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)

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 pre-diabetes
- less than 140 mg/dl means normal

An abnormal reading following the OGTT means the patient has impaired glucose tolerance (Donnelly et al 2000).

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).

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.

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 glycolsis 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).

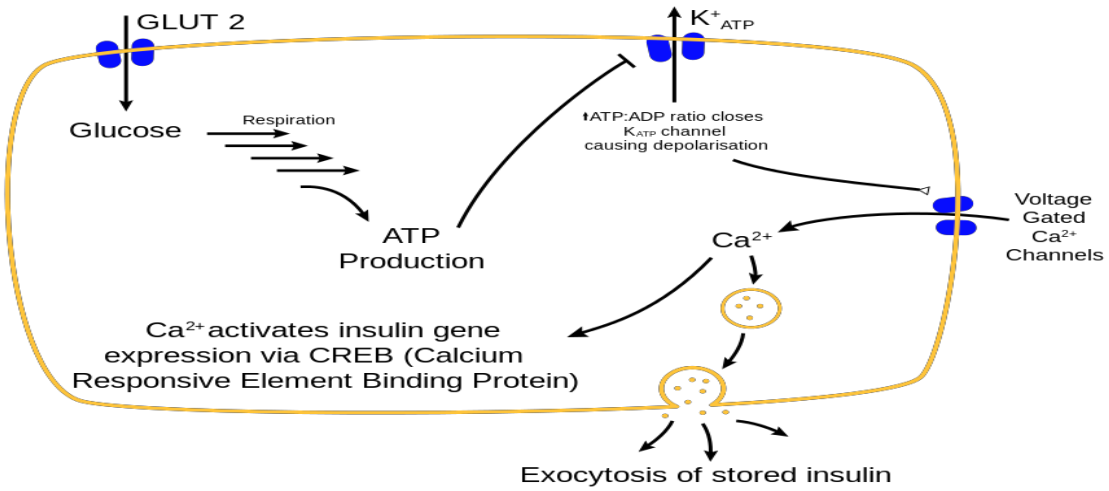

Figure 1.3: Mechanism of Insulin Secretion

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).

K_{ATP} channel independent pathway (amplification pathway)

Glucose can stimulate insulin secretion in pancreatic cells under conditions where K 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).

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), stimulation ofcell 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).

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**)**.

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).

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).

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).

Effects of insulin on glucose uptake

Insulin stimulates glucose uptake in muscle and adipose tissue by translocation 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).

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).

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).

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).

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).

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). 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). 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 uthors 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 below 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.

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).

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.

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

Table 1.4: Medicinal Plants with reported Anti diabetic Effect

Aegle marmelos

Bael (Aegle marmelos Corr.) is an indigenous fruit of India belongs to family Rutaceae and it is commonly known as Bengal quince (John and Stevenson, 1979), Bilva, Indian quince,Golden apple, Holy fruit, Bel, Belwa, Sriphal,Stone apple and Maredo in India.Aegle marmelos (L.) tree is held sacred by hindus and offered in prayers of deities Lord Shiva and Parvati and thus the tree is also known by the name Shivaduma (The Tree of Shiva).It has tolerance to arid conditions (Chundawat, 1990) as well as high rainfall.Exploration undertaken in eastern Uttar Pradesh and adjoining urea of Bihar indicated wide range of variability in thorniness on stem, fruit shape, scull thickness and pulp characteristics.Promising lines in respect to high yield and quality fruits were identified (Rai et al., 1991).The Bael tree has its origin from Eastern ghats and central India.It‟s indigenous to Indian subcontinent and mainly found in tropical and sub-tropical regions.The tree is also found as a wild tree, in lower ranges of Himalyas up to an elevation of 500 meters.Bael is also found growing along foothills of Himalayas, Uttranchal, Jharkhand, Madhya Pradesh and the Deccan Plateau and along the east coast (Sharma et al., 2007). Bael fruit is a sub-tropical, deciduous tree and fruit is globuse with grey or yellowish hard woody shell.Inside this, there is soft yellow or orange colored mucilaginous pulp with numerous seeds. It has numerous seeds, which are densely covered with fibrous hairs and are embedded in a thick, gluey, aromatic pulp (Kaushik et al., 2002).Bael fruit is truly popular for its ability to combat constipation. Its medicinal properties have been described in the ancient medical treatise in Sanskrit in CharakaSamhita (Aiyer, 1956).

Figure 1.5: Flower of Eagle marmelos

Figure 1.6: Fruit of Eagle of marmelos

All the plant parts like leaves, roots, barks, seeds and fruits of Bael are important ingredients of several traditional formulations against various diseases and many bioactive compounds have also been isolated of it (Badam et al., 2002, Gupta and Tondon 2004).

Biological classification of Bael

The scientific classification of bael is as below

- Kingdom: Plantae (Angiosperms)
- Order: Sapindales
- Family: Rutaceae
- Subfamily: Aurantioideae
- Tribe: Aurantieae
- Genus: Aegle
- Species: *A. marmelos*
- Binomial name: *Aegle marmelos*

Various names of Bael

In different regions, it is known by varied names.

- English name: Wood apple
- Arabic: Safarjale
- Bengali: Bael
- Hindi: Bael
- Marathi: Belaache zaad
- Tamil: Vilvamaran
- Sinhala: Beli
- Gujarati: Billu
- Malyalam: Koolam
- Urdu: Bael
- Indonesia: Maja
- Thai: Matum

DISTRIBUTION

Aegle marmelos is a subtropical plant which can grow up to an altitude of 1200 m from the sea level. It grows well in the dry forests of hilly and plain areas. A. marmelos can adapt a wide range of habitat and can be cultivated worldwide. It is native to India and has its origin from Eastern Ghats and central India. This tree is mentioned in the pre-historic writings dating back to 800 B.C. The Chinese Buddhist pilgrim, Hiuen Tsiang, when came to India (1629 A.D.), noticed the presence of this tree in India. It is cultivated throughout India and due to mythological importance; it is mainly grown near the temples. It grows wild in dry forests on hills and plains of central and southern India, Sub-Himalayan tracts from Jhelum eastwards to West Bengal, The Deccan Plateau, the East coast, and also found in Andaman Islands. It found almost in all the states of India such as in Andhra Pradesh, Bihar, Himachal Pradesh, Jammu and www.ijpab.com 10 Nidhi Sharma et al Int. J. Pure App. Biosci. 1 (6): 7-13 (2013) ISSN: 2320 – 7051 Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. In West Bengal, there are 13 types of fruits in Aegle marmelos based on the fruit's morphology. The fruits were grouped under five categories; oval, flat, spherical, oblong and pear shaped and in each group three subgroups (small, medium, big) were separated. It is also cultivated in Nepal, Myanmar, Tibet, Ceylon, Vietnam, Laos, Cambodia, Sri Lanka, Bangladesh, Thailand, Indonesia, Malaysia, the drier areas of Java, Fiji and to a limited extent on Northern Luzon of Philippine Islands where it first fruited in 191412,13,14. It is grown in Surinam and Trinidad, and some gardens of Egypt.

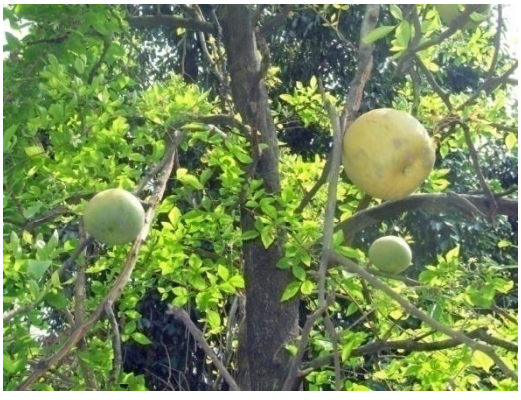

Figure 1.7: Bael tree

Botanical Description

Aegle marmelos is a slow-growing, medium sized tree, up to 12-15 m tall with short trunk. Its bark is thick, soft, flaking and spreading. Branches are spiny in some varieties, the lower ones are drooping. A clear, gummy sap, resembling gum arabic, exudes from wounded branches and hangs down in long strands, becoming gradually solid. Taste of this gum is sweet at first but later irritating to the throat. The leaves are deciduous, alternate, borne singly or in 2's or 3's oval, pointed, shallowly toothed leaflets, 4-10 cm long, 2-5 cm wide and the terminal one with a long petiole. New foliage is glossy and pinkishmaroon. Mature leaves emit a disagreeable odour when bruised. Flowers are fragrant having sweet aroma and blooms in clusters of 4 to 7 along the young branchlets. Each flower has 4 curved fleshy petals which are green outside and yellowish inside, and 50 or more greenish-yellow stamens. Shapes of the fruits can vary with varieties and can have round, pyriform, oval, or oblong shapes having 5-20 cm diameter. Fruit may have a thin, hard, woody or soft rind. It is dotted with minute oil glands which are aromatic. Inside the fruit, there is a hard central core and 8 to 20 faintly defined triangular segments, with thin, darkorange walls. These segments are filled with aromatic, pale orange, pasty, sweet, resinous, more or less astringent pulp. 10 to 15 seeds are embedded in the fruit pulp. Seeds are flattened-oblong, about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying.

The plant

Aegle marmelos is a slow-growing, medium sized tree, 25 to 30 feet tall. The stem is short, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. Young suckers bear many stiff, straight spines. There are sharp, axial one inch long spikes on this tree. The leaflets are oval or lancet shaped, 4-10 cm long, 2-5 cm wide. Leaves composed of 3 to 5 leaflets in it. The lateral leaflets are without petiole and the terminal one has a long one. The petiole is 1 to 2.5 inch long. Mature leaves emit a peculiar fragrance when bruised. Flowers occurs in clusters of 4 to 7 along the young branchlets, have 4 recurved, fleshy petals. The flowers are greenish white in color with a peculiar fragrant. Flowering occurs during the month of May and June. Fruit is spherical or oval in shape with a diameter of 2 to 4 inch. Shell is thin, hard and woody in nature. It is greenish when unripe and upon ripening it turns into yellowish color. The pulp of the fruit has 8 to 15 segments. The pulp is yellow, soft, pasty, sweet, resinous

and fragrant. Fruition occurs in the month of May and June. The seeds are embedded in the pulp. The seeds are small (nearly 1 cm in length), hard, flattened-oblong, bearing woolly hairs and each enclosed in a sac of adhesive (Lambole et al., 2010).

Phytochemicals

Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils. Roots and fruits contain coumarins such as scoparone, scopoletin, umbelliferone, marmesin and skimmin. Fruits, in addition, contain xanthotoxol, imperatorin and alloimperatorin and alkaloids like aegeline and marmeline identified as N-2- hydroxy-2-[4 - (3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide. b- sitosterol and its glycoside are also present in the fruits. Roots and stem barks contain a coumarin - aegelinol. Roots also contain psoralen, xanthotoxin, 6, 7- dimethoxy coumarin, tembamide, mermin and skimmianine. Leaves contain the alkaloids - O-(3,3- dimethyl allyl)-halfordinol, N-2-ethoxy-2 (4-methoxy phenyl) ethyl cinnamide, N-2-methoxy-2-(4-3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide, N- 2- [4-(3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide, N-2-hydroxy-2-[4-(3',3'- dimethyl allyloxy) phenyl] ethyl cinnamide, N-4-methoxy steryl cinnamide and N-2-hydroxy-2-(4- hydroxy phenyl) ethyl cinnamide. Mermesinin, rutin and b-sitosterol - b-Dglucoside are also present in the leaves. 2 A series of phenylethyl cinnamides, which included new compounds named anhydromarmeline (1), aegelinosides A and B were isolated from Aegle marmelos leaves as alfaglucosidase inhibitors. The structures of new compounds were characterized by spectroscopic data and chemical degradation of compounds isolated, anhydroaegeline (2) revealed the most potent inhibitory effect against alfaglucosidase with IC50 value of 35.8 lM. The present result also supports ethnopharmacological use of A. marmelos as a remedy for diabetes mellitus.3 A rare alkaloid, shahidine (1), having an unstable oxazoline core has been isolated as a major constituent from the fresh leaves of Aegle marmelos. It is moisture-sensitive, and found to be the parent compound of aegeline and other amides; however, it is stable in dimethyl sulfoxide. Its structure was established by spectroscopic analysis. Biogenetically, oxazolines may be considered as the precursor of hydroxy amides and oxazoles found in plants. Shahidine (1) showed activity against a few Gram-positive bacteria.4 From dry leaves of Aegle marmelos, four new alkaloids, N- 2-[4-(3′, 3′-dimethylallyloxy)phenyl] ethyl cinnamide, N-2- hydroxy-2-[4-(3′,3′-dimethylallyloxy)phenyl] ethyl cinnamide, N-4-methoxystyryl cinnamide and N-2- hydroxy-2-(4-hydroxyphenyl) ethyl cinnamide were isolated and characterized. Also isolated were aegeline and a purple compound whose structure has not

yet been established.5 From the unripe fruits of Aegle marmelos, a new alkaloid named marmeline was isolated and identified as N-2-hydroxy-2-[4-(3′,3′- dimethylallyloxy)phenyl] ethyl cinnamide. Aegline, imperatorin, alloimperatorin and xanthotoxol were also present.6 The purified polysaccharide isolated from the cambium layer of a young bael (Aegle marmelos) tree contains galactose, arabinose, rhamnose, xylose, and glucose in the molar ratios of 10.0:9.8:1.4:1.9:1. Methylation analysis and Smith degradation studies established the linkages of the different monosaccharide residues. The anomeric configurations of the various sugar units were determined by oxidation of the acetylated polysaccharide with chromium (VI) trioxide. The oligosaccharides isolated from the polysaccharide by graded hydrolysis were characterized. The structural significance of these results is discussed.7 The crude carbohydrate material isolated from bael (Aegle marmelos) seeds was resolved into four fractions. The homogeneous fraction contained 38.5% of carbohydrate and 60.6% of protein, and its carbohydrate moiety consisted of glucose, galactose, rhamnose, and arabinose in the molar ratios of 40:3:1:2. The linkages among various monosaccharide residues were established through methylation analysis and Smith-degradation studies. The anomeric configurations of the glycosyl groups and the structure at the glycosyl-amino acid junction were also determined. From the results of these experiments, a partial structure of the glycoprotein has been proposed.8 Purified hemicellulose isolated from a young bael (Aegle marmelos) tree with 2.5m sodium hydroxide contained d-xylose and 4-O-methyl-dglucoronic acid in the molar ratio of 7.43:1; traces of glucose, galactose, rhamnose, and arabinose were also present. The linkages between the monosaccharide units were determined by methylation analysis of a hemicellulose fraction (II A) and carboxyl-reduced, hemicellulose II A, and the results were corroborated by those from periodate oxidation and Smith degradation. The anomeric configurations of the d-xylopyranosyl residues were determined by chromium (VI) trioxide oxidation of the acetylated, carboxyl-reduced hemicellulose, and the aldobiouronic acid obtained from graded hydrolysis was characterized. These experiments clearly revealed the structure of this hemicellulose. The homogeneous, neutral polysaccharide isolated from the crude polysaccharide of the fruit pulp from bael (Aegle marmelos) contains arabinose, galactose, and glucose in the molar ratios of 2:3:14. The linkages among the different monosaccharide residues were established through methylation analysis and Smith-degradation studies of the polysaccharide. The anomeric configurations of the different glycosyl groups were determined by study of the chromium trioxide oxidation of the acetylated polysaccharide. Results of these

experiments have been discussed in order to assess the structure of the neutral polysaccharide.10 A new 7- geranyloxycoumarin [7-(2,6-dihydroxy-7-methoxy-7- methyl-3-octaenyloxy) coumarin] named marmenol (1) has been isolated from the leaves of methanolic extract of Aegle marmelos belonging to the family Rutaceae. In addition to marmenol, several known compounds have also been obtained for the first time from the same source. They include: praealtin D, trans-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, N-pcis- and trans-coumaroyltyramine, montanine, and rutaretin. The structures of marmenol and known constituents were established with the help of NMR spectroscopy. However, structure of 1 was further confirmed via 2-D NMR experiments.11 Antifungal constituents, 2-isopropenyl-4-methyl-1-oxacyclopenta[b]anthracene-5,10-dione and (+)-4- (20- hydroxy-30-methylbut-30-enyloxy)-8H-[1,3]dioxolo[4,5- h]chromen-8-one in addition to known compounds imperatorin, b-sitosterol, plumbagin, 1-methyl-2-(30- methyl-but-20-enyloxy)-anthraquinone, b-sitosterol glucoside, stigmasterol, vanillin and salicin were isolated during phytochemical investigation on seeds of Aegle marmelos Correa.

Cultural practices of bael

Climate and soil

Though bael is a fruit crop of subtropical origin, it has got a wider adaptability and can perform equally well in tropical, arid and semi-arid regions. Aegle marmelos is said to do best on rich, well-drained soil, but it has grown well and fruited on the oolitic limestone of southern Florida. It also grows well in swampy, alkaline or stony soils having pH range from 5 to 8. This tree requires pronounced dry season to give fruit. In India it has the reputation of thriving where other fruit trees cannot survive (Hiremarh et al., 1993).

Planting

Rainy season is the best time for planting. However, planting can also be done in spring season if irrigation facilities are available. Dig the planting pits of 1m × 1m ×1m size at least one month prior to onset of monsoon. Keep the planting pits open for 20-25 days thereafter; fill each pit with a mixture of top soil and 10-15 kg of FYM. This may be followed by irrigation to settle down the soil in pits. If depression takes place due to irrigation, add pit filling mixture to the pit.

Plant the bael sapling at the center of pit and provide support to the plant. Make a basin around it and irrigate gently. Do mulching with dry leaves to conserve moisture.

Irrigation

Young plants need to be watered regularly in summer and one month interval in winter for their rapid vegetative growth and establishment. In bearing trees irrigation is not required in dry summer, as it sheds leaves and resists hot dry summers. Irrigation can be applied at the time of new leaf emergence.

Harvesting and yield

Budded and grafted plants start fruiting after 4-5 years of planting whereas, seedlings after 8-10 years of planting. Bael Fruit takes around 8-10 months to mature and 10-12 months for ripening after fruit set. Bael is climacteric fruit that can be ripened, off the tree, if harvested at proper maturity stage. Maturity can be judged by the change in skull colour from dark green to yellowish green. Mature fruit should be harvested individually with 5 cm fruit stalk. A full grown (10-12 years old) budded or grafted bael tree produces on an average 150-200 fruits under good management practices. The fruits can be stored at room temperature for two weeks. At 10ºC, it can be kept up to three months. The average yield is 300-400 fruits per tree (Parmer 1982).

Fruit Cultivars of Aegle marmelos

There are no standardized names for A. marmelos cultivar. They are given names on the basis of locality where these are found. Fruits of different cultivars were of different shapes and sizes, such as spherical, oblong, cylindrical, pear-shaped and flat. Fruit weight also varied in different cultivars. The percentages of peel, seeds and contents of other fibres also varied. There are reports available on the cultivars of bael mainly from Uttar Pradesh and Bihar states of India 22, 23. Around twelve cultivars, viz., ‘Basti No.1’, ‘Gonda No.1’, ‘2’ and ‘3’, ‘Kagzi Etawah’, ‘Sewan Large’, ‘Deoria Large’, ‘Chakaiya’, ‘Lamba’, and ‘Baghel’ has been reported. ‘Kagzi Etawah’, ‘Sewan Large’, ‘Deoria Large’ and ‘Mirzapuri’, have been found to be superior and better than the other varieties in case of taste and qualities. S.K. Roy in 1975 studied 24 cultivars from four different locations in India- Agra, Calcutta, Delhi and Varanasi and mainly focused on their extreme variability. He selected and evaluated the different cultivars for high sugar content and low levels of mucilage, tannins and other phenolics 24. 'Kaghzi' is one of the esteemed large cultivar with thin rind and few seeds. Dr. L.B. Singh and his coworkers at the Horticultural Research Institute, Saharanpur, India, surveyed bael fruit trees in Uttar Pradesh and screened

about 100 seedlings, selected as the most promising for commercial planting are 'Mirzapuri', 'Darogaji', 'Ojha', 'Rampuri', 'Azamati', 'Khamaria'. Out of all these, the best rated was 'Mirzapuri', with very thin rind, breakable with slight pressure of the thumb, pulp of fine texture, free of gum, of excellent flavor and contain less amount of seeds. Four cultivars viz., 'Narendra Bael-4' 'Narendra Bael-5', 'Narendra Bael-7' and 'Narendra Bael-9' have been identified and studied by Srivastava and Singh from "Narendra Deo University of Agriculture and Technology", Faizabad, in 2004. They experimented to evaluate these commercially important cultivars and found that the heaviest fruit weight was recorded in 'Narendra Bael-7', whereas minimum fruit weight, fruit length and fruit breadth recorded was of 'Narendra BaeI-4'. Fibre content and seed/fruit were recorded minimum in 'Narendra Bael-9' and maximum in 'Narendra Bael-5'. Maximum total soluble solid, ascorbic acid and total sugar content recorded in 'Narendra Bael-5'. Though, minimum total soluble solids, ascorbic acid and total sugars were recorded in 'Narendra Bael-7'25. A number of cultivars have been selected recently which are the best among the others with regards to yield and fruit quality. These are; 'NB 5'—Fruit size is medium, round in shape having smooth surface at maturity, low mucilage, moderately fibrous and have soft flesh with excellent taste. 'NB 6'—Fruit size is medium, round with smooth surface, and have thin rind, few seeds, soft flesh, low mucilage and mild acidic. 'Pant Shivani'—Mid season cultivar with ovoid oblong shape, size 2 kg, colour lemon yellow on ripening, fiber and mucilage content medium, rind medium thick, pulp light yellow with very good taste and pleasant flavour. 'Pant Aparna'—late cultivar with small fruit size (0.6 - 0.8 kg), globose shape, and seed, mucilage, fibre and acidity are low. Its Flesh is yellow, sweet, tasty and having good flavour.

Traditional use

Bael species act as a climate purifier by absorbing poisonous gas from the atmosphere. The products obtained from Bael are highly nutritive as well as therapeutic. The juice of the fruit gives comfort from constipation and dyspepsia. The fruits are used against viral and intestinal parasites. The fruits are used to prepare squashes and cold drinks. The unripe fruits can be used after roasting. It can be used for the treatment of tuberculosis and gynecological disorders.

Bael leaves are useful in jaundice and in the treatment of wounds. The extract of leaves is beneficial in the treatment of leucorrhoea, conjunctivitis and deafness. Fruits give feeling of freshness and energy. It is used as carminative and astringent. It finds good utility in thyroid

related disorder. The other uses reported in cardiac stimulant, swollen joints, pregnancy trouble, typhoid and comma. The dried powder used in the treatment of irritable bowel syndrome (Sharma, et al., 2007).

Bael fruit products

Various process technology for production of value added preserved products from bael fruit. Fresh bael fruit can be stored for 15 days at 30oC when harvested at full maturity, for 1 week at 30oC when harvested ripe, for 3 months at 9oC. Fruit pulp can be stored for 6 months, when stored in heat-sealed containers. Fruit powder can be stored for a year when packed in 400 gauge polypropylene pouches and stored under dark, cool place, while fruit jam, squash and preserve can be stored for several months (ITDG, 2000). The bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, and terpenoids and has innumerable traditional medicinal uses (Karunanayake et al., 1984; Singh, 1986; Nagaraju and Rao, 1990). Thus value added products can be produced by using above process technology to reduce post harvest losses, increase shelf life, value addition and increase the income.

Bael products and uses:

A large number of bael processed products (Preserve, candy, panjiri, toffee, jam etc.) are prepared and some scientist and researcher are already worked on their processed products (Rakesh et al., 2005).

Preserve and candy

Preserve and candy are prepared from mature (tender green fruit), hole or large pieces of fruits in which sugar is impregnated till it becomes tender and transparent minimum fruit portion and minimum total soluble solids in preserves should be 55 and 70%, respectively (Lal et al., 1960). Fruits in general contain more than 75% water and get spoiled quickly if not stored properly. Removal of water from fruits is known to help in longer period of storage. The osmotic dehydration techniques not only enables the storage of fruits for a longer period but also preserve the flavor, color and texture of the product to a great extent and prevents its microbial spoilage (Bongirwar, 1997). “A fruit of its pieces impregnated with sugar or glucose syrup, sub piquantly drained free of syrup and dried is known as candied fruit”. The total sugar content of the

impregnated fruit is kept at about 75% to prevent fermentation. In case of bael candy, the fruit slices are drained subsequently free of syrup and dried at 55-60oC for 8-10 hrs in oven.

Bael fruit squash

An ideal composition of bael fruit squash was found to be 50 per cent extracted pulp, 50° Brix and 1 per cent acidity. The squash was chemically preserved by addition of 300 ppm SO2 (Roy and Singh, 1979). Fruit beverages commercially contain at least 25 per cent fruit pulp or juice and 40-50 per cent TSS, besides 1 per cent acid (Srivastav and Kumar, 1993). The squash from bael fruit pulp was prepared by adjusting the TSS and by adding the preservatives like sodium metabisulphite @ 350 ppm SO2 (Bhat and Kaul, 2006), and sodium benzoate @ 1g/litre (Verma and Gehlot, 2006). The squash was then filled in sterilized bottles, crowned and pasteurized at 80 oC for 30 minute fallowed by cooling and wax sealing to insure air tightness (Kenghe, 2008).

Bael RTS (Ready To Serve)

The ripe fruit were washed with tap water and broken by striking against hard object. The fruit pulp along with its seeds and fibres was scooped with the help of stainless steel spoon.Amount of water equal to the weight of pulp was added. The mixture of pulp and water was then heated up to 80°C for 1 minute and cooled. Pulp free from seeds and fibres was then obtained by passing through 20 mesh stainless steel sieve.The extracted bael pulp was improved by adjusting the TSS by addition of sugar and acidity by the addition of citric acid (Chand and Gehlot, 2006).

Toffee

Fruit toffees generally are more nutritious than ordinary toffees, and bael fruit pulp will provide even better toffees because of its nutritional and medicinal properties. Bael fruit toffees was successfully prepared by mixing 40 parts of cane sugar, 4.5 parts of glucose, 10 parts of skim milk powder and 6 parts of hydrogenated fat to 100 parts of extracted pulp. The final moisture content of the toffee was kept at 8.5 per cent (Roy and Singh, 1979).

Slab

It is also known as leather or paper. Ripe fruits are used in its preparation. Wash ripe fruits and collect fruit pulp by breaking fruits and removing its hard shell. At 200-300ml of water for each one kg of fruit pulp, mix well and heat it up to 800C. Collect fruit pulp free of seeds and fibers by straining heated mass through stainless steel sieve. Add sugar, citric acid and potassium meta-bisulphite (KMS) to this pulp so that treated pulp contains 35% total soluble solids, 0.5% total

acidity and 0.07% KMS. Boil treated pulp and spread on aluminum trays smeared with butter. Dry at 55-600C for 15-16 hrs to a moisture content of 14.5%. Cut slaves of dried pulp in aluminum trays, wrap in butter paper and pack in polyethylene bags).

Addition of up to 10 per cent sugar to the Int.J.Curr.Microbiol.App.Sci (2017) 6(3): 1870-1887

1883 extracted pulp was found to be ideal before drying the pulp to a moisture content of 14.5

Per cent (Roy and Singh, 1979)

Pharmacological properties

Acute and sub-acute toxicity studies:

Acute and sub-acute toxicity studies: This study was designed to elucidate the toxicity of the widely used plant Aegle marmelos in rats. The total alcoholic, total aqueous, whole aqueous and methanolic extracts isolated from the leaves of A. marmelos and studied their toxic effects. Acute, subacute and LD50 values were determined in experimental rats. The dead animals were obtained from primary screening studies, LD50 value determination experiments and acute studies subjected to postmortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted and any apparent and significant features or differences from the norm were recorded. Following the chronic administration of A. marmelos for 14 days, the vital organs such as heart, liver, kidney, testis, spleen and brain were carefully evaluated by histopathological studies and any apparent and significant changes or differences from the norm were studied. From the acute administration of A. marmelos, the LD50 values were determined using graphical method. The hearts stopped in systolic stand-still in the acute experiments. There were no remarkable changes noticed in the histopathological studies after 50 mg/kg body wt of the extracts of A. marmelos when administered intraperitoneally for 14 days successively. Pathologically, neither gross abnormalities nor histopathological changes were observed. After calculation of LD50 values using graphical methods, we found a broad therapeutic window and a high therapeutic index value for A. marmelos extracts. Intraperitoneal administration of the extracts of the leaves of A. marmelos at doses of 50, 70, 90 and 100 mg/kg body wt for 14 consecutive days to male and female Wistar rats did not induce any short-term toxicity. Collectively, these data demonstrate that the extracts of the leaves of A. marmelos have a high margin of drug safety.

Contractile activity:

The effect of the alcoholic extract of the leaves of Aegle marmelos Corr. on guinea pig isolated ileum and tracheal chain was investigated, as this plant is used traditionally to treat asthma and related afflictions. These effects were investigated using the isolated organ bath method. 1 mg/ml and 2 mg/ml doses of the alcoholic extract of this plant produced a positive relaxant effect in isolated guinea pig ileum and tracheal chain, respectively. In addition, they antagonized the contractions, which are produced by histamine. Because the alcoholic extracts elicited the antagonistic effect against histamine and also relaxed the histamine-induced contractions, it can be concluded that relaxations induced by A. marmelos in both guinea pig ileum and tracheal chain were due to the depression of H1- receptors. Since we observed a complete relaxation of the guinea pig ileum and tracheal chain produced by the extract, we investigated its antagonistic effect against histamine. These results were due to the presence of one or more antihistaminic constituents present in the alcoholic extract of this plant, therefore supporting to the traditional use of A. marmelos in asthmatic complaints.

Anti-microfilarial activity:

Methanolic extract of roots of vitex negundo L. and extracts of leaves of vitex negundo L. Ricinus communis L. and Aegle marmelos corr. were explored for possible antifilarial effect against Brugia malayi microfilariae. It was observed that among the herbal extract, root extract of vitex negundo L and leaves extract of Aegle marmelos Corr. At 100 ng/ml concentration showed complete loss of motility of microfilariae after 48 hrs of incubation. Thin layer chromatography of the extracts revealed the presence of alkaloids, saponins and flavonoids in the roots of vitex negundo L and coumarin in the leaves of Aegle marmelos Corr.

Antifungal activity:

A new anthraquinone, 1-methyl-2-(3′- methyl-but-2′-enyloxy)-anthraquinone (1) has been isolated from seeds of Aegle marmelos Correa and was characterized on the basis of spectral analysis (UV, IR, 1H NMR, 13C NMR, 2D NMR and mass spectroscopy). The compound exhibited significant antifungal activity against pathogenic strains of Aspergillus species and Candida albicans in disc diffusion assay (MIC value of 6.25 µg/disc), microbroth dilution and percent spore germination inhibition assays (MIC value of 31.25–62.5 µg/ml).16 The antifungal activity of essential oil isolated from the leaves of bael (Aegle marmelos (L.) Correa ex Roxb;

Rutaceae) has been evaluate using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500 ppm. However, the most resistant fungus, Fusarium udum was inhibited 80% at 400ppm. Kinetic studies showed concentration as well as time dependant complex inhibition of spore germination by essential oil.

Analgesic:

The methanol extract of leaves of Aegle marmelos at a dose level of 200 and 300 mg/kg showed significant analgesic activity on acetic acid-induced writhing and tail flick test in mice.18 Anti-inflammatory, antipyretic and analgesic: The serial extracts of the leaves of Aegle marmelos Corr. were investigated for anti-inflammatory property. The analgesic and antipyretic properties were also evaluated. The most of the extracts derived from the plant Aegle marmelos caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats. The extracts also produced marked analgesic activity by reduction the early and late phases of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts. This study was established anti-inflammatory, antinociceptive and antipyretic activities of the leaves of Aegle marmelos.

Hypoglycaemic effects:

The hypoglycaemic effect of the water extract of the fruits of Aegle marmelos was examined in streptozotocin-induced diabetic Wistar rats. Oral administration of the water extract (125 and 250 mg kg−1) twice a day for 4 weeks resulted in significant reductions in blood glucose, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and _-tocopherol and a significant elevation in plasma reduced glutathione and Vitamin C in diabetic rats. The effect of the extract at a dose of 250 mg kg−1 was more effective than glibenclamide in restoring the values of these parameters. The results of this study clearly showed the hypoglycaemic activity of the fruit extract.20 The aqueous extract of Aegle marmelos seeds was administered orally at different doses (100, 250 and 500 mg/kg) to normal as well as sub (fasting blood glucose (FBG) normal; glucose tolerance abnormal) and mild (FBG 120–250 mg/dl) diabetic rats. The dose of 250 mg/kg was found to be most effective dose and it decreases blood glucose level (BGL) by 35.1% in normal healthy rats after 6 h of administration. The same dose also showed a marked reduction in BGL of 41.2% in sub and 33.2% in mild diabetic rats in glucose tolerance test (GTT) after 2 h. Treatment of severely

(FBG >250 mg/dl) diabetic rats for 14 days with a dose of 250 mg/kg reduces the fasting blood glucose by 60.84% and urine sugar by 75% than their pretreatment levels. It brought about fall in level of total cholesterol (TC) by 25.49% with increase of 33.43% in high density lipoprotein (HDL) and decrease of 53.97 and 45.77% in low density lipoprotein (LDL) and triglyceride (TG), respectively. These results clearly indicate that aqueous seed extract of Aegle marmelos possess antidiabetic and hypolipidemic effects in diabetic rats.

Antidyslipidemic activity:

From the leaves of A. marmelos an alkaloidal-amide, Aegeline 2, was isolated and found to ivit have antihyperglycemic activity as evidenced by lowering the blood glucose levels by 12.9% and 16.9% at 5 and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight. Aegeline 2 has also significantly decreased the plasma triglyceride (Tg) levels by 55% ($P < 0.001$), total cholesterol (TC) by 24% ($P < 0.05$), and free fatty acids (FFA) by 24%, accompanied with increase in HDL-C by 28% and HDL-C/TC ratio by 66% in dyslipidemic hamster model at the dose of 50 mg/kg body weight. The reasonable mapping of compound 2 to validated pharmacophoric hypothesis and 3D QSAR model with an estimated activity (283 nM) suggest that the compound 2 might be a b3-AR agonist.

Immunomodulatory activity:

The aim of the present study was to investigate the immunomodulatory action of methanolic extract of Aegle marmelos fruit (FEAM) in experimental model of immunity. Methods: Cellular immunity was carried out by neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by mice lethality test and indirect haemagglutination assay. FEAM dose was selected by Stair case method (up and down) and administered at 100 and 500 mg/kg orally. The Ocimum sanctum (OSE, 100 mg/kg, p.o) was used as standard. FEAM at 100 and 500 mg/kg produced significant increases in adhesion of neutrophils and an increase in phagocytic index in carbon clearance assay. Both high and low doses of FEAM significantly prevented the mortality induced by bovine Pasteurella multocida in mice. Treatment of animals with FEAM and OSE significantly increased the circulating antibody titre in indirect haemagglunation test. Among the different doses, low one was more effective in cellular immunity models than the high. However, all the doses exhibited similar protection in humoral immunity procedures. From the above findings, it is concluded that FEAM possesses

potential for augmenting immune activity by cellular and humoral mediated mechanisms more at low dose (100 mg/kg) than high dose (500 mg/kg).

Antiproliferative activity:

In the present paper we show that extracts from Aegle marmelos Correa are able to inhibit the in vitro proliferation of human tumor cell lines, including the leukemic K562, T-lymphoid Jurkat, Blymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDAMB- 231 cell lines. Molecules present within the studied Aegle marmelos C. extracts were identified by gas-chromatography/mass- spectrometry analysis; three derivatives (butyl p-tolyl sulfide, 6- methyl-4-chromanone and butylated hydroxyanisole) were found to exhibit strong activity in inhibiting in vitro cell growth of human K562 cells. The antiproliferative activity of these compounds was found to be comparable to that of known antitumor agents, including cisplatin, chromomycin, cytosine arabinoside and 5-fluorouracil. In addition, the antiproliferative activity of butyl-p-tolyl sulfide, 6-methyl-4-chromanone and 5-methoxypsolaren was associated to activation of the differentiation pattern of K562 cells.

Wound healing activity:

Effect of topical and intraperitoneal administration of methanolic extract of Aegle marmelos ointment and injection was studied respectively on two types of wound models in rats, the excision and the incision wound model. Both the injection and the ointment of the methanolic extract of Aegle marmelos produced a significant response in both of the wound type tested. In the excision model the extract treated wounds were found to epithelialize faster and the rate of wound contration was higher, as compared to control wounds. The extract facilitated the healing process as evidenced by increase in the tensile strength in the incision model. The results were also comparable to those of a standard drug nitrofurazone.

Anti-fertility:

Fifty percent ethanolic extract from the leaves of A. marmelos was prepared. The effect of A. marmelos leaf extract on the reproductive system of male albino rats was investigated at three different doses, namely, 100, 200 and 300 mg−1 kg −1 day−1 for each rat for 60 days. Recovery was also investigated after a withdrawal of 120 days. All the major accessory sex organs shed weight post administration of the extract. There was a marked reduction in motility and density of the sperm derived from cauda epididymis of the treated animals. A. marmelos

reduced fertility of male rats by 100% at the 300-mg dose level. Serum testosterone levels also decreased significantly in all the experimental groups. The protein, glycogen and lipid peroxidation content of the testes was significantly reduced at the highest dose level; a highly significant increase in testicular cholesterol was observed along with a highly significant reduction in the sialic acid contents of testes, epididymis and seminal vesicles. Blood tests did not point to distress in any of the vital organs. Withdrawal of the extract restored all the altered parameters including organ weights, fertility, testosterone levels and tissue biochemistry to control levels after 120 days. The leaf extract of A. marmelos suppresses fertility in male rats. Complete recovery of fertility was observed following the withdrawal of drug. Absence of any deleterious effect on the vital organs points to the safe use of the extract.

Insecticidal activity:

Experiments were carried out to determine the potential of using essential oil from leaves of Aegle marmelos to control insect infestation of stored gram from Callosobruchus chinensis (L.) (Bruchidae) and wheat from Rhyzopertha dominica (F.) (Bostrychidae), Sitophilus oryzae (L.) (Curculionidae) and Tribolium castaneum (Herbst) (Tenebrionidae). After introducing the test insects, stored gram and wheat samples were fumigated with essential oil of Aegle marmelos at 500 µg/mL (ppm). The oil significantly enhanced feeding deterrence in insects and reduced the grain damage as well as weight loss in fumigated gram and wheat samples infested with all insects except T. castaneum. The essential oil at different doses significantly reduced oviposition and adult emergence of C. chinensis in treated cowpea seeds. The oil protected stored gram from C. chinensis and wheat from R. dominica and S. oryzae for two years. Limonene (88 %) was found to be the major component in the oil through GC-MS analysis. Regression analysis of data on individuals in treated cowpea confirmed that significant reduction of oviposition and adult emergence of C. chinensis decreased with increase in doses. The findings emphasize the efficacy of A. marmelos oil as fumigant against insect infestations of stored grains and strengthen the possibility of using it as an alternative to synthetic chemicals for preserving stored grains.

Miscellaneous:

The effect of the aqueous, alcoholic and petroleum ether extracts of A.marmelos have been studied for t h e hypoglycaemic and other pharmacological actions. The aqueous and alcoholic extracts at 500 mg/kg dose produced hypoglycaemia in normal fasted rabbits, but t h e

petroleum ether extract did not. The aqueous extract revealed cardiac stimulant, smooth-muscle relaxant and uterine stimulant properties. The alcoholic extract showed cardiac depressant, smooth muscle relaxant and uterine relaxant properties. Daily administration for six weeks showed necrosis and congestion of liver and kidney, with both the extracts but more pronounced with the aqueous extract.

Health benefits of Bael

1. **Bael for Tuberculosis**: In Ayurveda, it is used for the treatment of tuberculosis.
2. **Bael for Gynecological disorders**: The regular consumption of Bael helps to prevent gynecological related issues.
3. **Bael for Urinary diseases**: Use of bel leads you to overcome the problems of urinary diseases.
4. **Bael for Diabetes prevention**: It has bitter pungent, full of antioxidants and helps to stimulate the pancreas to secrete insulin, which leads to lowering of blood sugar. The leaves can be used against diabetes.
5. **Bael for Digestive disorders**: It supports intestinal biological formulations and protects the digestive system from ulceration, reduces the frequency of Irritable Bowel Syndrome (IBS), intestinal spasm thus beneficial in treating of dirrrhea, dysentery, and other infections of Elementary canal.
6. **Bael for Fever prevention**: The leaf juice with honey is helpful in prevention of fever.
7. **Bael for Epilepsy**: Flowers are uses as epilepsy tonic.
8. **Bael Nutritional facts**: It is rich in alakloids, polysaccharides, antioxidants, beta carotene, vitamin C, Vitamin B, and many other bio-chemical substances. It also contains tannins, calcium, phosphorous, iron, protein and fiber. The 100 gram of Bael contains the following nutrients: Calorific value (137 Kcal), Moisture (61.5g), Protein (1.8g), Fat (.3g), Minerals (1.7g), Fiber (2.9g), Carb (31.8mg), Calcium (85mg), Phosphorous (50mg), Iron (.7mg), Beta carotene (55 UG), Thiamine (.13mg), Niacin (1.1mg), Vitamin C (8 mg), Potassium (600mg) and Copper (.21 mg).
9. **Bael for Piles treatment**: The extract of unripe bel fruit is helpful in curing of piles and hemorrhoids.

Medicinal benefits of Bael

- Bel is known for its medicinal values because of the presence of many bio-chemical substances such as alkaloids, antioxidants, polysaccharides and essential oils.
- The fleshy inner product of bael is curative, pesticidal, nutritive as well as therapeutic in nature, which is used for the treatment of many diseases and disorders.
- Wood apple leaves are of therapeutic value and used in lowering of blood cholesterol.
- It is anti-inflammatory in nature. Its extracts when applied on the exposed area, help to cure inflammation.
- Bael's roots are antidiarrhoetic, antidote to snake venom and anti-inflammatory for healing.

Uses of Bael

- Its juice is used to make drink and squashes, especially in summer season because of its sweet and pleasant nature.
- Bael tender leaves are used as salads.
- It cleans the atmosphere as it helps to purify the atmosphere by absorbing the harmful gases from it.
- It is used to increase appetite.
- Its extract oil is used to cure respiratory problems.
- It is used in the preparation of candy, squash, toffee, pulp powder, and other eatable products.
- Bel juice is useful in curing of constipation because of its laxative properties.
- Bel juice gives great comfort in heartburn, acidity, hyperacidity and indigestion.
- If you are suffering from intestinal parasites, it is advisable to drink bael juice because of its antidote nature.
- Aegle marmelos juice is good for heart and brain. Bel juice mixed with Ghee, is beneficial in prevention of heart disease. It is also used as heart tonic.
- Chewing of raw leaves of Bel help to solve many gastric problems.
- Bael juice is rich in vitamin C, and good for scurvy treatment.

Bel precaution

Though, Bael is a beneficial Ayurvedic plant, but it has some side effects too. Before eating the fruit, one should take caution in the below given conditions.

- **Abdominal disorders**: Excess of bael consumption leads to abdominal disorders.
- **Constipation**: If you are taking more quantity of the fruit, it may lead to constipation.
- **Pregnancy**: One shouldn't take Bel during pregnancy.
- **Breast feeding**: Avoid during breast feeding.
- **Surgery**: It shouldn't be used in surgery.
- **Diabetes**: When taking for lowering of sugar in blood, one should observe sugar level carefully as it may down sugar too low.
- **Hypertension**: One should avoid taking this fruit if having high blood pressure.

Figure 1.8: Bael juice

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 *Aegle marmelos* in long evans rats.

- To determine the anti diabetic efficacy of the plant *Aegle marmelos*.

Chapter: 03

Methods and Materials

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.

Plant material collection

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

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.

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.

Extraction procedure

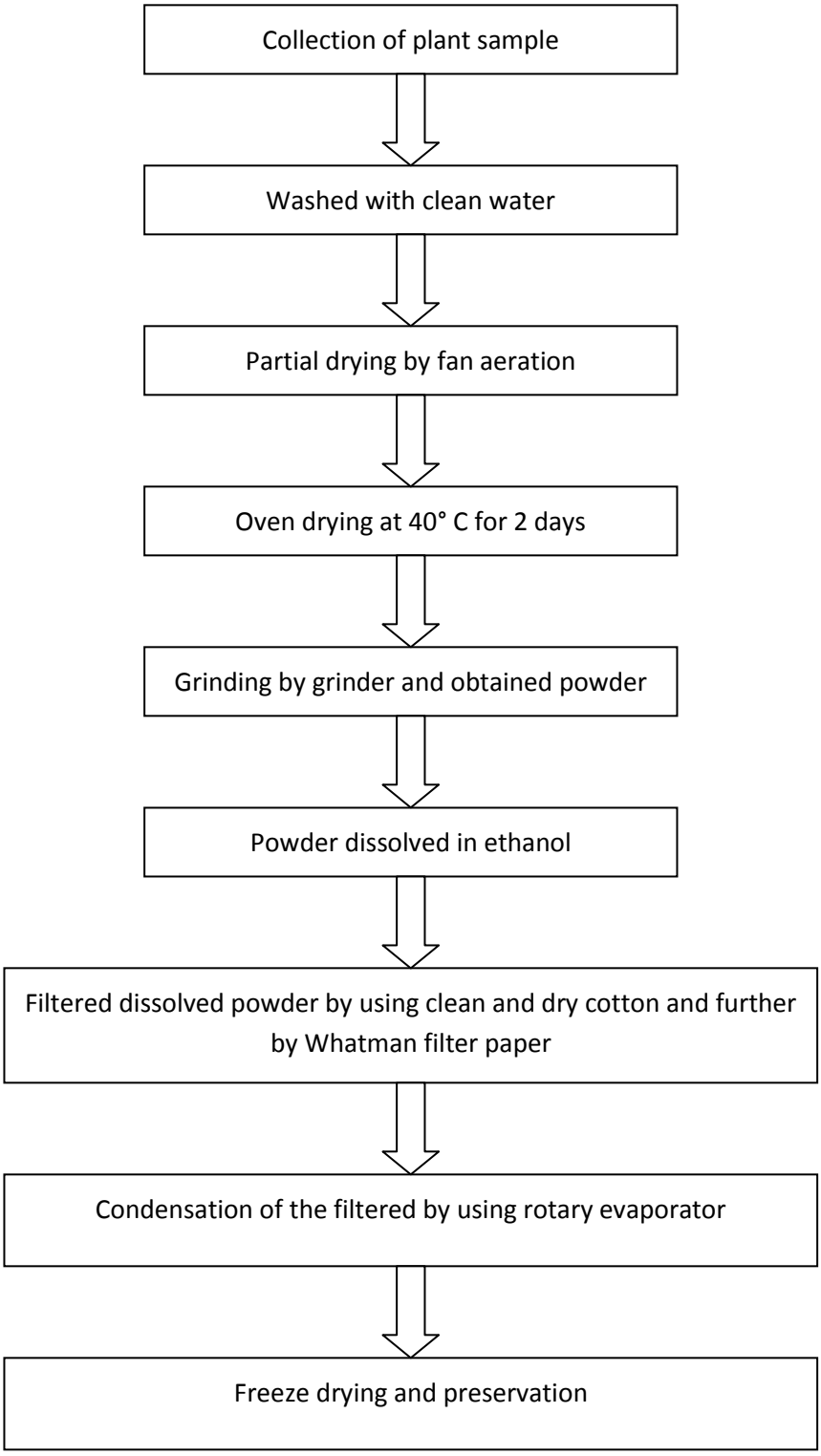

Figure 3.1: General Plant Extraction Procedure

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 across 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 behavioural, leaming, ageing (visual acuity less affected than that of albino strains), addiction-especially to alcohol-studies (M.D. Mordechai Hallak, 2002).

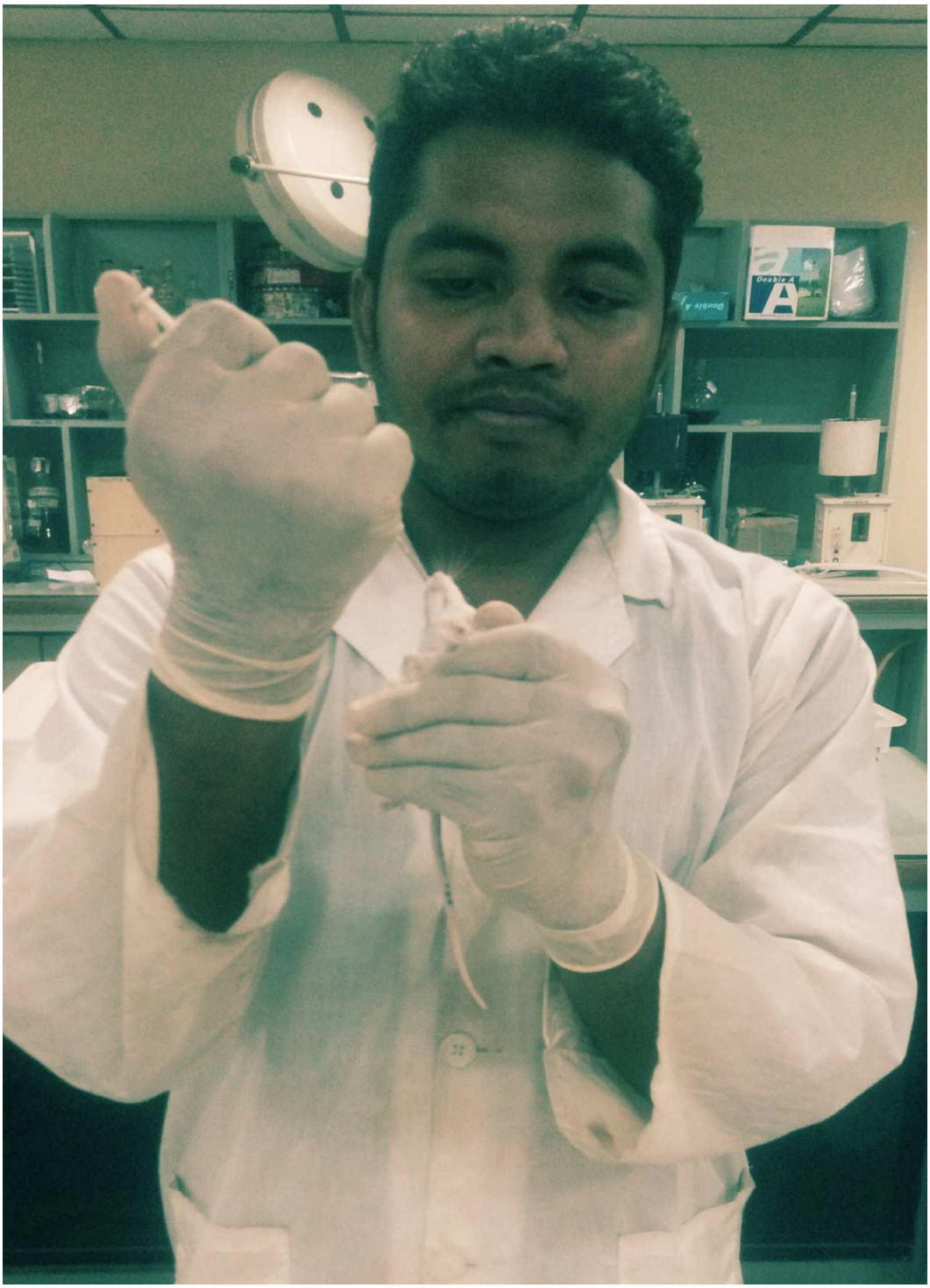

Figure: Feeding

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.

Screening for the Possible Inhibition of Carbohydrate Cbsorption by Plant Material

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.

Procedure

Rats were fasted for 20 hours 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 H2SO4 (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.

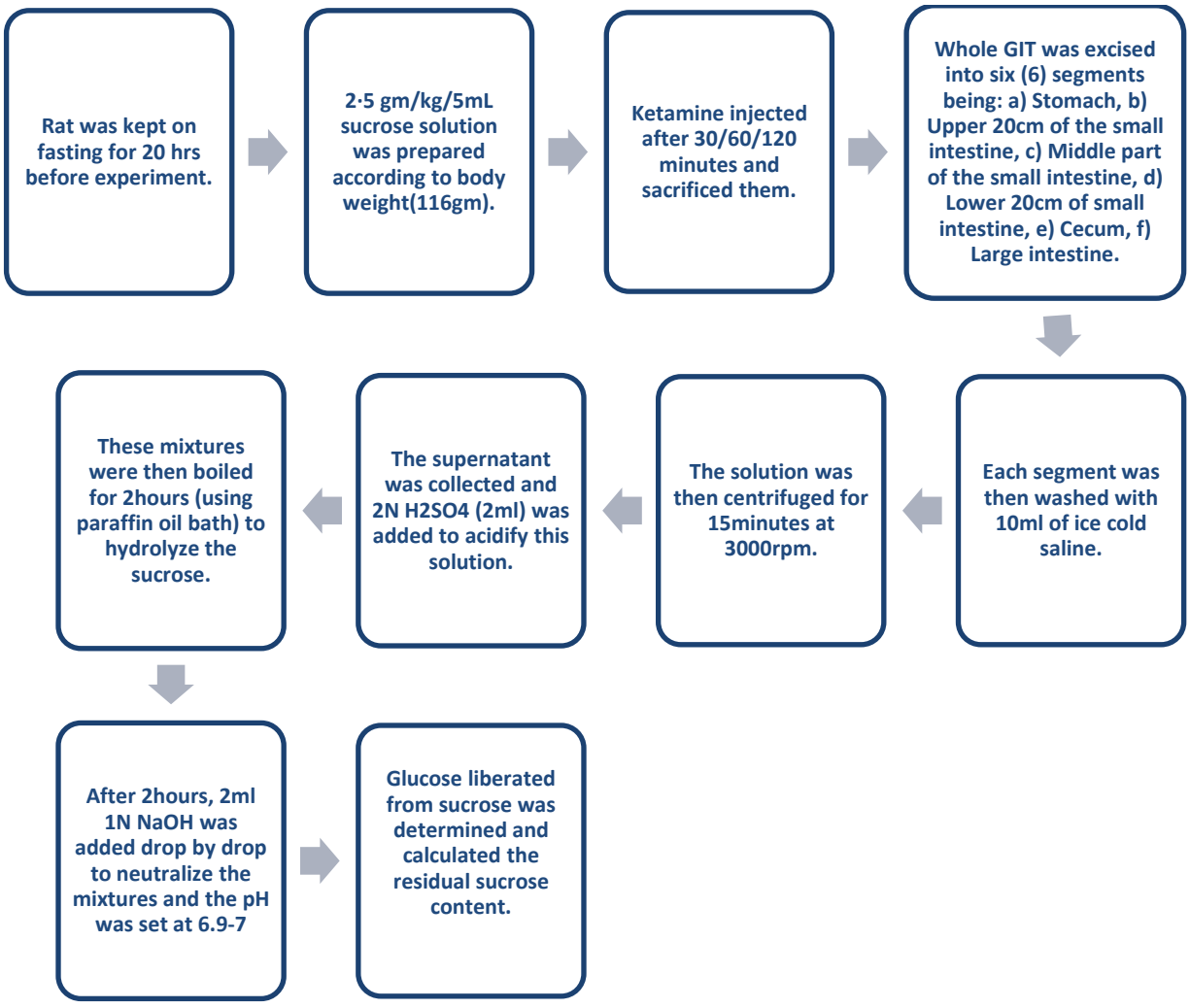

Figure 3.4: Steps in Six Segments

Chapter: 04

Results

Statistical Analysis

Statistical tests were conducted using Statistical Package for Social Science Software (SPSS) ver. 20 (IBM, Inc., Chicago, IL, USA). Results are presented as means±SEM. Data from experimental groups were compared using unpaired Student's t test and the Mann–Whitney U test, as required. Experiments with data being collected at several time intervals, were analyzed using repeated measures ANOVA followed by Bonferroni adjustment ensuring an error margin within ≤5%. One-way ANOVA was carried out to maintain an acceptable error margin of 5%. A two-tailed *P*-value of <0·05 was considered statistically significant.

Results

Effect of *Aegle marmelos* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with ***Aegle marmelos*** (500mg/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 ($p<0.05$) after oral sucrose loading in Long Evans 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 ***Aegle marmelos*** (500 mg/kg/body weight). Values are means and standard deviations represented by vertical bars ($n = 8$). Mean values were significantly different from those of control rats ($p < 0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

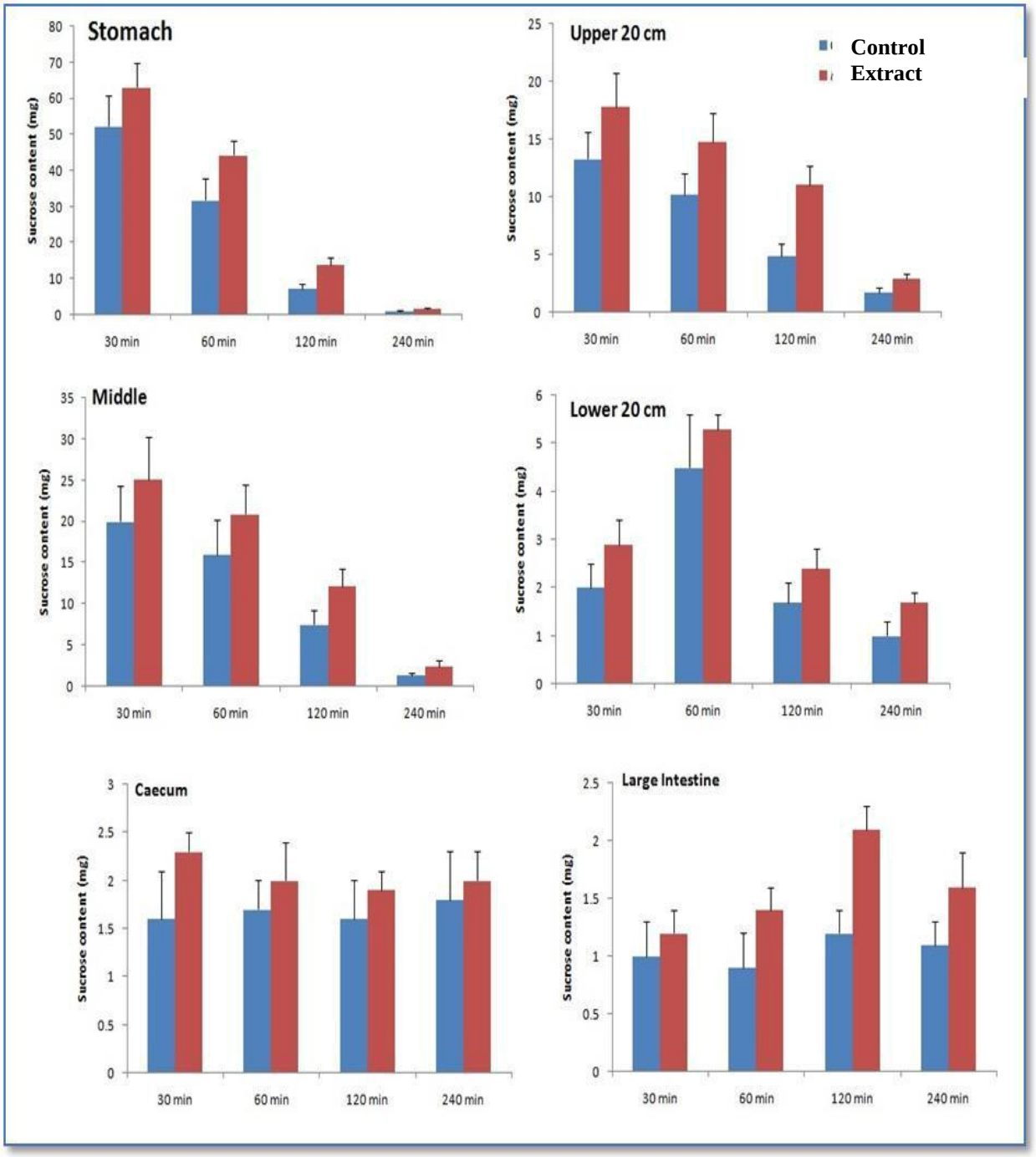

Figure 4.1: Effects of ethanol extract of ***A. Marmelos*** on gastrointestinal sucrose content

Chapter: 05

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 ***Aegle marmelos*** on normal rats. Additionally, unpublished, preliminary screening data, of this plant, showed promising hypoglycemic activity. Oral treatment with the defatted ethanolic pulp extract showed hypoglycemic activity in normal rats. However, the tissue level mechanism of action of ***Aegle marmelos*** 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 ***Aegle marmelos***, 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 ***Aegle marmelos*** 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.

So, our results can be fully attributed to the significant increase amount of unabsorbed sucrose was remained in 6 different parts of intestine which validates anti-hyperglycemic activity of ***Aegle marmelos***.

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 ***Aegle marmelos*** in diabetic models.

Conclusions

Aegle marmelos 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 ***Aegle marmelos.***

Chapter: 06

Reference

6.1 References

Abdel-Wahab YH, O'Harte FP, Mooney MH, Conlon JM & Flatt PR 1999 N-terminal glycation of cholecystokinine-8 abolishes its insulinotropic action on clonal B-cells. Biochemistry *et Biophysica* 1452 60-67.

Agrawal S, Kulkarni GT, Sharma VN (2010). A comparative study on the antioxidant activity of methanol extracts of acacia. Adv. Nat. Appl. Sci., 4(1): 78-84.

Ahmad M, Abdel-Wahab YHA, Tate R 2000 Effect of type-selective inhibitors on cyclic nucleotide phosphodiesterase activity and insulin secretion in the clonal insulin

secreting cell line BRIN-BD11. *Br J Pharmacol* 129 1228-1234.

Ajgaonkar SS 1979 Herbal drugs in the treatment of diabetes. *Int Diabetes Fed Bull* 24 10-17

Akhtar MS & Shah MV 1993 Elemental constituents of antidiabetic screening of a folklore medicinal plant prescription. *Ind J Toxicology, Occupational and Envt Health* 2 46.

Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC & Flores-Saenz JL 1998 Study of the anti-hyperglycemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology* 61 101–110.

Alberti KG, Zimmet PZ 1998 the WHO Consultation Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation *Diab Med* 15 pp.539-553.

Al-Habori M & Rahman A 1998 Antidiabetic and hypocholesterolaemic effects of Fenugreek. *Phytotherapy Research* 12 233-242.

American Diabetes Association (ADA) 1995 The pharmacological treatment of hyperglycemia in NIDDM. *Diabetes Care* 18 1510-8.

American Diabetes Association (ADA) 1997 Clinical practice recommendation, Screening for diabetes. *Diabetes Care* 20 22-24.

American Diabetes Association (ADA) 2001 Report of the expert committee on the diagnosis and classification of diabetes mellitus, *Diabetes Care* 24 S5-S20.

Amos AF, McCarty DJ & Zimmet P 1997 The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetes Med* (suppl 5), S1-S-85

Amos S, Akah PA, Odukwe CJ, Gamaniel KS, Wambede C (1999). The pharmacological effects of an aqueous extract from Acacia nilotica seeds. Phytother. Res., 13: 683-685

Ashcroft FM & Ashcroft SJM 1992 Mechanism of insulin secretion. In Insulin, Molecular Biology of pathology (Eds, Ashcroft, F.M. and Ashcroft, S.J.M.), Oxford University Press, Oxford. pp 97-150.

Ashcroft FM, Williams B, Smith PA and Fewtrell CMS 1992 Ion channels involved in the regulation of nutrient-stimulated insulin secretion. In *Nutrient regulation of insulin secretion*, (Ed, Flatt, P.R). Portland Press, London, pp193-212.

Aspinwall CA 2000 Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca^{2+} stores in insulin-stimulated insulin secretion in beta cells. *J Biol Chem* 275 22331 22338.

Atta-Ur-Rahman & Khurshid Zaman 1989 Medicinal plants with hypoglycaemic activity. *Journal of Ethnopharmacology* 26 1-55. Bailey C 1992 Biguanides & NIDDM. *Diabetes Care* 15 755-772.

Bailey C, Mynett K & Page T 1992b Importance of the intestine as a site of metformine-stimulated glucose utilization. *Br J Pharmacol* 112 671-675.

Bailey C, Wilcock C & Day C1992a Effect of metformin on glucose metabolism in the splanchic bed. *Br J Pharmacol* 105 1009-1013.

Bailey CJ & Day C 1989 Traditional plant medicines as treatment for diabetes. *Diabetes Care* 12 553-564.

Bailey CJ & Flatt PR 1986 Animal models of diabetes. In *Recent advances in diabetes*, Nattrass M (ed). Churchill Livingstone, Edinburgh. pp77-89.

Bailey CJ & Flatt PR 1986 Antidiabetic drugs, new developments. *Industrial Biotechnology* 6 139-142.

Banso A (2009). Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica. J. Med. Plants Res., 3: 082-085.

Baravkar AA, Kale RN, Patil RN, Sawant SD (2008). Pharmaceutical and biological evaluation of formulated cream of methanolic extract of Acacia nilotica leaves. Res. J. Pharm. Technol., 1(4): 481-483.

Cheta D 1998 Animal models of type 1 (insulin-dependent) diabetes mellitus. Journal of Pediatric. *Endocrinological Metabolism* 11 11-9.

Chiasson JL, Joss RG & Wolever TM 1996 The effect of acarbose on insulin sensitivity in subjects with impaired glucose tolerance. *Diabetes Care* 19 1190-1193.

Chifundera K, Baluku B & Mashimango B 1993 Phytochemical screening and molluscicidal potency of some Zairean medicinal plants. *Pharmacol Res* 28(4) 333-40.

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (6th Ed.). New Delhi NISCIR, 2002, 2.

Donnelly R, Emslie-Smith AM, Gardner ID & Morris AD 2000 ABC of arterial and venous disease: vascular complications of diabetes. *British Medical Journal* 320 1062- 1066.

Dorzbach E & Muller R 1971 The insulin therapy: lehmann's Publishing Company, Munich.

El-Tahir A, Satti GM, Khalid SA (1999). Antiplasmodial activity of selected sudanese medicinal plants with emphasis on Acacia nilotica. Phytother. Res., 13: 474-478.

Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH, Akhtar MS (1999). Studies on antihypertensive and antispasmodic activities of methanol extract of Acacia nilotica pods. Phytother. Res., 13: 665- 669.

Jigam AA, Akanya HO, Dauda BEN, Okogun JO. Polygalloyltannin isolated from the roots of Acacia nilotica Del. (Leguminoseae) is effective against Plasmodium berghei in mice. Journal of Medicinal Plants Research. 2010; 4(12):1169-1175.

Kalaivani T, Rajasekaran C, Suthindhiran K, Mathew L (2010b). Free radical scavenging, cytotoxic and hemolytic activities from leaves of Acacia nilotica (l.) wild. ex. delile subsp. indica (benth.) brenan. Evid. Based Complement. Alternat. Med., 2011: 274741.

Kritikar KR, Basu BD. Indian Medicinal plants with illustrations (2nd Ed.), Uttaranchal Oriental Press. 2003; 4:1289-92.

Meena PD, Kaushik P, Shukla S, Soni AK, Kumar M, Kumar A (2006). Anticancer and antimutagenic properties of Acacia nilotica (Linn.) on 7, 12-dimethylbenz(a) anthracene-induced skin papillomagenesis in Swiss albino mice. Asian Pac. J. Can. Prev., 7: 627-632.

Manoj K. Acacia nilotica Linn. as a phytomedicine: An overview. Int jour of Drug Discovery and Herbal Research (IJDDHR). 2015; 5(1):843-848.

Res. J. Pharm. Technol., 1(4): 481-483. Crowch CM, Okello EJ (2009). Kinetics of acetylcholinesterase inhibitory activities by aqueous extracts of Acacia nilotica (L.) and Rhamnus prinoides. Afr. J. Pharm. Pharmacol., 3(10): 469-475.

Shittu GA (2010). In vitro antimicrobial and phytochemical activities of Acacia nilotica leaf extract. J. Med. Plants Res., 4(12): 1232-1234.

Singh BN, Singh BR, Singh, RL, Prakash D, Sarma BK, Singh HB (2009a). Antioxidant and anti-quorum sensing activities of green pod of Acacia nilotica L. Food Chem. Toxicol., 47: 778-786.

WHO 1978 Resolution: Drug policies and management: Medicinal plants, WHO document, WHA, pp 31-33.

WHO Expert Committee on diabetes mellitus: Second Report 1980, Tech Rep Ser 46; Geneva: Word Health Organization. p61.

WHO study Group on Diabetes Mellitus 1985 Geneva, World Health Organization, (WHO Tech. Repot Series No 727.

rad AJ & Greene D 1977 Diabetic polyneuropathy: The importance of insulin deficiency, hyperglycemia and alterations in myolinositol metabolism in its pathogenesis. *New England Journal of Medicine* 295 1416-1421.

Winocour PD 1989 The role of platelets in the pathogenesis of diabetic vascular disease. In: Complications of Diabetes Mellitus. Molecular and Cellular Biology of Diabetes Mellitus, VoI III. Draznin B, Melmed S, LeRoith D (eds). *Alan R. Liss Inc*, New York pp 37-47.

Wollheim CB & Sharp GW 1981 Regulation of insulin release by calcium. *Physiological Reviews* 61 914-973.

World Health Organization 1980 Second Report of the WHO Expert Committee on Diabetes Mellitus. Technical report series. 646-66.

World Health Organization Expert Committee on Diabetes Mellitus 1980 Second report. Technical report series 646. World Health Organization, Geneva.

Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White M F , Herrera PL, Accili D & Efstratiadis A 2002 Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor. *J Clin Invest* 110 1011–1019.

Yada T 1994 Action mechanisms of amino acids in pancreatic B-cells. In *Insulin secretion and Pancreatic B-cell Research*. Flatt PR & Lenzen S (Eds), Smith-Gordon and Company Limited, London, pp 129-143.