PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIDIABETIC ACTIVITY OF ROOTS OF M*elia azedarach* Linn.,

A dissertation submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

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This is to certify that the dissertation entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIDIABETIC ACTIVITY OF ROOTS OF *MELIA AZEDARACH* Linn.,"** submitted by **M.NIVEDHA, Reg. No: 261720654** to The Tamil Nadu Dr. M.G.R. Medical University, examination is evaluated.

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Dedicated to my beloved family and Almighty god

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Introduction

1. INTRODUCTION

"Nature, in the broadest sense, is the natural, physical, or material world (or) universe". Humans are part of nature; human activity is often understood as a separate category from other natural phenomena.

"Nature itself is the best physician"

- Hippocrates.

Nature always stands as a golden mark exemplifies the outstanding phenomenon of symbiosis. Plants are indispensable to man. The three important necessities of life namely food, clothing, shelter & most of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health care.

The human being appears to be afflicted with more diseases than any other animal species. There can be little doubt then that he, very early, sought, to alleviate his sufferings from injury and disease by taking advantage of plants growing around him.

"Marundhena Vaentaavaam Yaakkaikku arundhiyadhu

Atradhu poatri unin"

- Thiruvalluvar.

It states, "No medicine is necessary for him who eats after assuring (himself) that what he has (already) eaten has been digested.

The history of herbal medicines is as old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt & Greece long before the beginning of the Christian era.

Most of the medicinally active substances identified in the 19th & the twentieth centuries were used in the form of crude extract. In China, many medicinal plants had been in use since 5000 B.C.

Indians also worked meticulously to examine & classify the herbs which they came across, into groups called gunas. Charaka made fifty groups of 10 herbs each of which, according to him, would suffice an ordinary physician's need. Similarly, sushrutha arranged 760 herbs in 7 distinct sets based on some of their common properties. A large portion of the Indian population even today depends on the ISM - ayurveda, "An ancient science of life". The well known treatises in Ayurveda are charaka samhita & Sushrutha samhita.

The important of the extraction method & alcohol as an extractant was reported by Le' mery. William Withering in 1785 published an account of some of the medicinal properties of foxglove leaves based on ten years of experimentation. Percolation process was used for the crude drugs. In the 19th century, the term 'Materia Medica' was used for the subject now known as "Pharmacognosy". While studying sarsaparilla, it was seydler, a German scientist, who coined the term "Pharmacognosy" in 1815 in the title of his work "Analecta Pharmacognostical". Pharmacognosy is derived from two Greek words viz. Pharmacon (a drug) and Gignosco (to acquire the knowledge of). Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal & related products of crude or primary type obtained from plant, animal & mineral origins.[1]

Traditional & Alternative system of medicine:-

- Traditional Chinese medicine & kampoh system
- Ayurveda- Indian system of medicine
- Siddha system of medicine
- Unani system of medicine
- Homeopathic system of medicine
- Acupressure & acupuncture
- Naturopathy and yoga
- Bach flower remedies

- Aromatherapy
- Hydrotherapy
- Native American healing practices
- Tibetian system of medicine.

India has a very long, safe & continuous usage of many herbal drugs in the officially recognized alternative systems of health. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine. Millions of Indians use herbal drugs regularly, as, spices, home-remedies, health foods as well as over-the-counter (OTC) as self- medication or also as drugs prescribed in the non-allopathic systems. There are more than 500,000 non-allopathic practitioners are trained in the medical college of their respective systems of health & are registered with the official council which monitor professionalism. Hence, these systems are not folklore or traditional herbal practices.[2]

Medicinal plants[3-8]:-

The term "Medicinal plants" include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.

Recently, WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plant.

Importance of some herbs with their medicinal values:-

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.
- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to

grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.

- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.
- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.
- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as Chirayta, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.
- Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.
- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.
- Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.
- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.
- Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.
- Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.
- Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon,

Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.

- Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.
- Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.
- Certain aromatic plants such as Aloe, Golden seal, Barberry and Chirayata are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.
- Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne (Lal Mirch, Myrrh, Camphor and Guggul.
- A wide variety of herbs including Giloe, Golden seal, Aloe and Barberry are used as tonics. They can also be nutritive and rejuvenate a healthy as well as diseased individual.
- Honey, turmeric, marshmallow and liquorice can effectively treat a fresh cut and wound. They are termed as vulnerary herbs.

Examples of herbal medicines with proven Anti diabetic activity/ plant part used[9]:-

Roots -Asiatic ginseng, Ashvagandha, winter cherry, *Acontium carmichaeii*, *Oriza sativum*

Leaves,Stems – Sapphire-berry (Asiatic sweet-leaf)

Fruits/Flowers- *Musa paradisiaca* (Banana)

Bark – *Ficus bengalensis* (Banyan tree)

Leaves -Barbados, Betel, Betel vine, Black tea, Common fig,Wattakaka volubilis, Abrus precatoriusL , Aloe vera and *Aloe barbadensis*, *Mangifera indica*, Gudmar , Adulsa, *Piper longum*

Leaf extract -Bilwa, bael fruit

Fruit pulp - *Annona reticulata* (Custard apple)

Husks extract -Desert Indian wheat, Ispghul

Whole plant -Prickly chaff flower, *Tinospora cardifolia*

Fruit -Anemarans, *Atractylodes japonica*, *Dioscorea japonica*, Nagarmotha, *Capsicum annum*, *Gandoderma lucidium*

Rhizomes- *Zingiber officinalis*

Aerial part -*Coptis chinensis*, Brahmi

Seeds -*Galega officinalis, Lathyrus japonica*, *Trigonella foenum graecum*

Plant extract -*Acacia Arabica*, *Tinospora cordifolia*

Paste -*Allium cepa, Allium sativum*

Fruit pulp,seed leaves,whole plant -*Momordica charantia*

Extracts -*Azadirachta indica*

Root extarct – *Annona squamosa*

Extarct - *Nigella sativa*

Dried stem - Arjunsa

Oleo gum resin incision of stem bark -Guggul

Flower -Jaswand

Gummy exudation of stem and bark -Babhul

Root leaves –*Withania somnifera*

Stem roots -*Tinospora cardifolia*

Roots,stolen –*Glycyrrhiza glabra*

Mature fruit and dried seeds –*Syzygium cumini*

Entire herb -*Ocimum sanctum*

Fruits -*Terminalia chebula, Terminalia bellerica*

Dried ripe seeds -*Areca catechu*

Matured fruits and dried seed- *Syzygium cumini.*

DIABETES MELLITUS[10-19]:-

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production.

The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. In the United States, DM is the leading cause of end-stage renal disease (ESRD), nontraumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be likely a leading cause of morbidity and mortality in the future.

Diabetes is the most common endocrine disorder that affects more than 194 million people worldwide. If nothing is done to control this disease, the number will exceed 333 million by 2025(6.3% of population).

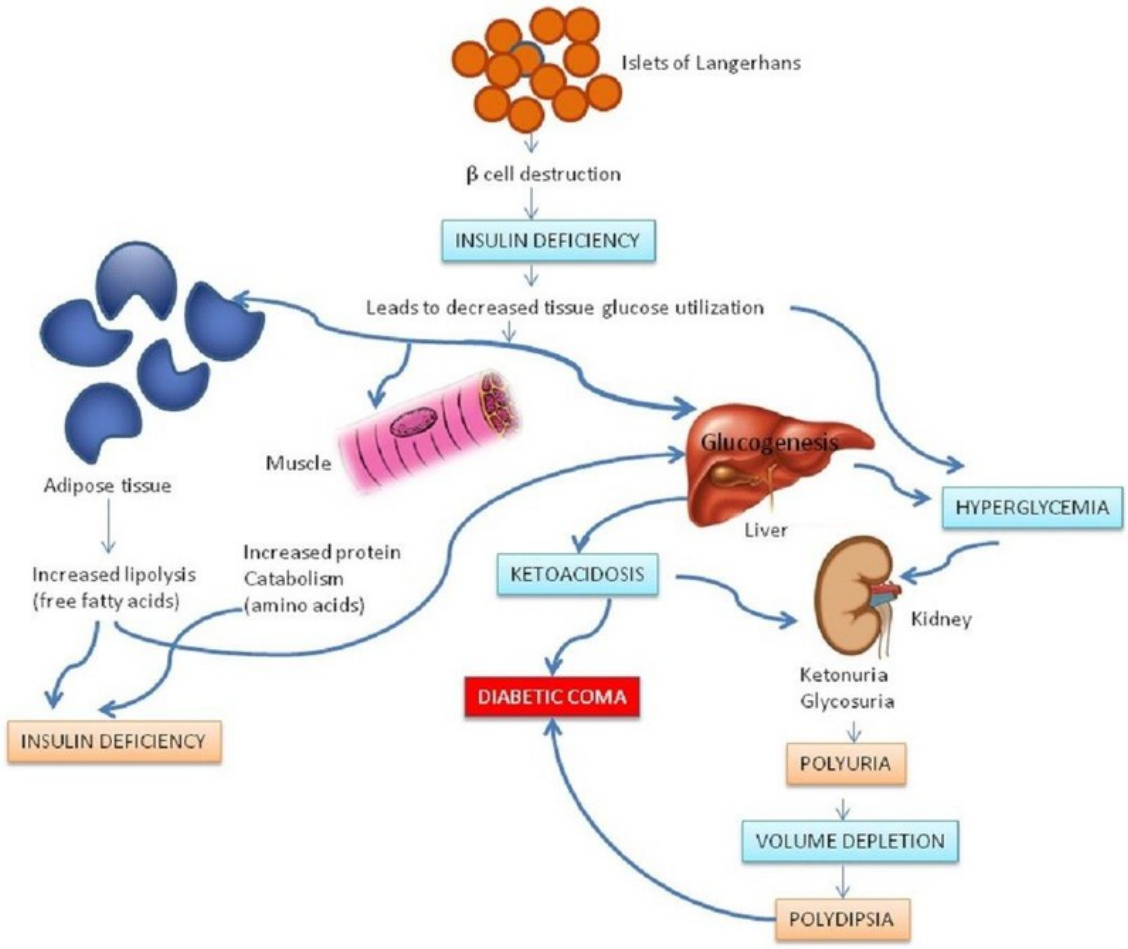


Figure: 1 Flow chart for Diabetes Mellitus

ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS:-

1. Type 1 diabetes (beta cell destruction, usually leading to absolute insulin deficiency)

    A. Immune-mediated

    B. Idiopathic

2. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

3. Other specific types of diabetes

A. Genetic defects of beta cell development or function characterized by mutations in:

a. Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1)

b. Glucokinase (MODY 2)

c. HNF-1α (MODY 3)

d. Insulin promoter factor-1 (IPF-1; MODY 5)

e. HNF-1β (MODY 5)

f. NeuroD1 (MODY 6)

g. Mitochondrial DNA

h. Subunits of ATP-sensitive potassium channel

i. Proinsulin or insulin

j. Other pancreatic islet regulators/proteins such as KLF11, PAX4, BLK, GATA4, GATA6, SLC2A2 (GLUT2), RFX6, GLIS3.

(MODY, maturity-onset diabetes of the young)

B. Genetic defects in insulin action

a. Type A insulin resistance

b. Leprechaunism

c. Rabson-Mendenhall syndrome

d. Lipodystrophy syndromes

C. Disease of the exocrine pancreas- pancreatitis, pancreatectomy, neo-plasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carbonyl ester lipase.

D. Endocrinopathies- acromegaly, cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma.

E. Drug or chemical induced- glucocorticoids, vacor (a rodenticide), pentamide, nicotinic acid, diazoxide, thiazides, etc

F. Infections- congenital rubella, cytomegalovirus, coxsackievirus

G. Uncommon forms of immune mediated diabetes – "stiff-person'' syndrome, anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes- Turner's syndrome, Down's syndrome

4. Gestational diabetes mellitus

CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS:-

• Symptoms of diabetes plus random blood glucose concentration ≥ 11.1 mmol/L (200 mg/dl) or

• Fasting plasma glucose ≥7.0 mmol/L (126 mg/dl) or

• Hemoglobin $A_{1c} \geq 6.5\%$ or

• 2-h plasma glucose ≥11.1 mmol/L (200 mg/dl) during an oral glucose tolerance test.

RISK FACTORS FOR TYPE 2 DIABETES MELLITUS:-

- Family history of diabetes (i.e., parent or sibling with type 2 diabetes)
- Obesity (BMI ≥25 kg/m^2 or ethnically relevant definition for overweight)
- Physical inactivity
- Race/ethnicity (e.g., African American, Latino, native American, Asian American, pacific islander)

- Previously identified with IFG, IGT, or an hemoglobinA_{1c} of 5.7–6.4%
- History of GDM or delivery of baby >4 kg (9 lb)
- Hypertension (blood pressure ≥140/90 mmHg)
- HDL cholesterol level <35 mg/dl (0.90 mmol/l) and/or a triglyceride level >250 mg/dl (2.82 mmol/l)
- Polycystic ovary syndrome or acanthosis nigricans
- History of cardiovascular disease

INSULIN BIOSYNTHESIS:-

Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as a single-chain 86-aminoacid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the amino-terminal signal peptide, giving rise to proinsulin. Proinsulin is structurally related to insulin-like growth factors I and II, which bind weakly to the insulin receptor.

Cleavage of an internal 31-residue fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells. Because C peptide is cleared more slowly than insulin, it is a useful marker of insulin secretion and allows discrimination of endogenous and exogenous sources of insulin in the evaluation of hypoglycemia. Pancreatic beta cells co-secrete islet amyloid polypeptide (IAPP) or amylin, a 37-amino-acid peptide, along with insulin.

The role of IAPP in normal physiology is incompletely defined, but it is the major component of the amyloid fibrils found in the islets of patients with type 2 diabetes, and an analogue is sometimes used in treating type 1 and type 2 DM. Human insulin is produced by recombinant DNA technology; structural alterations at one or more amino acid residues modify its physical and pharmacologic characteristics.

INSULIN SECRETION:-

- Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastro-intestinal peptides, and neurotransmitters also influence insulin secretion.
- Glucose levels >3.9 mmol/L (70 mg/dl) stimulate insulin synthesis, primarily by enhancing protein translation and processing. Glucose stimulation of insulin secretion begins with its transport into the beta cell by a facilitative glucose transporter.
- Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibits the activity of an ATP-sensitive K+ channel.
- This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g., sulfonylureas, meglitinides); the other is an inwardly rectifying K+ channel protein. Inhibition of this K+ channel induces beta cell membrane depolarization, which opens voltage-dependent calcium channels (leading to an influx of calcium) and stimulates insulin secretion. Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min, superimposed upon greater amplitude oscillations of about 80–150 min.
- Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose-stimulated insulin secretion and suppress glucagon secretion. Glucagon-like peptide 1 (GLP-1), the most potent incretin, is released from L cells in the small intestine and stimulates insulin secretion only when the blood glucose is above the fasting level. Incretin analogues or pharmacologic agents that prolong the activity of endogenous GLP-1 enhance insulin secretion.

INSULIN ACTION:-

Once insulin is secreted into the portal venous system, ~50% is removed and degraded by the liver. Unextracted insulin enters the 2403systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the recruitment of intracellular signaling molecules, such as insulin receptor substrates (IRS).

IRS and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin. As an example, activation of the phosphatidylinositol-3′-kinase (PI-3-kinase) pathway stimulates translocation of a facilitative glucose transporter (e.g., GLUT4) to the cell surface, an event that is crucial for glucose uptake by skeletal muscle and fat. Activation of other insulin receptor signaling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes in insulin-responsive cells.

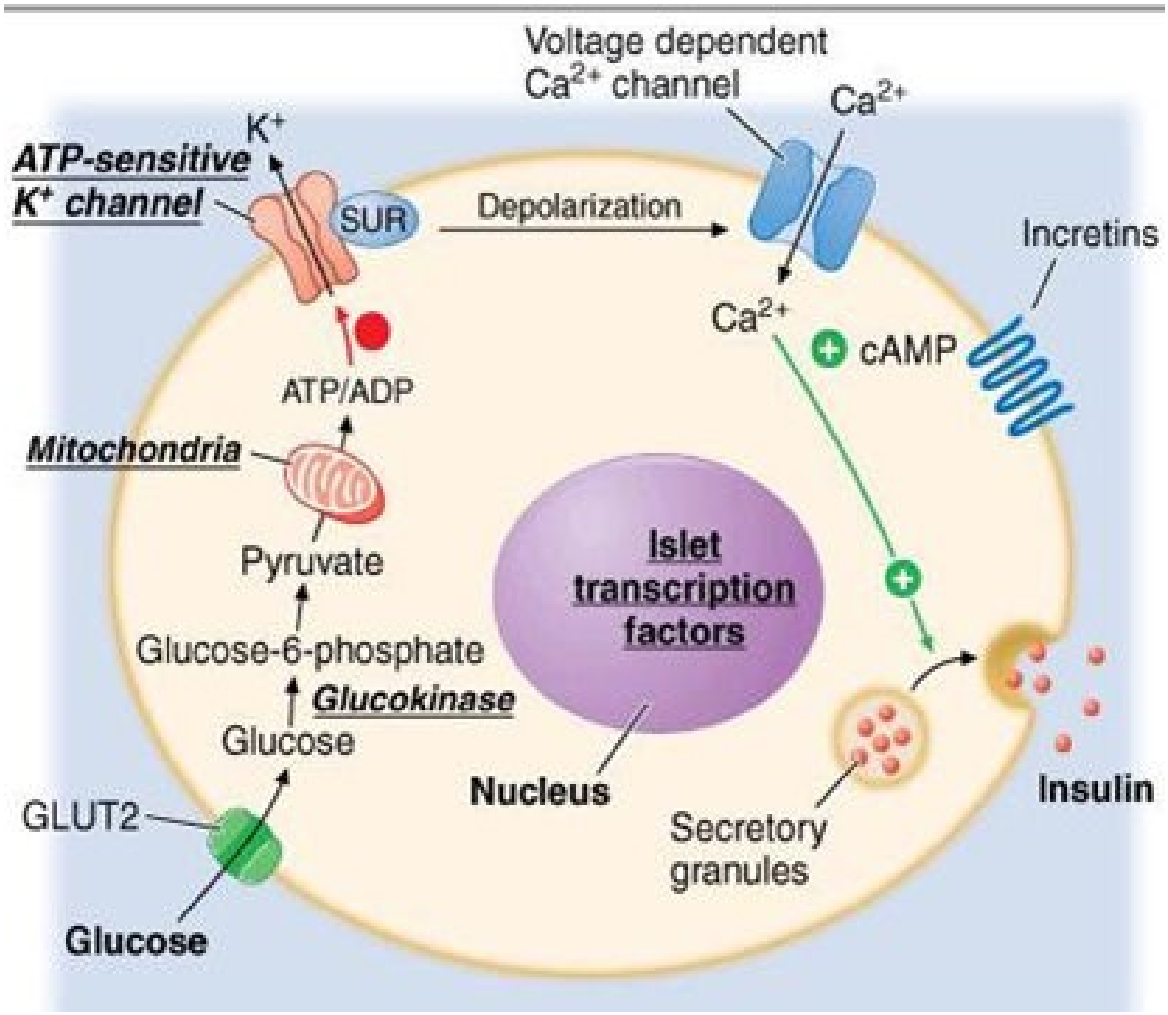


Figure: 2 Mechanisms of glucose-stimulated insulin secretion and abnormalities in diabetes

PATHOPHYSIOLOGY OF TYPE 1 DM:-

Although other islet cell types (alpha cells [glucagonproducing], delta cells [somatostatin-producing], or PP cells [pancreatic polypeptide-producing]) are functionally and embryologically similar to beta cells and express most of the same proteins as beta cells, they are spared from the autoimmune destruction. Pathologically, the pancreatic islets have a modest infiltration of lymphocytes (a process termed insulitis).

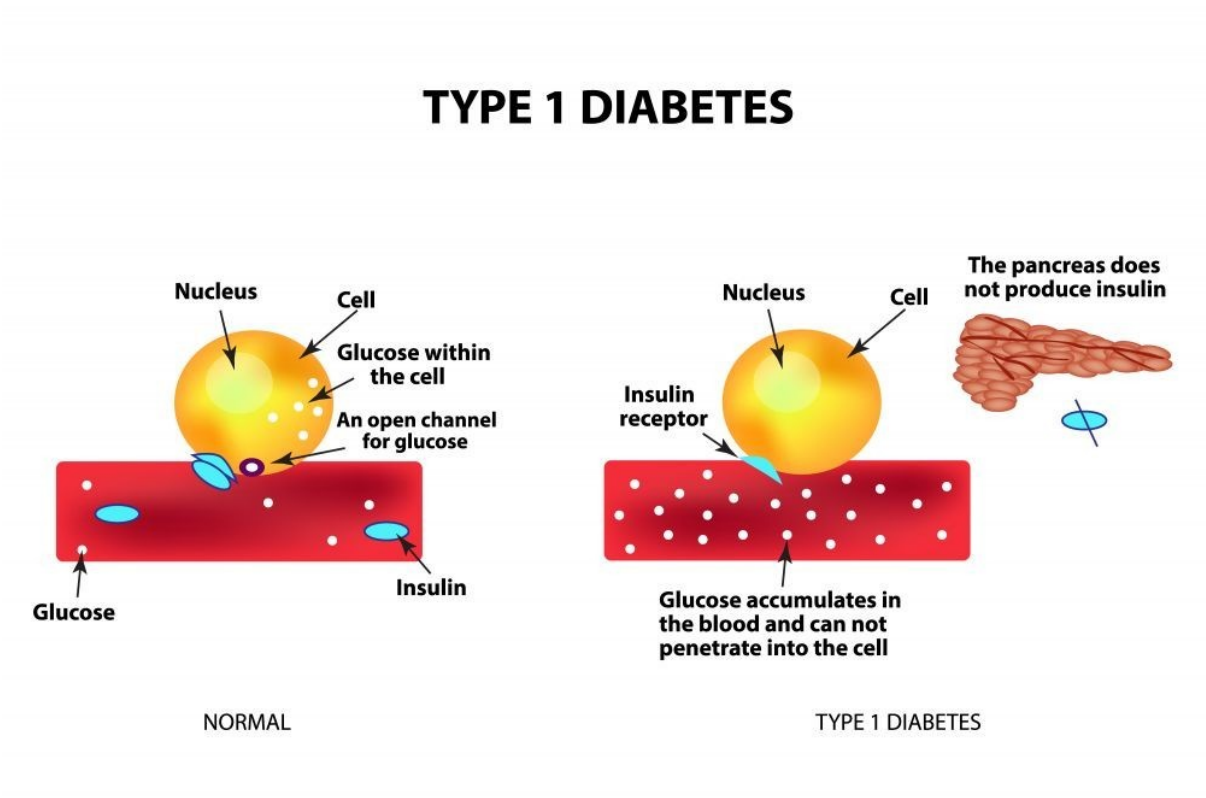


Figure: 3 Mechanism of type 1 Diabetes Mellitus

After beta cells are destroyed, it is thought that the inflammatory process abates and the islets become atrophic. Studies of the autoimmune process in humans and in animal models of type 1 DM (NOD mouse and BB rat) have identified the following abnormalities in the humoral and cellular arms of the immune system:

(1) Islet cell autoantibodies;

(2) Activated lymphocytes in the islets, peripancreatic lymph nodes, and systemic circulation;

(3) T lymphocytes that proliferate when stimulated with islet proteins; and

(4) Release of cytokines within the insulitis.

- ✓ Beta cells seem to be particularly susceptible to the toxic effect of some cytokines (tumor necrosis factor α [TNF-α], interferon γ, and interleukin 1 [IL-1]). The precise mechanisms of beta cell death are not known but may involve formation of nitric oxide metabolites, apoptosis, and direct CD8+ T cell cytotoxicity.
- ✓ The islet destruction is mediated by T lymphocytes rather than islet autoantibodies, as these antibodies do not generally react with the cell surface of islet cells and are not capable of transferring DM to animals.
- ✓ Efforts to suppress the autoimmune process at the time of diagnosis of diabetes have largely been ineffective or only temporarily effective in slowing beta cell destruction. Pancreatic islet molecules targeted by the autoimmune process include insulin, glutamic acid decarboxylase (GAD; the biosynthetic enzyme for the neurotransmitter GABA), ICA-512/IA-2 (homology with tyrosine phosphatases), and a beta cell–specific zinc transporter (ZnT-8). Most of the autoantigens are not beta cell–specific, which raises the question of how the beta cells are selectively destroyed.
- ✓ Current theories favor initiation of an autoimmune process directed at one beta cell molecule, which then spreads to other islet molecules as the immune process destroys beta cells and creates a series of secondary autoantigens.
- ✓ The beta cells of individuals who develop type 1 DM do not differ from beta cells of normal individuals because islets transplanted from a genetically identical twin are destroyed by a recurrence of the autoimmune process of type 1 DM.

PATHOPHYSIOLOGY OF TYPE 2 DM:-

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 DM (≥80% of

patients are obese). In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state. IGT, characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues. Although both insulin resistance and impaired insulin secretion contribute to the pathogenesis of type 2 DM, the relative contribution of each varies from individual to individual.

GUIDELINES FOR ONGOING, COMPREHENSIVE MEDICAL CARE FOR PATIENTS WITH DIABETES

- Optimal and individualized glycemic control
- Self-monitoring of blood glucose (individualized frequency)
- HbA1c testing (2–4 times/year)
- Patient education in diabetes management (annual); diabetes-self management education and support
- Medical nutrition therapy and education (annual)
- Eye examination (annual or biannual)
- Foot examination (1–2 times/year by physician; daily by patient)
- Screening for diabetic nephropathy (annual)
- Blood pressure measurement (quarterly)
- Lipid profile and serum creatinine (estimate GFR) (annual)
- Influenza/pneumococcal/hepatitis B immunizations
- Consider antiplatelet therapy

Abbreviations: GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}.

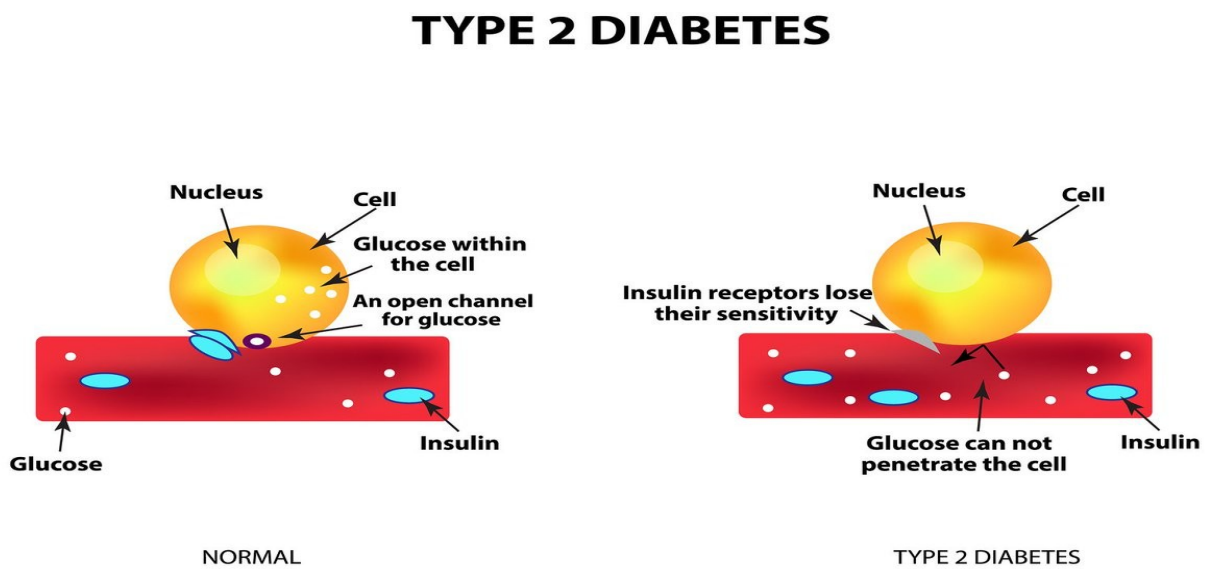


Figure: 4 Mechanism of type 2 Diabetes Mellitus in cell

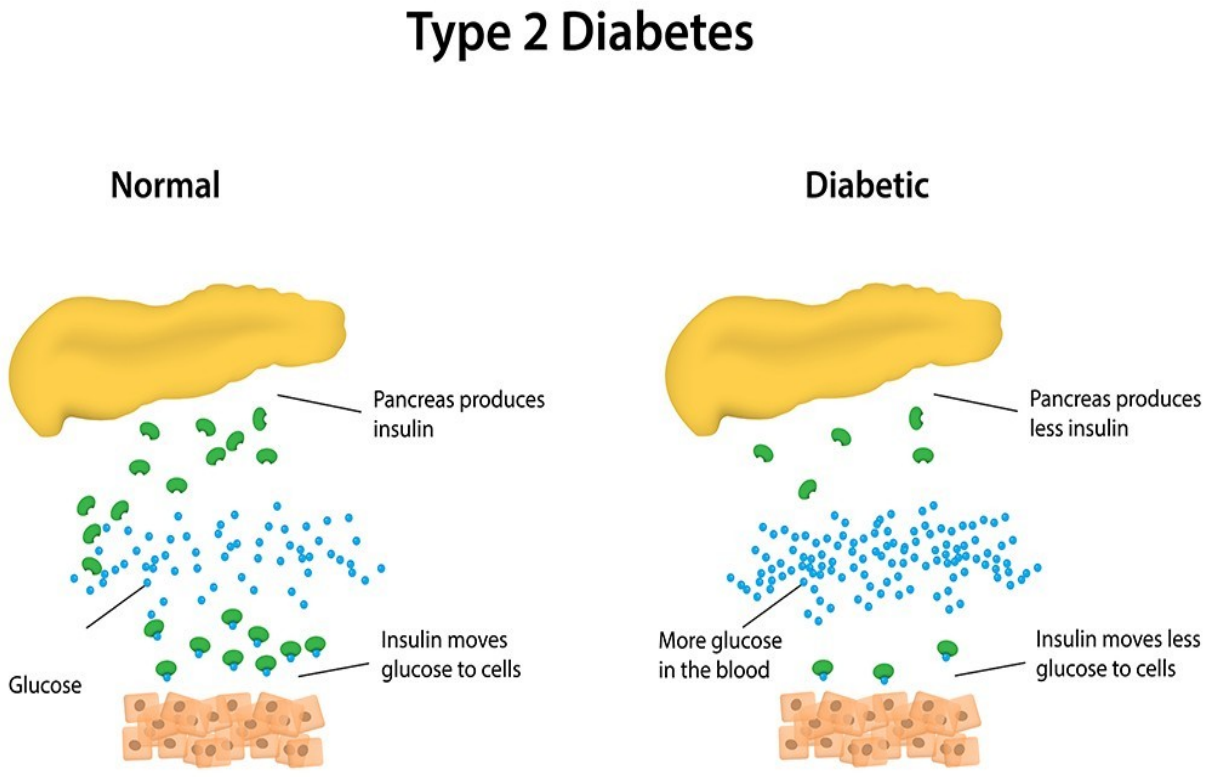


Figure: 5 Mechanism of type 2 Diabetes Mellitus

Table: 1 AGENT USED FOR TREATMENT OF TYPE 1 OR TYPE 2 DIABETES

ORAL	M. O. A	EXAMPLES	ADVAN TAGE	DIS-ADVAN TAGE	CONTRA-INDICATION
Biguanides	↓Hepatic glucose production	Metformin	Weight neutral, do not cause hypoglycemia, inexpensive, extensive experience, ↓ CV events	Diarrhea, nausea, lactic acidosis	Serum creatinine >1.5 mg/dL (men) >1.4 mg/dL (women) (see text), CHF, radiographic contrast studies, hospitalized patients, acidosis
α-Glucosidase inhibitors	↓GI glucose absorption	Acarbose, miglitol, voglibose	Reduce postprandial glycemia	GI flatulence, liver function tests	Renal/liver disease
Dipeptidyl peptidase IV inhibitors	Prolong endogenous GLP-1 action	Alogliptin, Anagliptin, Gemigliptin, linagliptin, saxagliptin, sitagliptin, teneligliptin, vildagliptin	Well tolerated, do not cause hypo glycemia	-----	Reduced dose with renal disease; one associated with increase heart failure risk; possible association with ACE inhibitor–induced angioedema

Insulin secretagogues : Sulfonylureas	↑Insulin secretion	Glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glyburide, glyclopyramide	Short onset of action, lower postprandial glucose, inexpensive	Hypoglycemia, weight gain	Renal/liver disease
Insulin secretagogues : Non-sulfonylureas	↑Insulin secretion	Nateglinide, repaglinide, mitiglinide	Short onset of action, lower postprandial glucose	Hypo glycemia	Renal/liver disease
Sodium-glucose cotransporter 2 inhibitors	↑Urinary glucose excretion	Canagliflozin, dapagliflozin, empagliflozin	Insulin secretion and action independent	Urinary and vaginal infections, dehydration, exacerbate tendency to hyperkalemia	Limited clinical experience; moderate renal insufficiency
Thiazo-lidinediones	↓Insulin resistance, ↑ glucose utilization	Rosiglitazone, pioglitazone	Lower insulin requirements	Peripheral edema, CHF, weight gain, fractures, macular edema	CHF, liver disease
Parenteral Amylin agonists	Slow gastric emptying, ↓ glucagon	Pramlintide	Reduce postprandial glycemia, weight loss	Injection, nausea, ↑ risk of hypoglycemia with insulin	Agents that also slow GI motility

GLP-1 receptor agonists	↑ Insulin, ↓ glucagon, slow gastric emptying, satiety	Exenatide, liraglutide, dulaglutide	Weight loss, do not cause hypoglycemia	Injection, nausea, ↑ risk of hypoglycemia with insulin secretagogues	Renal disease, agents that also slow GI motility; medullary carcinoma of thyroid
Insulin	↑ Glucose utilization, ↓ hepatic glucose production, and other anabolic actions	Aspart, Glulisine, Lispro, Detemir, Glargine	Known safety profile	Injection, weight gain, hypoglycemia	-
Medical nutrition therapy and physical activity	↓ Insulin resistance, ↑ insulin secretion	Low-calorie, low-fat diet, exercise	Other health benefits	Compliance difficult, long-term success low	-

Abbreviations: M.O.A - Mechanism Of Action, ACE – Angiotensin Converting Enzyme, CHF -Congestive Heart Failure, CV – Cardiovascular, GI – Gastrointestinal, HbA1c - Hemoglobin A_{1c}.

ADVERSE EFFECTS OF THERAPY FOR DIABETES MELLITUS:-

As with any therapy, the benefits of efforts directed toward glycemic control must be balanced against the risks of treatment. Side effects of intensive treatment include an increased frequency of serious hypoglycemia, weight gain, increased economic costs, and greater demands on the patient. In the DCCT, quality of life was very similar in the intensive and standard therapy groups. The most serious complication of therapy for DM is hypoglycemia, and its treatment with oral glucose or glucagon injection. Severe, recurrent hypoglycemia warrants examination of treatment regimen and glycemic goal for the individual patient. Weight gain occurs with most (insulin, insulin secretagogues, thiazolidinediones) but not all (metformin, α-glucosidase inhibitors, GLP-1 receptor agonists, DPP-IV inhibitors) therapies. The weight gain is partially due to the anabolic effects of insulin and the reduction in glucosuria. As a result of recent controversies about the optimal glycemic goal and concerns about safety, the FDA now requires information about the cardiovascular safety profile as part of its evaluation of new treatments for type 2 DM.

DIABETES-RELATED COMPLICATIONS:-

Microvascular

a. Eye disease
   Retinopathy (nonproliferative/proliferative)
   Macular edema
b. Neuropathy
   Sensory and motor (mono- and polyneuropathy)
   Autonomic
   Nephropathy (albuminuria and declining renal function)

Macrovascular

- Coronary heart disease
- Peripheral arterial disease

- Cerebrovascular disease

Other

- Gastrointestinal (gastroparesis, diarrhea)
- Genitourinary (uropathy/sexual dysfunction)
- Dermatologic
- Infectious
- Cataracts
- Glaucoma
- Cheiroarthropathya
- Periodontal disease
- Hearing loss

Other comorbid conditions associated with diabetes (relationship to hyperglycemia is uncertain): depression, obstructive sleep apnea, fatty liver disease, hip fracture, osteoporosis (in type 1 diabetes), cognitive impairment or dementia, low testosterone in men.

Review of literature

2. REVIEW OF LITERATURE

- **PHARMACOGNOSTICAL REVIEW**

1. Mydeen fathima begam k., (2014) reported the Seed histochemistry of *Melia azedarach* Linn.[20]
2. Shweta vekaria et al, (2014) reported the pharmacognostic evaluation of root of Mahanimba (*Melia azedarach* Linn).[21]
3. Garima Mishra et al, (2013) reported the *Melia azedarach* Linn*:* A review.[22]
4. Sabira sultana et al, (2012) reported the Comprehensive review on ethanobotanical uses, phytochemistry and pharmacological properties of *Melia azedarach* Linn.[23]
5. Italo chiffelle G. et al, (2009) studied the physical & chemical characterization of *Melia azedarach* Linn fruit and leaf for use as botanical insecticide.[24]

- **PHYTOCHEMICAL REVIEW**

6. Kaushal Kanwer shekhawat et al, (2017) reported the Phyto-morphological overview of medicinal plant: *Melia azedarach* Linn.[25]
7. Shweta R. vekariya et al, (2016) studied the physico-chemical, phyto-chemical & HPTLC analysis of root of *Melia azedarach* Linn.[26]
8. Fang zhou et al, (2016) reported the Four new Tirucallane triterpenoids from the fruits of *Melia azedarach* Linn and their cytotoxic activities.[27]
9. Noor s. Jaafar et al,(2016) reported the Qualitative phytochemical comparison between flavonoids and phenolic acids contents of leaves and

fruits of *Melia azedarach* Linn (family: meliaceae) cultivated in iraq by HPLC and HPTLC.[28]

10. Bharat Pokhrel et al, (2015) reported the phytochemical screening, antimicrobial and antioxidant activity of *Melia azedarach* Linn leaves in methanol solvent. [29]
11. Krishnaiah G.M. et al, (2014) studied the Phytochemical Studies and GC-MS Analysis of the Leaf Extracts of *Melia azedarach* Linn. [30]
12. Dr. Sumathi A., (2013) reported the evaluation of physicochemical and phytochemical parameters of *Melia azedarach* Linn leaves (Family: Meliaceae).[31]
13. Mohammed Fazil Ahmed et al, (2012) reported the Phytochemical Studies and Hepatoprotective activity of *Melia azedarach* Linn, against CCl4 induced Hepatotoxicity in rats.[32]
14. Vijayakumar S. et al, (2012) studied the Histological and physiochemical standardization of *Melia azedarach* Linn. bark.[33]

- **PHARMACOLOGICAL REVIEW**

15. Martin dade et al, (2018) reported the repellent & lethal activities of extracts from fruits of chinaberry against Triatoma infestans.[34]
16. Kuniaki nerome et al, (2018) reported the functional growth inhibition of influenza A and B viruses by liquid and powder components of leaves from the sub tropical plant *Melia azedarach* Linn.[35]
17. Martha Ervina et al, (2018) reported the review: *Melia azedarach* Linn. as a potent anticancer drug.[36]
18. Vijayalakshmi S. et al, (2018) studied the antimicrobial activities of mountain neem (*Melia azedarach*) in the areas of wollega university, nekemte, ethiopia.[37]

19. Daniel seifu et al, (2017) reported the Antidiabetic and gastric emptying inhibitory effect of herbal *Melia azedarach* Linn leaf extract in rodant model of diabetes type 2 mellitus.[38]

20. Sumarawati T. et al, (2017) studied the Anticancer Mechanism of *Melia azedarach* Linn, Doxorubicin and Cyclosphamide Combination against Breast Cancer in Mice.[39]

21. Muhammad Khawar Abbas et al, (2017) reported the Antifungal, Antioxidant and Phytochemical Screening of *Melia azedarach* Flower Extracts by Using Different Solvents.[40]

22. Ishaq H., (2016) studied the Anxiolytic and antidepressant activity of different methanolic extracts of *Melia azedarach* Linn.[41]

23. Prashant kumar et al, (2014) reported the Anti-hyperglycemic effect of the leaves of *Melia azedarach* on alloxan induced diabetic rats.[42]

24. Senthil Rajan Dharmalingam et al, (2014) reported the Anti-Urolithiatic Activity of *Melia azedarach* Linn Leaf Extract in Ethylene Glycol-Induced Urolithiasis in Male Albino Rats.[43]

25. Azam M. M.et al, (2013) reported the Pharmacological potentials of *Melia azedarach* Linn –A review.[44]

26. Deepika Sharma et al, (2013) reported the preliminary and pharmacological profile of *Melia azedarach* Linn*:* An overview.[45]

27. Vijayanand s. et al, (2011) studied the Evaluation of antidiabetic activity of *Melia azadirachta* on alloxan induced diabetic rats.[46]

28. Ramya S. et al, (2009) reported the In Vitro Antibacterial Prospective of Crude Leaf Extracts of *Melia azedarach* L. against Selected Bacterial Strains.[47]

29. María C. Carpinella et al, (2003) studied the Antifungal Effects of Different Organic Extracts from *Melia azedarach* on Phytopathogenic Fungi and Their Isolated Active Components.[48]

30. Zakir-ur-rahman et al, (1991) studied the Toxicological studies of *Melia azedarach* Linn (flowers and berries).[49]

31. Hanifa moursi S.A et al, (1985) studied the effect of *Melia azedarach* fruits on gipsing-restraint stress induced ulcers in rats.[50]

Ethno botanical survey

3. ETHNO BOTANICAL SURVEY

Figure No: 6. *Melia azedarach*

Figure No: 7. *Melia azedarach* tree

Figure No: 8. Leaves and Flowers

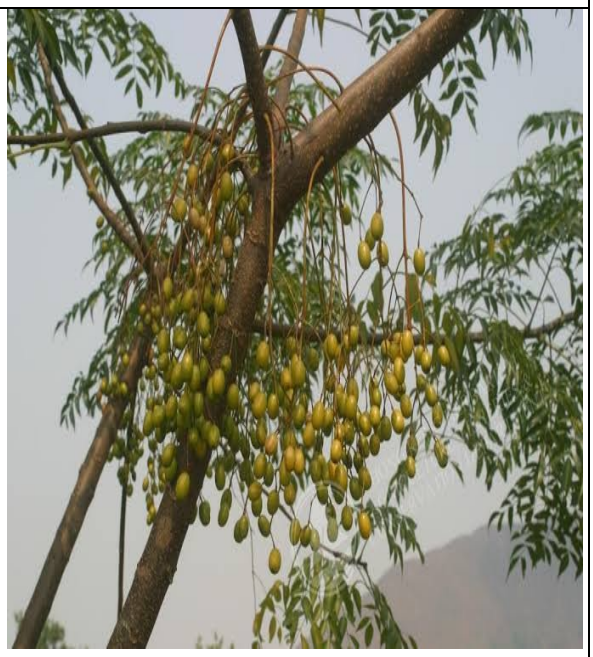


Figure No: 9. Fruits

Figure No: 10. Stem and roots

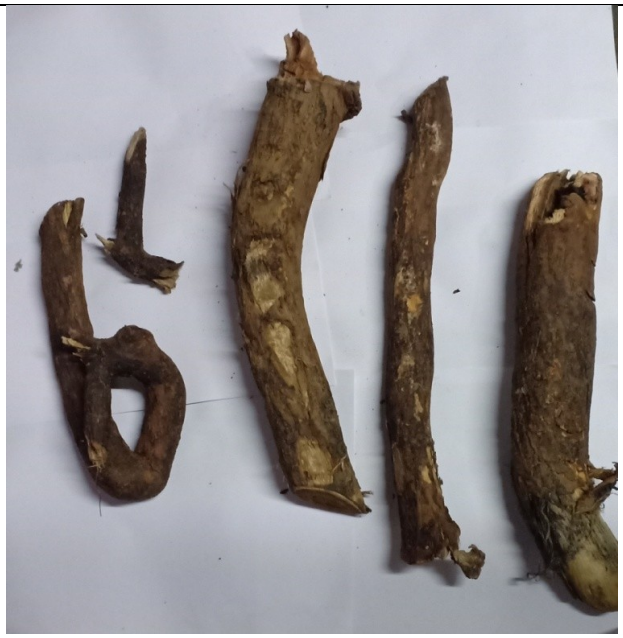


Figure No: 11. Roots of *Melia azedarach*

- **PLANT PROFILE**

Plant Form : Tree

Plant name : *Melia azedarach Linn*

Common names : Chinaberry

Synonyms : *M. dubia cavanilles, M. composita willd, M. sempervirens*

Family : Meliaceae

Flower colour : Purple, violet, white

Flower arrangement : Panicle

Leaf type : Compound

Leaf arrangement : Alternate

Mono/Dicot : Dicot plant

- **TAXONOMICAL STATUS[51]**

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Sapindales

Family - Meliaceae

Genus - Melia

Species - *M. azedarach*

Binomial name - *Melia azedarach* Linn.

- **Veracular names[52]**

Tamil – Malaivembu, Tittam

Telugu – Kondavepa, Yerri-vepa

Assam - Khammaga

Bengali – Ghoranim

English – Persian lilac

Gujarati – Barkanlimbodo

Hindi – Bakayan, Mahanima

Kannada – Turakabevu, Arebevu

Malayalam – Malaveppu, Simaveppu

Marathi – Bakananimb

Sanskrit – Mahanimbah

- **Plant Parts Used**[51]

Leaf, flower, seed, oil, root, young branches, fruit and bark.

- **Monograph**

Bengali name: Ghoda Neem.

Common name: Chinaberry tree, Texas umbrella,

Scientific name: *Melia azedarach* Linn.,

Family: Meliaceae.

- **Morphology**

M. azedarach L. is a small to medium sized deciduous tree. It grows to a height of 5 to 15m tall and 30 to 60cm in diameter. The plant is characterized by the presence of a spreading, dense and dark green crown. Its bark is dark brown in color, relatively smooth, and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green and relatively pale. Flowers are white with purple stripes and are characterized by the presence of a typical fragrance. Fruits or berries are yellow, round, smooth, and fleshy. Dried fruits are hard with 4 to 5 seeds.

Duration: Perennial.

Growth habit: Multi-branched tree.

Bangladesh nativity: Native.

- **Tree Propagation and Keeping**

With an extensive and deep root system, the neem can grow also in marginal and leached soils, up to an altitude of 1500 m. The tree requires annual rainfall from 500 to 1150 mm; it tolerates drought but cannot withstand water-logged areas and poorly drained soils. Neem is propagated from seeds or cuttings. Young seeds germinate readily in 14 to 21 days at 19 to 22° C. The neem tree will grow in full sun to partial shade, better in a well-drained soil mix. The tree grows well in pots and its size is easily controlled by

pruning. Even though very drought tolerant in nature, it is more sensible when still in pots. Trees in pots should be watered regularly and the soil should dry slightly before watering again. The plants can be fertilized monthly to increase growth speed. Watering should be reduced and fertilization avoided during cold months. All repotting and pruning should be done at this time.

- **Distribution**

It is native in Pakistan, India, Indochina, Southeast Asia and Australia. It is widespread and naturalized in most of the tropics and subtropical countries. The genus Melia includes four other species, occurring from Southeast Asia to northern Australia.

- **PHYTOCHEMICAL CONSTITUENTS[52]**

Terpenoids, Flavonoids, Steroids, Acids, Anthraquinones, Saponins, Alkaloids, Tannins and Limonoids.

- **ETHNOBOTANICAL USES**

Leaf – Amenorrhoea, bronchitis, cardiac diseases, cough, inflammation, leprosy, scabies.

Fruit - Skin diseases.

Flower – Dysentery, Fever.

Seed – Fever, helminthiasis, typhoid

Root – Amenorrhoea, asthma, **diabetes**, fever, cough, Skin diseases, ulcer, leprosy, vomiting & wound.

Rationale for selection

4. RATIONALE FOR SELECTION

- The plant *Melia azedarach* Linn, belonging to the family Meliaceae was selected for the present work.
- The traditionally claimed properties associated with the plant used in claimed were amenorrhoea, asthma, burning sensation, bronchitis, cough, **diabetes**, dysentery, fever, general debility helmintic, leprosy, leucoderma, lumbago, sciatica, skin diseases, ulcer, vomit, wound, cardiac diseases, hysteria, inflammation, scabies, typhoid.
- Traditional claim reveals that root of *Melia azedarach* is having anti-diabetic activity [52]. Leaves, Flowers, Fruits, Seeds and barks were scientifically validated for anti-diabetic activity.
- The anti-diabetic activity was not scientifically validated on roots of *Melia azedarach.*
- So this plant has been selected for anti-diabetic activity.

Aim and Objectives

5. AIM & OBJECTIVES

- The aim and objective of the study was to evaluate the pharmacognostical, phytochemical & pharmacological profile on the roots of *Melia azedarach* Linn.
- Collection & authentication of the plant specimen.
- Establishing pharmacognostical profile of the plant.
- Extraction of the plant material by using soxhlet apparatus with solvents of the increasing polarity such as Petroleum ether > Chloroform > Ethyl acetate > Ethanol > Aqueous (cold maceration).
- Phytochemical screening & determination of bioactive constituents.
- Evaluation of antidiabetic activity by *In vitro* & *In vitro* models.

Plan of Work

6. PLAN OF WORK

1. PHARMACOGNOSTICAL STUDIES

- Collection of plant material
- Authentication
- Macroscopical studies
- Microscopical studies
- Powder Microscopy
- Linear measurement
- Histochemical analysis
- Physiochemical constants

a) Ash values

Total ash values

Water soluble ash value

Acid insoluble ash value

Sulphated ash value

b) Extractive values

Water soluble extractive values

Alcohol soluble extractive values

Non volatile Ether soluble extractive values

c) Determination of crude fibre content

d) Determination of loss on drying

e) Determination of swelling index

f) Determination of foaming index

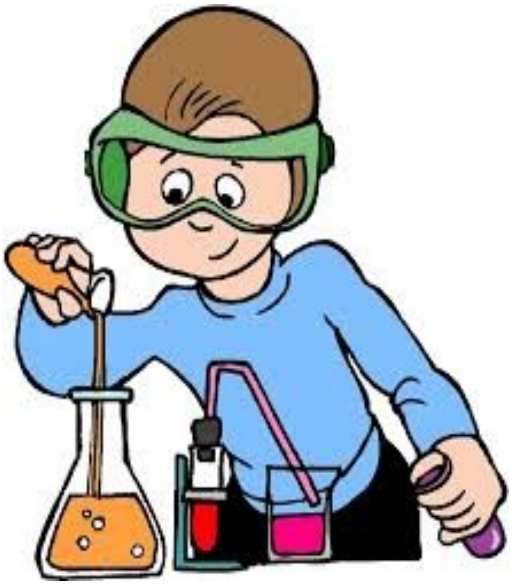
- Qualitative & Quantitative estimation of heavy metals & inorganic elements.

2. PHYTOCHEMICAL STUDIES

- Preparation of Extracts.
- Preliminary phytochemical screening of powder & extracts.
- Fluorescence analysis of powder & extracts.
- Qualitative & Quantitative estimation of phytoconstituents.

3. PHARMACOLOGICAL STUDIES

- *In vitro* evaluation of anti-diabetic activity
  - α – amylase inhibition assay
  - Non enzymatic glycosylation of haemoglobin method
- *In vivo* evaluation of anti-diabetic activity
  - Streptozotocin induced diabetes in rats
  - Measurement blood glucose level
  - Measurement of body weight.
  - Histopathological examination of pancreas.



Materials and methods

7. MATERIALS & METHODS

PHARMACOGNOSTICAL STUDIES

Collection & authentication of plant material

The roots of *Melia azedarach* Linn was collected during August 2018, from Sunguvarchatram, Kanchipuram, Tamilnadu, India.

Identification and Authentication of plant material

The identification & authentication of the sample was carried out by Dr. K. N. Sunil Kumar, R.O. and HOD Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai – 106. It was identified as *Melia azedarach* L, Meliaceae family (M23081801A).

MACROSCOPY

Macroscopical characters include organoleptic characters and various morphological features of the plant were studied. Hence this observation is primary importance before any further testing can be carried out. Organoleptic characters such as color, odour, taste and nature are studied. Morphological characters such as size, shape, surface, fracture and thickness were observed for the determination of safety, efficacy and purity of crude drugs. Fresh roots of *Melia azedarach* Linn were collected & different organoleptic features viz. colour, type, odour, taste were observed. These parameters are considered useful in the qualitative control of the crude drug & evaluated as per standard WHO guidelines.

MICROSCOPY[53-59]

Staining method:

Fixation of plant material;-

The sample or roots was cut fixed in FAA solution (formalin 5ml+Acetic acid 5ml+90ml of 70% ethanol). The specimen was dehydrated after 24 hours of fixing. The roots were graded with series of tertiary butyl alcohol as per the standard method.

Infiltration of the specimen;-

It was carried out by gradual addition of 58-60ºC of melting pointed paraffin wax until Tertiary butyl alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning;-

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12µ. Dewaxing of the sections was done by customary procedures. The sections were stained Toludine blue. Since toludine blue in a polychromatic stain, the sections were stained as per the method published by 'O' Brein et al. The staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to subrein, violet to mucilage and blue to the protein bodies. Whenever, necessary sections were also stained with safranin, fast green and iodine for starch.

PHOTO MICROGRAPHS

Microscopic descriptions of tissues were supplemented with photo micrographs whenever necessary. Photographs of different magnifications were taken with Nikon lap photo 2 microscopic units. For normal observations bright field was used. For the study of crystals & lignified cells, polarized light was employed. Since, these structures have bi refringent property under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books.

POWDER MICROSCOPY

Washed, Shade dried root of *Melia azedarach* Linn were coarsely powdered well (with the help of grinder), then the powder was passed through the sieve no. 60 & used for powder analysis & organoleptic characters such as nature, colour, odour & taste were studied. Powder was stained with 1% phloroglucinol in 90% ethanol, N/50 iodine, Conc. Hydrochloric acid and glycerin. Slides were observed under the microscope.

LINEAR MEASUREMENTS

Measurement of length & width of fibres in powdered roots of *Melia azedarach* Linn

The roots of *Melia azedarach* Linn were powdered by means of a wood grinder. This powder was then passed through sieve no.60 and used for quantitative microscopy. Powder analysis of the root powder showed the presence of fibers; hence we proceeded measuring the dimensions of the fibers.

The first step involved was the calibration of the eye piece micrometer using the stage micrometer. For determining calibration factor, the eye piece was removed from the microscope, the lens was unscrewed and in the ridge the eyepiece was placed. The lens was then placed. The stage micrometer was then placed on the stage of the microscope and focused under high power with the eyepiece coincides with which division of stage micrometer and calculate the calibration factor using the formula:

$$\text{Each division of eyepiece micrometer} = \frac{\text{No of divisions of stage micrometer}}{\text{No of divisions of eyepiece micrometer}} \times 10$$

The stage micrometer was replaced with the slide containing the powdered drug. For the preparation of the slide, a little quantity of powder first boiled with chloral hydrate solution. The cleared powder was removed in a watch glass and stained with one drop each of phloroglucinol and concentrated hydrochloric acid. A little of this powder was then taken on a slide mounted in dilute glycerin and observed under low power. The width and length of stained fibres was measured by focusing them on the lines of the eyepiece micrometer. Note the no. of divisions covered by the length and width of the fibres.

Measurement of length & width of starch in powdered roots of *Melia azedarach* Linn

The roots of *Melia azedarach* Linn were powdered by means of a wood grinder. This powder was then passed through sieve no.60 and used for quantitative microscopy. Powder analysis of the root powder also showed the presence of starch; hence we proceeded with measuring the length & width of starch.

The first step involved was the calibration of the eye piece micrometer using the stage micrometer. For determining calibration factor, the eye piece was removed from the microscope, the lens was unscrewed and in the ridge the eyepiece was placed. The lens was then placed. The stage micrometer was then placed on the stage of the microscope and focused under high power with the eyepiece coincides with which division of stage micrometer and calculate the calibration factor using the formula:

$$\text{Each division of eyepiece micrometer} = \frac{\text{No of divisions of stage micrometer}}{\text{No of divisions of eyepiece micrometer}} \times 10$$

The stage micrometer was replaced with the slide containing the powdered drug. For the preparation of the slide, a little quantity of powder first boiled with chloral hydrate solution. The cleared powder was removed in a watch glass and stained with one drop each of iodine solution and. A little of this powder was then taken on a slide mounted in dilute glycerin and observed under low power. The length and width of stained starch was measured by focusing them on the lines of the eyepiece micrometer. Note the no. of divisions covered by the length and width of the starch.

Calibration factor

One smallest division of stage = 0.01mm or 10µm

$$\text{Each division of eyepiece micrometer} = \frac{\text{No of divisions of stage micrometer}}{\text{No of divisions of eyepiece micrometer}} \times 10$$

HISTOCHEMICAL ANALYSIS[60-61]

Portions of fresh roots of the plant of *Melia azedarach* L. were used. The roots were soaked in water before taking the sections. The sections were stained using specific reagents (N/50 iodine, dilute ferric chloride, phloroglucinol & Con. HCL, picric acid, ortho toludine blue & dragendroff's reagent) to observe & locate starch, lignin, tannin, protein,

flavanoid & alkaloid respectively as per the protocols. The stained sections were then washed in water to remove the excess stain & observed under a microscope.

PHYSIO-CHEMICAL CONSTANTS[62-64]

Shade dried powdered plant material of the roots of *Melia azedarach* were used for the determination of the physio-chemical constants in accordance with the WHO (World Health Organization) guidelines.

DETERMINATION OF ASH VALUES:-

Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which compiles of inorganic salts, naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination of quality and purity of crude drug in the powdered form.

TOTAL ASH

Total ash is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot bath 30 minutes and cooled in the desiccator incinerate about 2 to 3g accurately weighed, of the ground drug in the tarred silica dish at a temperature not exceeding 450ºC until the sample was free from carbon, cooled in a desiccator and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

Percentage of Total ash value = (weight of total ash/weight of drug taken× 100)

WATER SOLUBLE ASH

It is the difference in weight between the total ash and the residue after treatment of the total ash in water.

Procedure:

Total ash obtained was boiled for 5 minutes with 25ml of water, insoluble matter were collected in an ashless filter paper, washed with hot water and ignite for 15 minutes at a temperature not exceeding 450ºC. Subtract the weight of the residue in mg from the weight of the total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

Percentage of Water soluble ash = (Weight of residue obtained/ Weight of the sample taken) *100

ACID INSOLUBLE ASH

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25ml of dilute hydrochloric acid was added. The insoluble matter was collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter to the original crucible dry on hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

Percentage of Acid soluble ash = (Weight of residue obtained/ Weight of the sample taken) *100

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These tests are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible was heated to redness for 10 minutes, allowed to cool in the desiccator and weigh. 2g of sample were accurately weighed, ignited gently then thoroughly chared. Cool, moistened the residue with 1ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25ºC until all black particles have disappeared. Crucible was allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow cooling and weighing. This process was repeated until two successive weighing differ by more than 0.5 mg.

Percentage of Sulphated ash = (Weight of residue obtained/ Weight of the sample taken) *100

DETERMINATION OF EXTRACTIVE VALUES:-

Extractive values are useful for the evaluation for phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in the crude drug.

Determination of water soluble extractive:

5g of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for 6 hours and allowed to stand for rest 18 hours. It was then filtered rapidly; taking precaution against loss of solvent and 25ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC for 1 hour in the hot air oven and cooled in a desiccator for 30 minutes and weighed. The process is repeated till a constant weight is obtained; the percentage of water soluble extractive value was calculated with reference to the air dried drug.

Percentage of water soluble extractive value = (Weight of the dried extract/ Weight of the sample taken) × 100.

Determination of alcohol soluble extractive:

5g of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for 6 hours and allowed to stand for rest 18 hours. It was then filtered rapidly; taking precaution against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105ºC for 1 hour in the hot air oven and cooled in a desiccator for 30 minutes and weighed. The process was repeated till a constant weight is obtained; the percentage of alcohol soluble extractive value was calculated with reference to the air dried drug.

Percentage of alcohol soluble extractive value = (Weight of the dried extract/ Weight of the sample taken) × 100.

Determination of non-volatile ether soluble extractive:

A suitable weighed quantity of the sample was transferred to an extraction thimble and extracted with solvent ether or petroleum ether (Boiling point 40 - 60ºc) in a soxhlet for 6 hours. The extract was filtered into a tarred evaporating dish, evaporated and dried at 105ºc to constant weight. The percentage of non volatile ether soluble extractive value with reference to the air dried drug was calculated.

Percentage of non-volatile ether soluble extractive value = (Weight of the dried extract/ Weight of the sample taken) × 100.

DETERMINATION OF CRUDE FIBRE CONTENT:-

About 2 g of a drug sample accurately weighed was extracted with ether. Then 200ml of 1.25% sulphuric acid was added to the extracted drug and the whole mixture boiled for 30 minutes under reflux in a 500ml flask.

The mixture was then filtered through a hardened filter and the residue was washed with boiling water until free of acid. The entire residue was rinsed back into the flask with 200ml boiling 1.25% sodium hydroxide solution and again boiled under reflux

for 30 minutes. The liquid was then quickly filtered through a tarred filter and the residue on the filter was washed with boiling water until neutral, dried at 110ºC to constant weight. The difference between the weight of the dried residue and that of the incinerated residue represents the weight of the crude fiber. It was expressed as the percentage of the original weight of the material.

LOSS ON DRYING:-

10g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Uses of high speed mill in preparing the samples were avoided. The sample in the tarred evaporating dish was placed in the drying chamber (105ºC) for 5 hours and weigh. Drying and weighing was continued every one hour interval until the difference between two excessive weights is not more than 0.25%. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.001g difference. Percentage moisture content was compared with respect to the air dried sample.

Percentage of moisture content = (Final weight of the sample/ Initial weight of the sample) × 100.

DETERMINATION OF SWELLING INDEX:-

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stopper measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10minutes for 1 hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

DETERMINATION OF FOAMING INDEX:-

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80-90ºC for about 30minutes. it was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The tubes were stoppered and shaked in a length wise motion for 15 seconds, two shakes per second. Allowed to stand for 15minutes and the height of the foam is measured. The results are assessed as follows.

If the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula:

Foaming index = 1000/a

Where, a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

QUALITATIVE AND QUANTITATIVE ESTIMATION OF HEAVY METALS AND INORGANIC ELEMENTS[65]:-

Plant materials play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non essential elements which may be beneficial or harmful to living things. Non essential elements like lead, arsenic, cyanide,

chromium, cadmium, aluminium, silver bring about toxic effects resulting in intoxification. Hence, qualitative and quantitative estimation of inorganic elements in the plant *Melia azedarach* Linn were carried out.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS:-

To the ash of the drug material 50%v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolve readily in strong acid and base, but after boiling it becomes insoluble.

Arsenic: Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (scheele's green) which on boiling gives a red precipitate of cupric oxide.

Borate: The mixture obtained by the addition of sulphuric acid ad alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Calcium: Solution of calcium salts, when treated with ammonium carbonate solution yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Carbonate: Carbonate, when treated with dilute acid effervescene, liberating carbon dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.

Copper: An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute HCL.

Lead: Strong solution of lead salts, when treated with HCL, yields a white precipitate. This is soluble in boiling water and is re-deposited as crystals when the solution is cooled.

Magnesium: Solution of magnesium salts, when boiled with ammonium carbonate solution yields white precipitate, but no precipitate is produced with ammonium chloride solution.

Mercury: Solution of mercury salts, when treated with sodium hydroxide solution, yield a yellow precipitate.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the aide of the test tube), a brown colour is produced at the junction of two liquid, indicating the presence of nitrates.

Phosphate: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.

Potassium: Moderately strong solution of potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

Sulphate: Solutions of sulphate, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide solution.

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS[66]:-

Inductive coupled plasma- optical emission spectroscopy (ICP-OES)

It is an excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly. This technique utilizes the plasma as an ion source or light emission source are capable of producing values.

QUANTITATIVE ESTIMATION OF HEAVY METALS

Instrumentation parameter:

Instrument Name: Inductively Coupled Plasma Optical Emission Spectrometry

Instrument Model: PE Optima 5300 DV ICP-OES

Optical system: Dual View – axial or radial.

Detector system: Charge coupled detector, (UV-VISIBLE detector which is maintaining at - 40°C) to detect the intensity of the emission line wavelength range from 165 to 782nm.

Torch (Light source): Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing in a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2mm inner diameter

Spray chamber: Scott type

Nebulizer: Cross flow Gem tip

Preparation of samples by acid digestion method

Weighed 50g of powdered mixture of powered drug treated with acid mixture of sulphuric acid: water in the ratio of 4: 1 in the kjeldhal flask and heated continuously till the solution is colourless. The sample mixture is then transferred in a 25ml volumetric flask and made upto the volume with distilled water. Blank solution is prepared as above without sample. The standards of lead, cadmium, mercury, arsenic, magnesium, potassium, iron, aluminium, calcium and copper are prepared as per the protocol and the calibration curve is developed for each of them.

Detection

Samples were analysed for the detection and quantification of lead, cadmium, mercury, arsenic, magnesium, potassium, iron, aluminium, calcium and copper by Inductive Coupled Plasma Optical Emission Spectrometry.

PHYTOCHEMICAL STUDIES[67-69]:-

Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with chromatographic technique like TLC. Therefore a complete investigation is required to characterize the phytoconstituents qualitatively and quantitatively.

EXTRACTION:-

Extraction is the process to bring out the metabolites into the extracting solvent depends upon its polarity. The first step was the preparation of successive solvent extracts. The dried coarsely powdered roots of *Melia azedarach* Linn was first extracted with petroleum ether (60-80·C) in soxhlet apparatus and then with solvents of increasing polarity like chloroform, Ethyl acetate and Ethanol at 60-70ºc. They were then followed with maceration in aqueous solvent. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed phyto chemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING:-

The chemical tests for various phytoconstituents in the dried powder and extracts of roots of *Melia azedarach* were carried out as described below and the results are recorded.

1. **Detection of Alkaloids**

Dragendorff's reagent

- The extracts and the powder were dissolved in 5ml of distilled water, to this 5ml of 2M hydrochloric acid was added until an acid reaction occurs, and then 1ml of Dragendroff's reagent was added and examined for an immediate formation of an orange red precipitate.

Mayer's reagent

- The extracts and the powder were mixed with the little amount of dilute hydrochloric acid and Mayer's reagent and examined for the formation of white precipitate.

Wagner's reagent

- The extracts and the powder were treated with a few drops of Wagner's reagent. Formation of brown or reddish brown precipitate indicates the presence of alkaloids.

2. **Detection of Carbohydrates**

Molisch's test

- The extracts and powder were treated with a few drops of alcoholic naphthol then add few drops of concentrated sulphuric acid through the sides of the test tube.
- Formation of purple to violet colour ring appears at the junction of the test tubes indicating the presence of Carbohydrates.

Fehling's test

- The extracts and the powder were treated with Fehling's A and Fehling's B solution and heated. Formation of red colouration indicates the presence of sugar.

3. Detection of Proteins and Amino acids

Biuret test

- The extract and the powder were treated with a few drops of Biuret reagent. Formation of violet colour indicates the presence of proteins.

Xanthoprotein test

- The extracts and the powder were treated with a few drops of conc. Nitric acid and boiled, yellow precipitate is formed. After cooling it, add 40% sodium hydroxide solution. Formation of orange colour indicates the presence of proteins.

Ninhydrin test

- The extracts and the powder were treated with a few drops of 0.25 % ninhydrin reagent and boiled for few minutes. Formation of blue colour indicates the presence of proteins.

4. **Detection of Glycosides**

Borntrager's test

- The extracts and the powder were boiled with 1ml of sulphuric acid for 5 minutes and filtered while hot. The filtrate was cooled and shaken with equal volume of chloroform. The lower layer of chloroform was separated and shaken with half of its volume of dilute ammonia. Formation of rose pink to red colour in the ammoniacal layer indicates the presence of glycosides.

Modified Borntrager's test:

- The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% of ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated

and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer

Test for hydroxy-anthraquinones

- The extracts and the powder were treated with a few drops of potassium hydroxide solution. Formation of red colour indicates the presence of glycosides.

5. Detection of Flavonoids

Shinoda test

- The extracts and the powder were treated with few piece of magnesium turnings and concentrated hydrochloric acid added drop wise. Formation of pink, scarlet, crimson red or occasionally green to blue colour which appear after a few minutes, indicates the presence of flavonoids.

Alkaline reagent test

- **T**he extracts and the powder were treated with a few drops of sodium hydroxide solution; formation of intense yellow colour indicates the presence of flavonoids.

6. Detection of Phenolic compounds

Ferric chloride test

- A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green colour.

7. **Detection of Tannins**

Ferric chloride test

- The extracts and the powder were treated with a few drops of ferric chloride solution. Formation of green colour indicates the presence of tannins.

Gelatin test

- The extracts and the powder were treated with a few drops of 1% gelatin solution containing 10% sodium chloride. Formation of precipitate indicates the presence of tannins.

Lead acetate test

- The extracts and the powder were mixed with basic lead acetate solution and examined for formation of white precipitate.

8. **Detection of Phytosterols**

Libermann-Burchard test

- The extracts and the powder were treated with a few drops of acetic anhydride, boiled and cooled. Then concentrated sulphuric acid is added along the sides of the test tube. A brown ring formation at the junction of two layers and upper layer turning green shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Salkowski test

- The extract was treated with few drops of concentrated sulphuric acid, red colour at lower layer indicates the presence of steroids and formation of yellow colour at lower layer indicates the presence of triterpenoids.

9. **Detection of Saponins**

Froth formation test

- The extracts and the powder were shaken well with water. Formation of stable froth indicates the presence of glycosides.

QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT[70-72]:-

1. DETERMINATION OF TOTAL FLAVONOID CONTENT

Ultra Violet spectroscopy method was carried out for the determination of total flavonoids content.

Preparation of standard stock solution

Accurately weighed 25mg of Quercetin standard transferred to 100ml of volumetric flask and dissolved with dimethyl sulfoxide (DMSO).

Preparation of test solution

The root extract was weighed accurately equal to the weight of standard Quercetin and transferred to 100ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The dilution was made with dimethyl sulfoxide.

Procedure

From the prepared solution of standard and test solution 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium chloride solution to every single concentration. Incubate the solution about 10 minutesute at ambient temperature. After 10 minutesute, standard and sample solution measure the absorbance of spectrophotometrically at 435nm with the standard and test sample solutions.

DETERMINATION OF TOTAL PHENOLIC CONTENT

Folin- Ciocalteu's assay

Methods

Ultra Violet spectroscopy method was carried out for the determination of total phenolic content.

Preparation of 16%Na_2Co_3

16gm w/w of sodium carbonate dissolved in 100ml of water.

Preparation of standard

40gm of Gallic acid standard transferred 100ml of standard flask. Add to this 50ml distilled water and shake well until the sample dissolved. Made volume with distilled water and withdrawn 10ml of the above solution into 100ml standard flask then make up to volume with distilled water.

Preparation of test solution

The 1gm of extract transferred to 250ml of flask, adds to this 50ml of water and heated on a water bath about 45minutes. Then the solution was cool and made up to volume with distilled water, the solution was centrifuged at 5000rpm about 20 minutes. Then 2ml of super saturation solution transferred to flask add to this 2.5ml of 7.5% sodium carbonate. After incubation at 40˚C for 30 minutes, the absorbance of the reaction mixtures was measured at 765 nm in a spectrometer. Three replicates were made for each test sample.

FLUORESCENCE ANALYSIS[73]

Fluorescence analysis was carried out in day light and in UV light. The root powder and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light and results were tabulated.

CHROMATOGRAPHY[74]

Chromatographic techniques are an important analytical tool in the separation, identification and estimation of different phytoconstituents present in the plant extract.

THIN LAYER CHROMATOGRAPHY

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is a reliable technique in which solute undergoes distribution between two phases, stationary phase and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

TLC Plate preparation

The plates are prepared using TLC spreader. 40g of silica G was mixed with 85ml of water to prepare homogenous suspension and poured in a spreader. 0.25mm thickness of plates is prepared, air dried until transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in a desiccator.

Selection of mobile phase

The solvent mixture was selected on the basis of the phytoconstituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents.

Solvent system:

The extracts were run in following mobile phases:

Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)

Toluene: ethyl acetate (93:7)

Chloroform: Methanol (27:3)

Detection was done under UV at 254nm and 365nm

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY – FINGER PRINT PROFILE

- HPTLC is one of the versatile chromatographic method which helps in identification of compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug, thus enabling an assessment of drug quality.
- HPTLC serves as a convenient tool for finding the distribution pattern of phytoconstituents which is unique to each plant. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution.
- HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

Instrument conditions

Sample used: Ethanol extract

Instrument: CAMAG HPTLC

HPTLC Applicator: CAMAG LINOMAT IV

HPTLC Scanner: CAMAG TLC SCANNER II

Sample dilution: 5mg/ml in methanol

Volume of injection: 20µl

Mobile phase: Toluene: Ethyl Acetate: Formic Acid (6:2.5:0.5 v/v/v)

Lamp: deuterium 254nm

Stationary phase: Silica Gel 60 F_{254}, Merck

Equipment

CAMAG HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC scanner II.

Chromatographic condition

The estimation has to be done using the following chromatographic conditions. Chromatography was performed on a 10×10 cm pre activated HPTLC silica gel G 60 F254 plate. Samples are applied to the plate as 6mm wide band with an automatic TLC applicator Linomat IV with Nitrogen flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner II. The plates were pre washed with solvent ethyl acetate.

PHARMACOLOGICAL STUDIES[75-79]

IN VITRO ANTIDIABETIC STUDIES:-

1. **α - amylase inhibition assay**

α - amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/l, pH 6.8) at a concentration of 0.1 mg/ml. Various concentrations of sample solutions (0.25 ml) were mixed with α-amylase solution (0.25 ml) and incubated at 37°C for 5 minutes. Then the reaction was initiated by adding 0.5 ml 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 minutes, the reaction was stopped by adding 0.5 ml DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100°C for 5 minutes. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. Acarbose was used as standard.

Percentage of inhibition was calculated by following equation:

%inhibition= [(Absorbance of sample – absorbance of control)/ Absorbance of sample]*100

2. Non-enzymatic glycosylation of haemoglobin method

This test is not important to detect diabetes. It is more important to judge the control of diabetes. The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose and form an abduct Alc. The greater the blood-glucose concentration, the greater is the amount of glucose-bound (called glycosylated) haemoglobin. Such glucose haemoglobin linkage is quite stable and lasts for 60 to 120 days (the life-span of red blood corpuscles).

Thus the amount of glycosylated haemoglobin is a sure guide to the concentration of glucose in the blood (i.e., the degree of control over the disease achieved). Amount of Glycosylated haemoglobin should not be more than 12%.

Antidiabetic activity of roots of *Melia azedarach* Linn were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4.1 ml each of above solution was mixed. Extracts were weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Acarbose was used as a standard drug for assay. Percentage of inhibition was calculated as,

%inhibition= [(Absorbance of sample – absorbance of control)/ Absorbance of sample]*100

***IN VIVO* ANTIDIABETIC STUDIES:-**

Experimental animals

The present study was conducted after obtaining approval from Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA (1917/ReBi/S/16/CPCSEA/25.10.2016) (25/18).

Acute toxicity studies

Acute toxic study has been carried out already by using OECD 423 (Acute Toxic Class Method). In this study there was no toxicity/death observed up to dose of 2500mg/kg. The acute toxicity study in rats showed that upto 2500mg/kg dose, the plant is safe for consumption and medicinal uses.

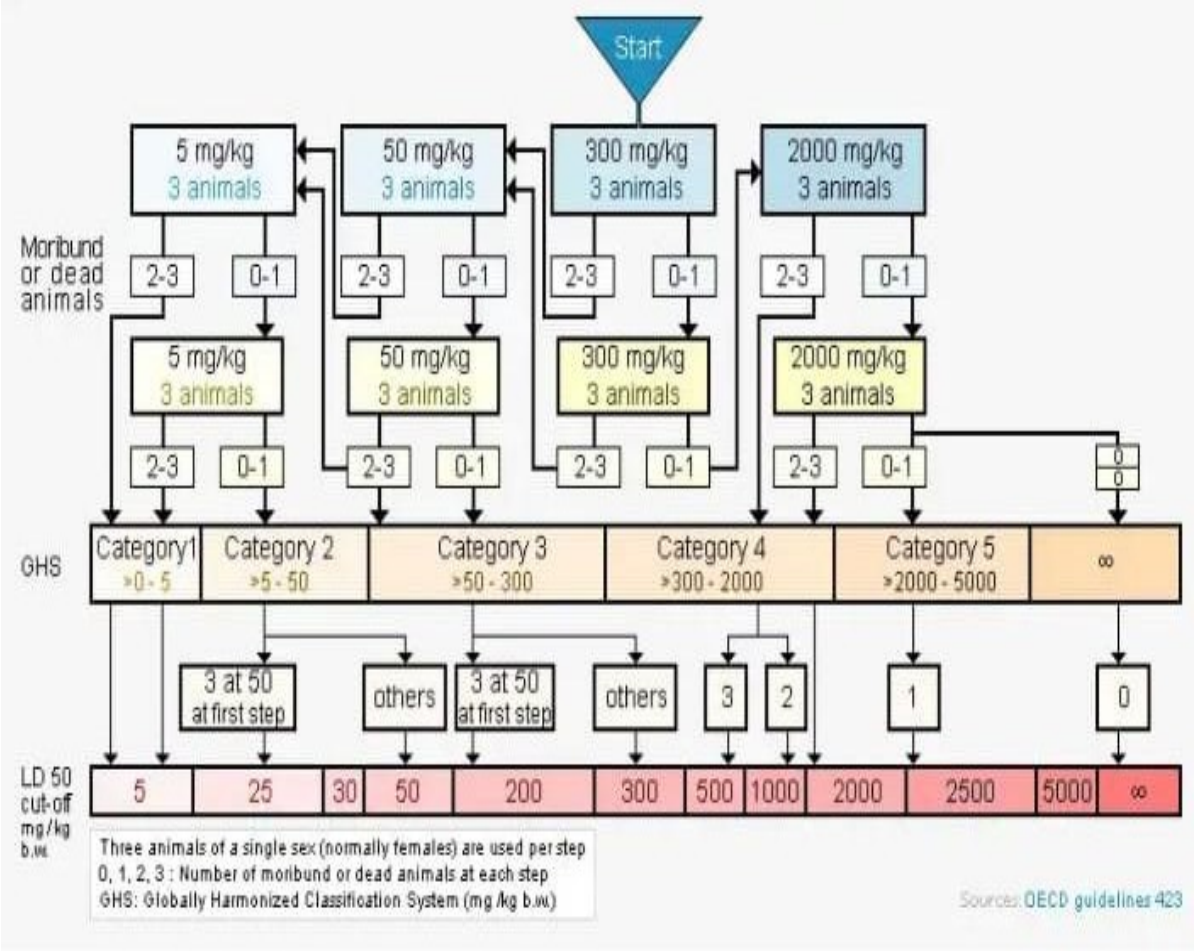


Figure No: 12 OECD Guidelines 423 for acute toxicity studies

Experimental design:

In the present experiment, 30 rats were used. The rats were divided into 5 groups. Six rats in each group.

Table: 2 Animal grouping for *in vivo* study

S.NO	GROUP	DRUG	TREATMENT	NO. OF ANIMALS
1	I	Normal Control	Normal saline	6
2	II	Diabetic control	Streptozotocin 60mg/kg	6
3	III	Standard	Glibenclamide 5mg/kg/p.o	6
4	IV	Test Dose 1	Plant extract – 200mg/kg/p.o	6
5	V	Test Dose 2	Plant extract – 400mg/kg/p.o	6
Total				30

Induction of diabetes:

Healthy adult Wistar albino rats of both sex with body weight around 150-220 g were used in the present study. Animals were maintained at 22·c with 12 hr light and dark cycle, fed with standard rat pellet diet. After initial determination of 12hr fasting blood glucose levels, animals were given single i.p injection of streptozotocin (STZ) at a dose of 60mg/kg (Freshly dissolved in saline) and blood glucose was monitored after 24 hr and thereafter at weekly intervals after 12hrs fasting.

Determination of antidiabetic activity and changes in body weight

Animals showing blood glucose levels 200mg/dl 48hr after STZ treatment were selected for study. Animals were treated orally for different duration at different doses (250mg/kg and 500mg/kg) of test extracts suspended in distilled water. Fasting blood glucose levels are monitored weekly along with untreated controls. Any reduction in blood sugar level in comparison to that of normal and diabetic controls was taken as antidiabetic activity. Changes in body weight of normal, diabetic controls and experimental animals were recorded at the same time, i.e. in fasting state.

ESTIMATION:-

On 0, 7^{th}, 14^{th}, 21^{st}, 28^{th} day 0.1ml blood collected from lateral tail vein. On 28 day blood was collected by cardiac puncture (2 to 5ml) for biochemical estimation.

PARAMETERS:

- Blood glucose level
- Body weight
- Plasma lipid level

After 28 days, animal will be sacrificed and pancreas will be isolated for histopathological studies.

HISTOPATHOLOGICAL STUDY:-

Small piece of pancreas were fixed in bouin's solution for 24 hours, dehydrated through graded concentration of ethanol, embedded in paraffin wax, sectioned at 5µm thickness which is stained with Mayer's hematoxylin and eosin and observed under light microscope.

Method of disposal-Biological disposal tie up with corporation of Chennai.

Statistical analysis

All results were expressed as mean ±SEM for each group. Statistical analysis was performed using one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons. The values were considered to be significantly different when $p<0.05$.

Results and discussion

8. RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Macroscopy:-

The dried root pieces were 30 to 35 cm long and 13 to 15 cm thick (varying in length and diameter).

Outer surface rough with adherent patches of rhytidome, fissures, longitudinal and transverse cracks, furrows and exfoliations at places; fracture splintery at bark region, fibrous in wood region.

Organoleptic characters

Colour – Externally: greyish brown,
Internally: buff in colour
Taste – Slightly bitter
Odour – Characteristic

Figure:13 Macroscopy of roots of *Melia azedarach*

Figure:14 Roots of *Melia azedarach*

MICROSCOPY

T.S of roots of *Melia azedarach* Linn

Detailed transverse section showed 15 -20 layers of tendentiously elongated compactly arranged suberised cork cells with tannin content; some of them show oil globules and rosette crystal of calcium oxalate. Cortex somewhat reduced made up of parenchyma cells.

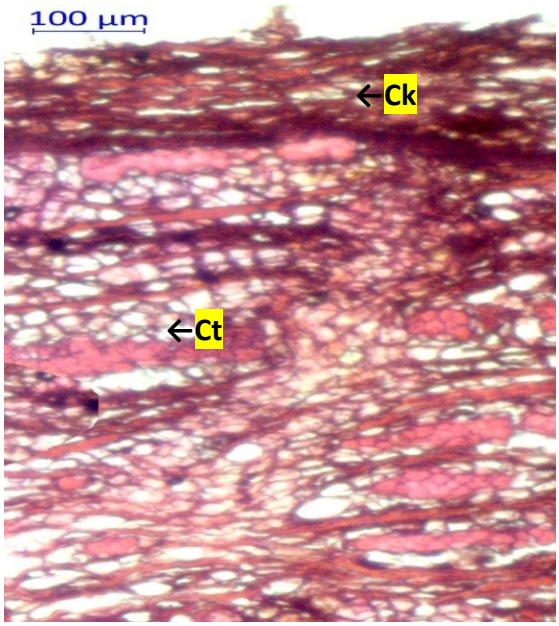


Figure: 15 Cork (ck) and cortex (ct)

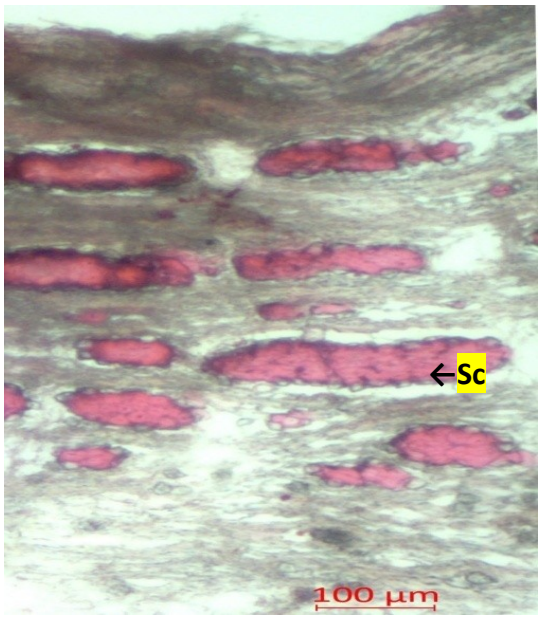


Figure: 16 Fibre groups (sc-sclereids)

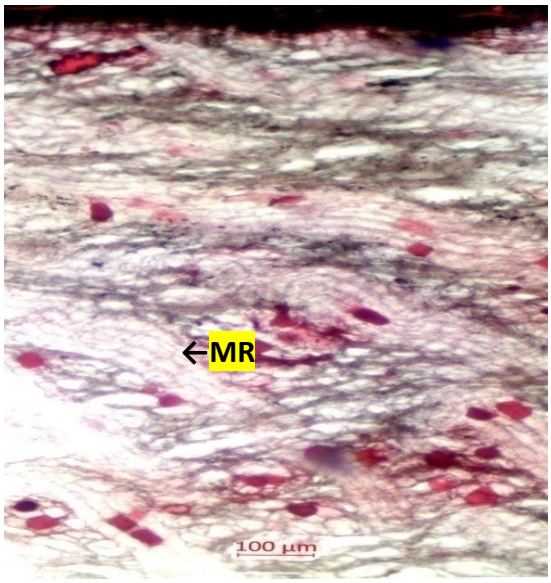


Figure: 17 Medullary rays (MR)

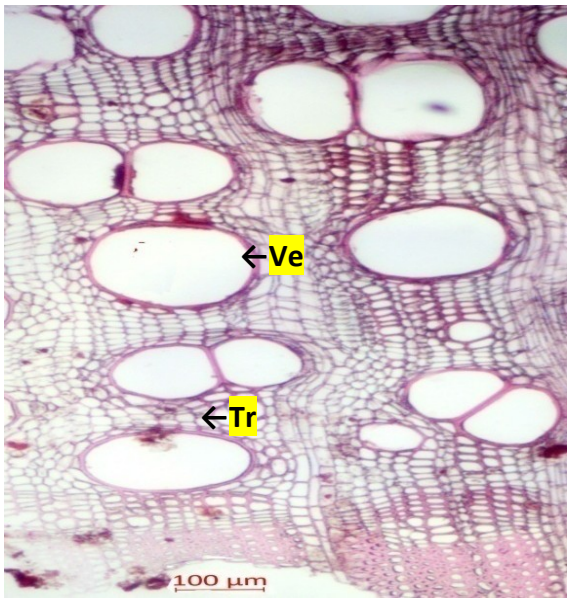


Figure: 18 Vessels (Ve) (Tr- Tracheid)

Medullary rays are multiserriate starting from the cortex and reaches up to inner layers of the xylem.

Vessels are borderdly pitted; xylem parenchyma trachieds and fibres are also seen; some of the xylem vessels show tylosis.

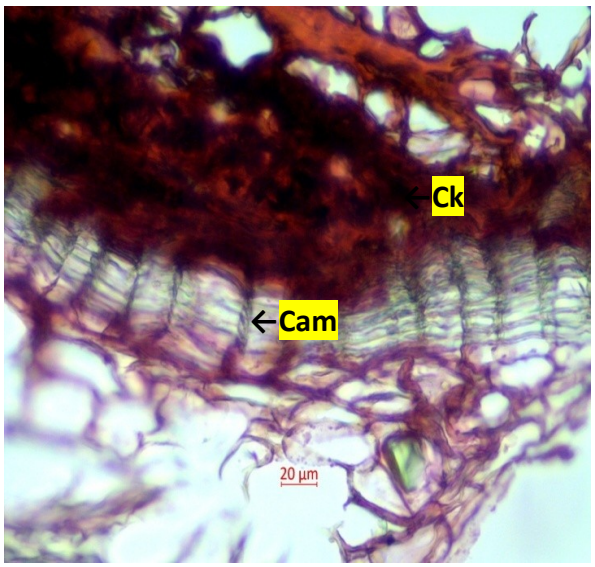


Figure: 19 Cork and cambium (cam)

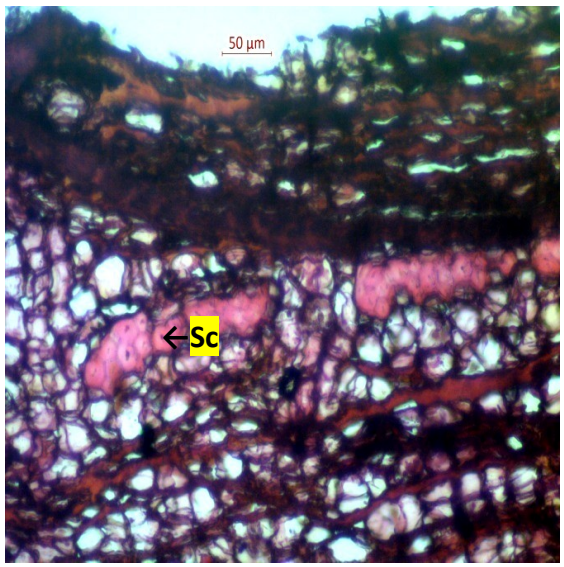


Figure: 20 Cortex with fiber groups

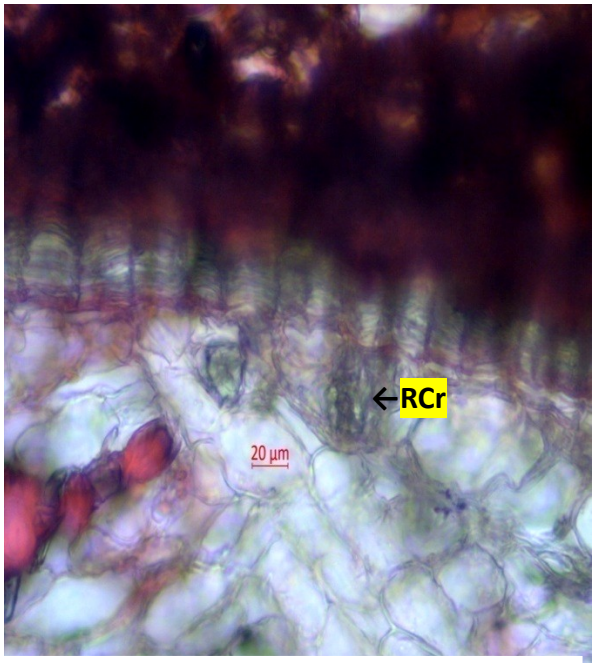


Figure: 21 Rosette crystals (Rcr) below cork cambium

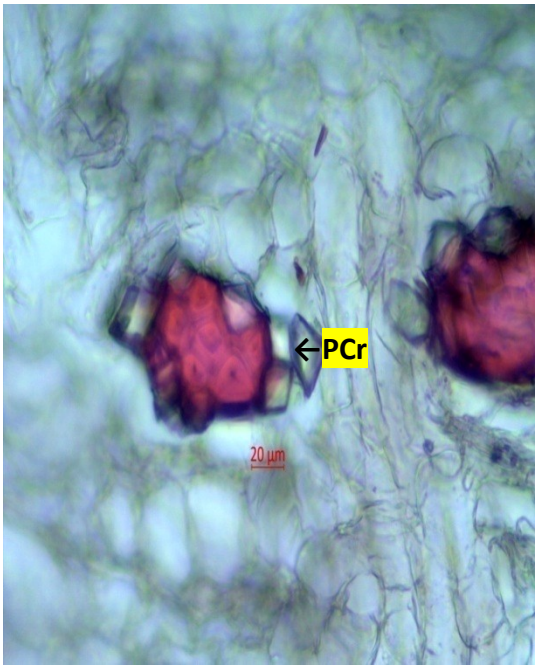


Figure: 22 Prismatic crystals around fibre group

6 to 8 celled pericyclic fibres are found distributed circularly in the cortex; rarely few solitary stone cells can be seen.

Vascular bundle occupies the major part of the root; phloem situated above the xylem and made up of sieve elements.

Simple and compound starch grains and oil globules found all over the parenchyma of medullary rays.

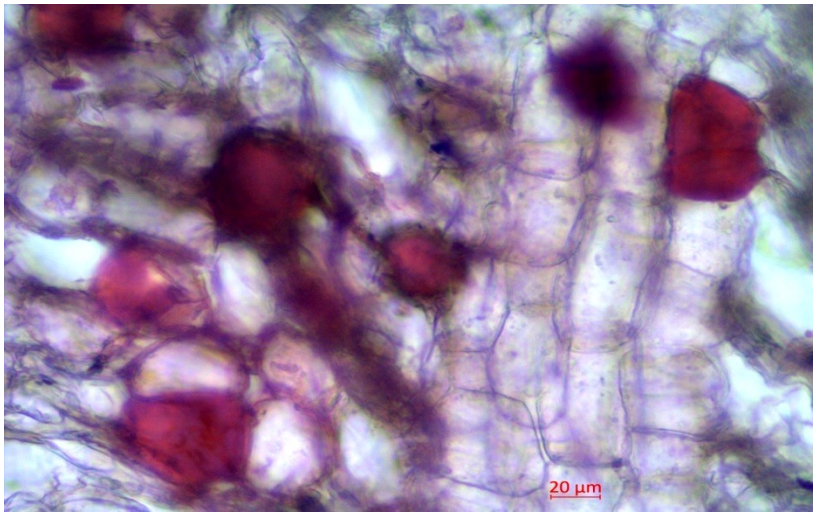


Figure: 23 Medullary rays

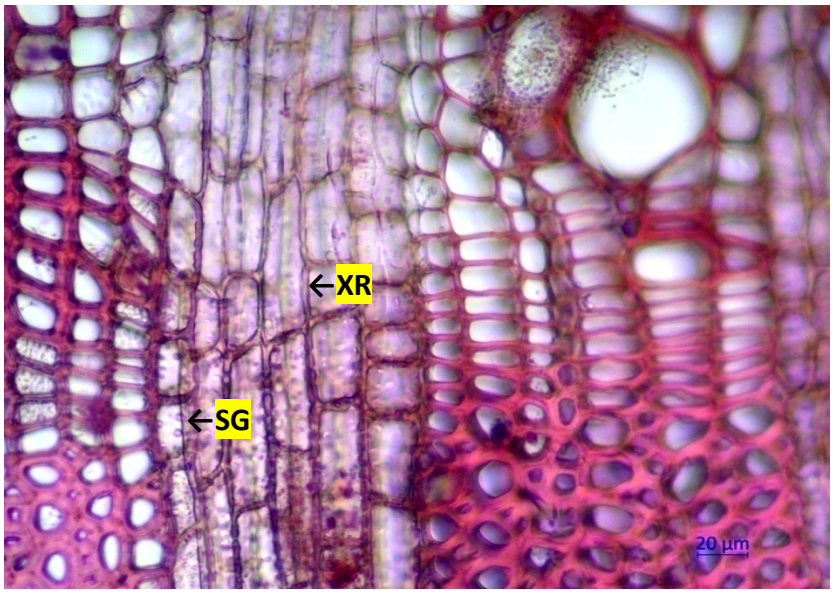


Figure: 24 Medullary rays and vascular elements (XR-Xylem rays) (SG-Starch grains)

Powder microscopy:

The powder was brown in colour with a bitter taste and showed the presence of cortex, pitted cells, fragments of medullary rays, fibers, crystal fibers and sclereids with prismatic crystals.

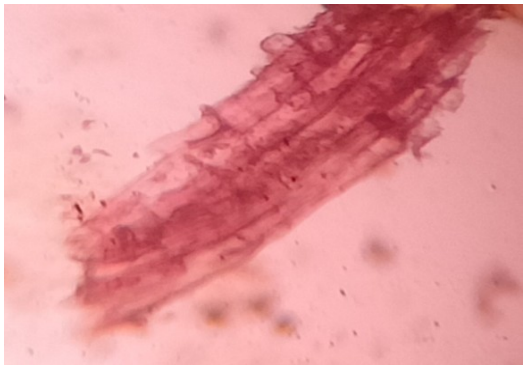


Figure: 25 Cork

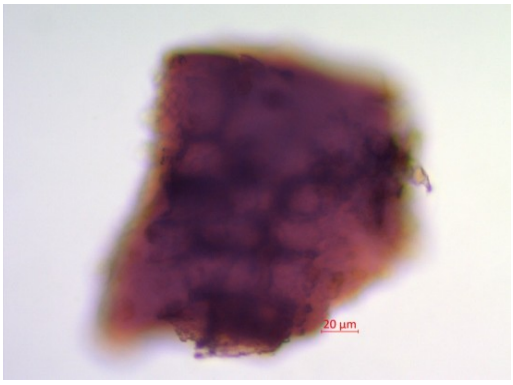


Figure: 26 Cork with tannin content

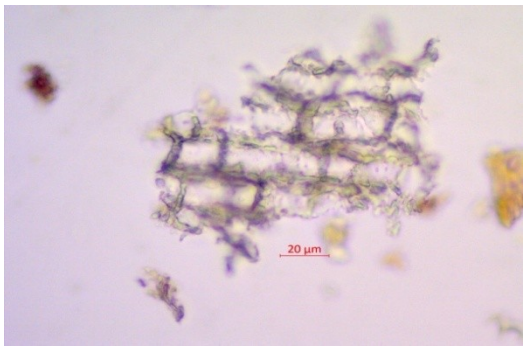


Figure: 27 Pitted cells

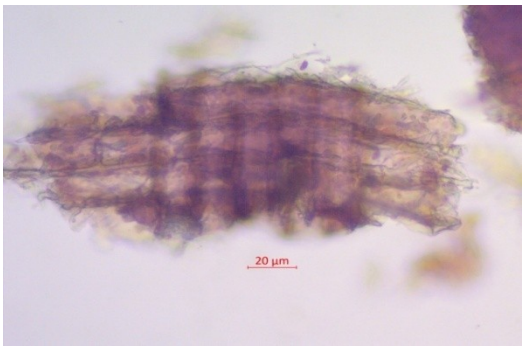


Figure: 28 Medullary rays

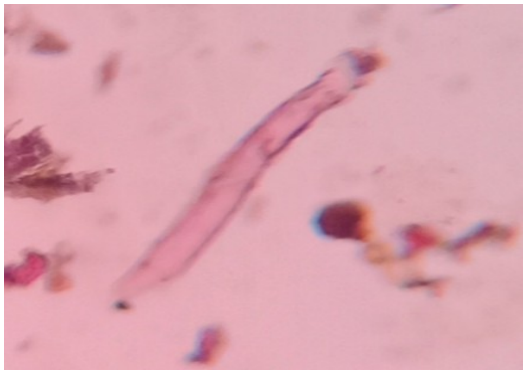


Figure: 29 Fibers

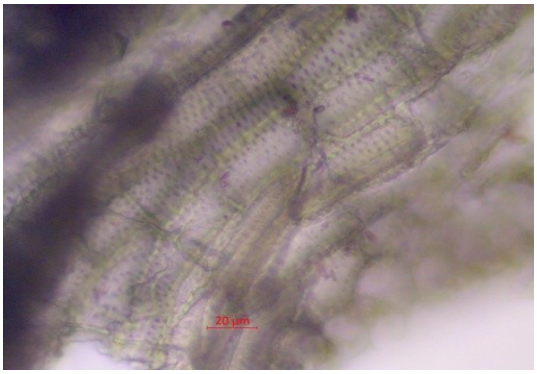


Figure: 30 Tracheids

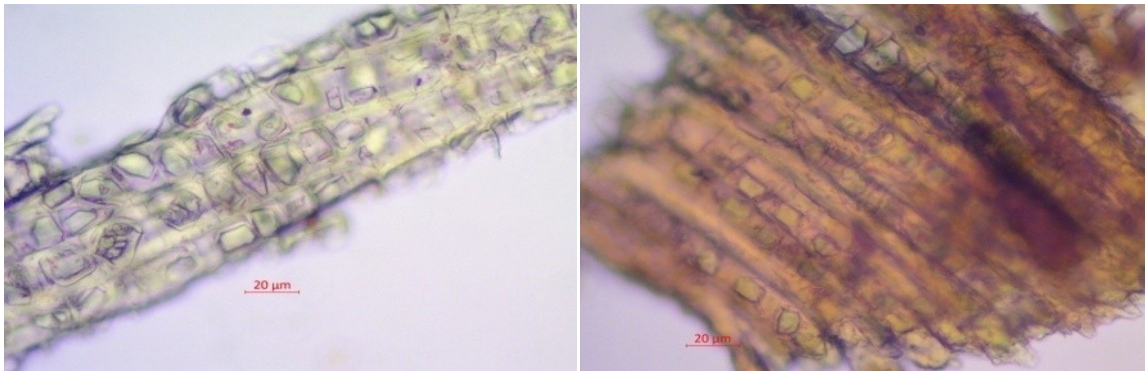


Figure: 31 Crystal Fibers

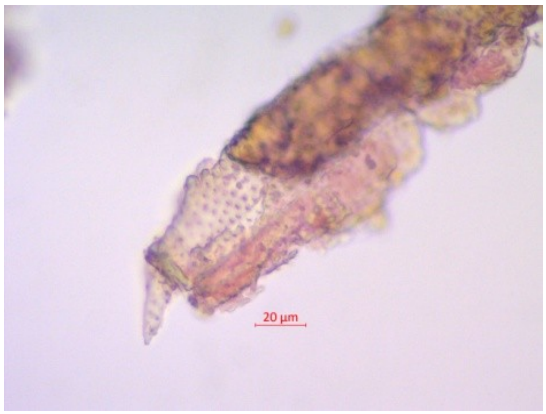


Figure: 32 Pitted vessels

Figure: 33 Sclereid with Crystal

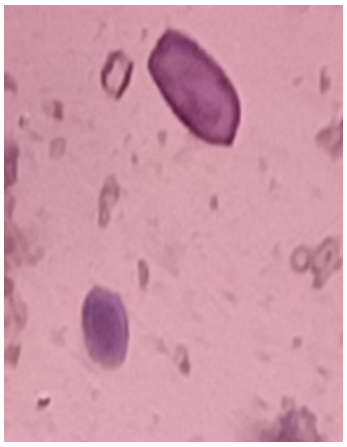


Figure:34 Starch grains

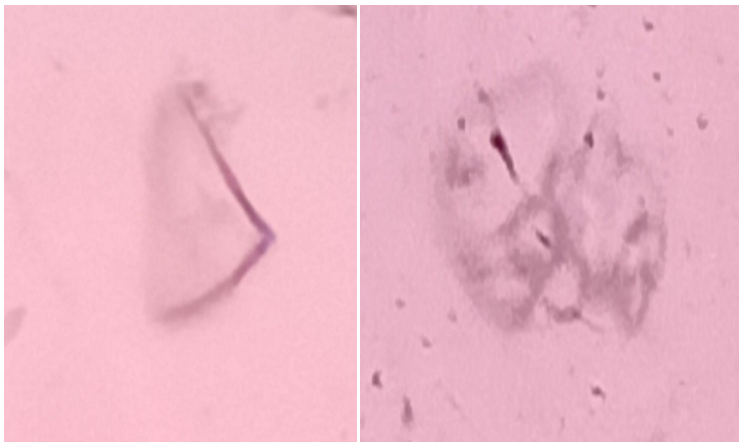


Figure: 35 Prismatic&Rosette crystals of calcium oxalate

LINEAR MEASUREMENT OF FIBRES AND STARCH GRAINS

The length and width of fibres and starch grains of powdered root was measured.

Calculation

Calibration factor

= No of divisions of stage micrometer/ No of divisions of eyepiece micrometer x 10

= 10/6 x 10

= 16.67µm

Table: 3 Linear measurements of fibres of *Melia azedarach* Linn

S.NO	PARAMETERS	MINIMUM (µm)	AVERAGE(µm)	MAXIMUM (µm)
1.	Length	166.6	234.91	333.2
2.	Width	8.33	25.82	58.31

Table: 4 Linear measurement of starch grains of *Melia azedarach* Linn

S.NO	PARAMETERS	MINIMUM (µm)	AVERAGE(µm)	MAXIMUM (µm)
1.	Length	8.33	15.83	24.99
2.	Width	8.33	12.5	16.66

HISTOCHEMICAL ANALYSIS

Table: 5 Histochemical studies of roots of *Melia azedarach* Linn

S.NO	REAGENTS	TEST FOR	OBSERVATION	HISTOLOGY	RESULTS
1.	Phloroglucinol + HCL	Lignin	Red	Xylem, Cortex	++
2.	N/50 Iodine solution	Starch	Blue	Near xylem vessels	++
3.	Dil. Ferric chloride	Tannin	Dark blue black	Cork	++
4.	Picric acid	Protein	No Yellow colour	--	--
5.	Dragendroff's reagent	Alkaloid	No Orange colour	--	--
6.	Glycerine + Water	Calcium oxalate crystals	Colourless crystals	Cork cambium, Fiber group	++

Note: ++ indicates Positive -- indicates Negative

PHYSIOCHEMICAL CONSTANTS

Table: 6 Physiochemical Parameters of powdered roots of *Melia azedarach* Linn

S.NO	PARAMETERS	VALUES(%w/w)
I.	**Ash value**	
1.	Total ash	3.99±0.84
2.	Water soluble ash	2.14±0.42
3.	Acid insoluble ash	1.23±0.18
4.	Sulphated ash	4.43±0.90
II.	**Extractive values**	
1.	Water soluble extractive	2.75±0.29
2.	Alcohol soluble extractive	4.05±0.39
3.	Non volatile Ether soluble extractive	1.598±0.20
III.	**Crude fibre content**	43.04±5.27
IV.	**Loss on drying**	4.67±0.41
V.	**Foaming index**	<100
VI.	**Swelling Index**	Nil
VII.	**Volatile oil content**	Nil

Values are expressed as Mean ± SD, n=6.

INORGANIC ELEMENTS & HEAVY METALS

Table: 7 Qualitative analysis of inorganic elements & heavy metals in *Melia azedarach*

S.NO	INORGANIC ELEMENTS	OBSERVATIONS
1.	Aluminium	+ (Presence)
2.	Borate	- (Absence)
3.	Calcium	+
4.	Carbonate	-
5.	Chloride	-
6.	Copper	+
7.	Iron	+
8.	Lead	+
9.	Magnesium	+
10.	Mercury	+
11.	Nitrate	-
12.	Phosphate	-
13.	Potassium	+
14.	Silver	-
15.	Sulphates	-
16.	Arsenic	-

Table: 8 Quantitative analysis of inorganic elements in *Melia azedarach* Linn

S.NO	INORGANIC ELEMENTS	TOTAL AMOUNT
1.	Aluminium	3.143
2.	Calcium	274
3.	Copper	0.752
4.	Iron	2.041
5.	Magnesium	36.034
6.	Potassium	132.9

Quantitative estimation of heavy metals by ICP OES Method

The quantification of the individual heavy metals was analyzed for powdered mixture of *Melia azedarach* Linn by ICP OES technique.

Table: 9 Quantitative estimation of Heavy metals

S.NO	ELEMENT	RESULTS(ppm)	SPECIFICATION AS PER WHO GUIDELINES
1.	Mercury	0.354	NMT 0.5
2.	Arsenic	Not detected	NMT 5.0
3.	Lead	0.308	NMT 10
4.	Cadmium	Not detected	NMT 0.3

PHYTOCHEMICAL STUDIES

Percentage yield:

Successive solvents extracts were prepared. The percentage yield, colour and nature of the extract were as follows.

Table: 10 Percentage yield of successive extract

S.NO	**EXTRACT**	**METHOD OF EXTRACTION**	**PHYSICAL NATURE**	**COLOUR**	**YIELD (%w/w)**
1	Petroleum ether	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Brown	1.8
2	Chloroform		Solid	Yellowish brown	3.1
3	Ethyl acetate		Semisolid	Reddish brown	2.1
4	Ethanol		Solid	Reddish brown	4.6
5	Aqueous		Semisolid with sticky	Reddish brown	2.9

PRELIMINARY PHYTOCHEMICAL SCREENING

The extracts were screened using various chemical reagents. The results are,

Table: 11 Preliminary phytochemical screening of roots of *Melia azedarach* Linn

S. NO	TESTS	Powder	PET. ETHER	CHLOROFORM	ETHYL ACETATE	ETHANOL	AQUEOUS
1.	Alkaloids	-	-	-	-	-	-
2.	Carbohydrates	+	+	+	-	-	+
3.	Protein	-	-	-	-	-	-
4.	Glycosides	-	-	-	-	-	-
5.	Flavonoids	+	+	+	+	+	+
6.	Phenolic compounds	+	+	+	+	+	-
7.	Tannins	+	-	-	-	+	-
8.	Steroids	+	+	+	+	+	+
9.	Saponin	-	-	-	-	-	+
10.	Terpenoids	+	+	+	+	+	-

Note: + indicates Positive - indicates Negative

Figure: 36 Preliminary phytochemical screening

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Melia azedarach Linn was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them estimation of total flavonoids and phenolics content in petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were decided to be taken as parameters.

Table: 12 Quantitative estimation of phytoconstituents of roots of *Melia azedarach*

S.NO	EXTRACTS	AMOUNT (% w/w)	
		FLAVONOID	PHENOLIC
1.	Pet. Ether	0.589	0.143
2.	Chloroform	1.38	0.263
3.	Ethyl acetate	1.14	0.646
4.	Ethanol	2.77	2.377
5.	Aqueous	2.124	0.896

FLUORESCENCE ANALYSIS

The powdered drug and extracts are examined under Short UV and Long UV.

Table: 13 Fluorescence analysis of root powder of *Melia azedarach* Linn

S.No	TREATMENT	DAY LIGHT	SHORT UV 254nm	LONG UV 365nm
1.	Powder	Reddish brown	Yellowish red	Reddish brown
2.	Powder + water	Reddish brown	Yellowish brown	Reddish brown
3.	Powder + NaOH	Brownish black	Green	Brown
4.	Powder + HCL	Yellowish brown	Yellowish green	Yellowish brown
5.	Powder + Acetic acid	Reddish brown	Reddish brown	Reddish brown
6.	Powder + Ethanol	Yellowish brown	Yellowish red	Yellowish red
7.	Powder + H2SO4	Reddish brown	**Fluorescence green**	Yellowish brown
8.	Powder + HNO3	Reddish brown	**Fluorescence green**	Brownish yellow
9.	Powder + Iodine	Reddish brown	**Fluorescence green**	Yellowish brown
10.	Powder + FeCl3	Yellowish brown	**Fluorescence green**	Yellowish orange
11.	Powder + 1N KOH	Brownish black	**Fluorescence green**	Reddish brown
12.	Powder+ Ammonia	Reddish brown	Yellowish brown	Brown

FLUORESCENCE ANALYSIS OF EXTRACTS

Table: 14 Fluorescence analysis of root extracts of *Melia azedarach* Linn

S.No	EXTRACTS	DAY LIGHT	SHORT UV 254nm	LONG UV 365nm
1.	Petroleum ether	Brown	**Fluorescence green**	Yellowish brown
2.	Chloroform	Yellowish brown	Brown	Brown
3.	Ethyl acetate	Reddish brown	Bluish black	Greenish black
4.	Ethanol	Reddish brown	Bluish black	Greenish black
5.	Aqueous	Reddish brown	Bluish black	Greenish black

The powdered roots and extracts of *Melia azedarach* L. showed presence of fluorescence characters.

THIN LAYER CHROMATOGRAPHY

Figure: 37 Visible light

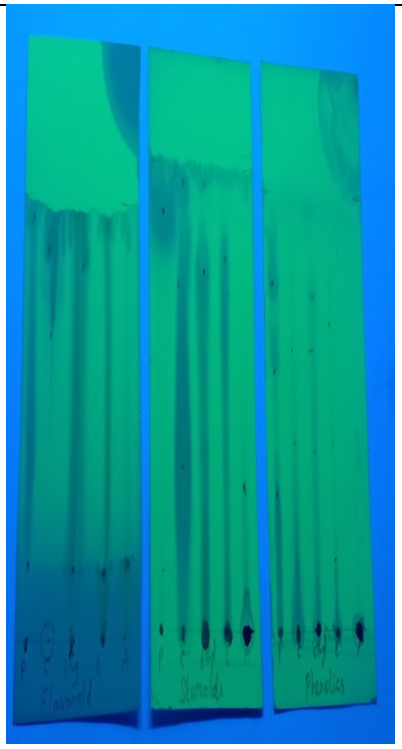


Figure: 38 Short UV 254nm

Figure: 39 Long UV 365nm

TLC was performed for five successive extracts by using suitable solvent systems to analyze flavonoids, phenolics compounds and steroids. Various spots were obtained and it was detected using UV.

Table: 15 TLC of root extracts of *Melia azedarach* Linn

S.NO	CHEMICAL CONSTITUENT	SOLVENT SYSTEM	EXTRACTS	NO. OF SPOTS	R_f VALUE
1.	Flavonoids	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26)	Pet.ether	2	0.16, 0.94
			Chloroform	2	0.67, 0.86
			Ethyl acetate	2	0.19, 0.84
			Ethanol	2	0.17, 0.94
			Aqueous	1	0.14
2.	Phenolics	Toluene: Ethyl acetate (93:7)	Pet.ether	3	0.29, 0.57, 0.77
			Chloroform	2	0.41, 0.61
			Ethyl acetate	3	0.39, 0.61, 0.74
			Ethanol	3	0.14, 0.4, 0.67
			Aqueous	-	-
3.	Steroids	Chloroform: Methanol (27:3)	Pet.ether	2	0.13, 0.88
			Chloroform	2	0.33, 0.72
			Ethyl acetate	2	0.75, 0.94
			Ethanol	1	0.79
			Aqueous	1	0.29

HPTLC FINGER PRINT PROFILE

HPTLC Finger print Data of Ethanolic Extracts of *Melia azedarach L.,*

High Performance Thin Layer Chromatography (HPTLC) finger printing was performed in the ethanolic extract of *Melia azedarach* Linn. The chromatographic conditions were carried as detailed in material and method of this study. There were 12 peaks observed with different R_f values and different heights. Percentage of areas was also obtained from the chromatogram.

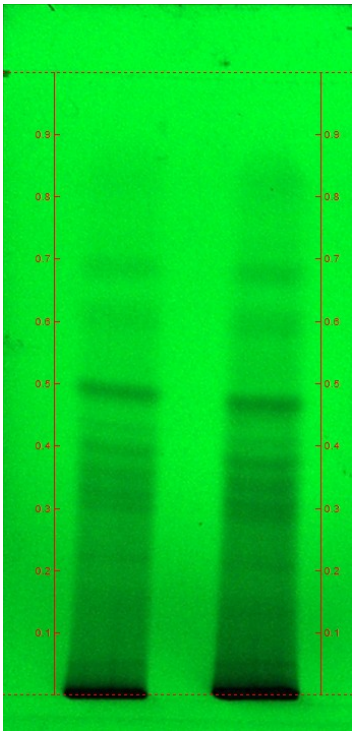


Figure: 40 HPTLC at λ = 254 nm

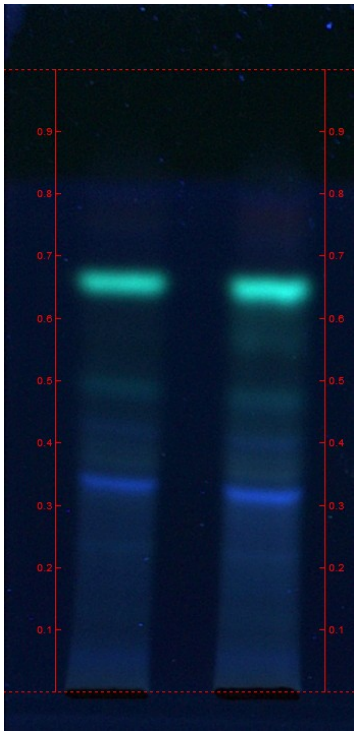


Figure: 41 HPTLC at λ = 366 nm

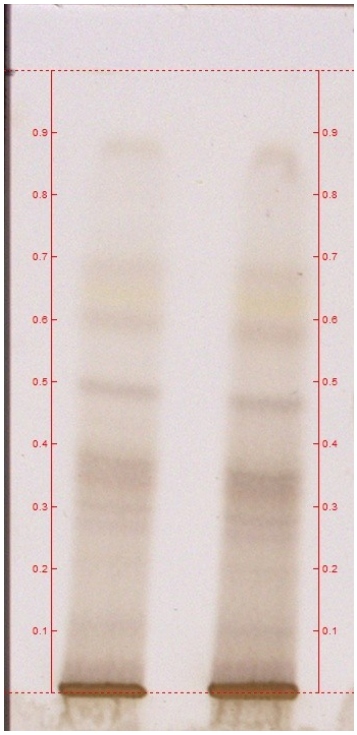


Figure: 42 HPTLC at λ = 520 nm

Color	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)
Dark	0.23	Blue	0.05	Brown	0.09
Dark	0.31	Blue	0.22	Brown	0.28
Dark	0.33	Blue	0.32	Brown	0.34
Dark	0.36	Green	0.41	Brown	0.47
Dark	0.40	Green	0.47	Yellow	0.58
Dark	0.49	Green	0.57	Brown	0.62
Dark	0.69	Green	0.65	Brown	0.86

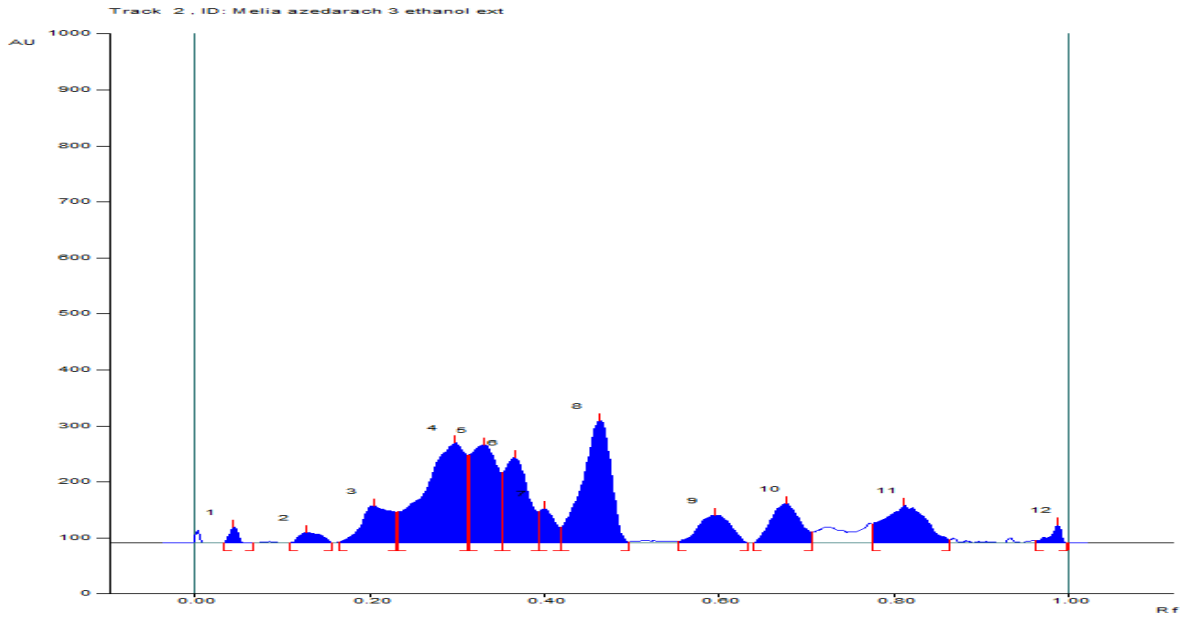


Figure: 43 HPTLC Chromatogram @ 254 nm

Table: 16 HPTLC Chromatogram @ 254 nm

Peak	Start Position Rf	Start Height AU	Max Position Rf	Max Height AU	Max %	End Position Rf	End Height AU	Area AU	Area %
1	0.03	0.6	0.04	27.8	2.50	0.07	0.0	244.0	0.71
2	0.11	0.1	0.13	18.3	1.64	0.16	0.2	445.5	1.30
3	0.16	1.5	0.20	66.0	5.92	0.23	54.4	2117.2	6.16
4	0.23	55.5	0.30	178.4	15.99	0.31	56.1	7895.3	22.96
5	0.31	156.6	0.33	175.1	15.7	0.35	24.8	5056.0	14.7
6	0.35	125.5	0.37	151.8	13.61	0.39	55.7	3845.4	11.18
7	0.39	56.2	0.40	60.8	5.45	0.42	26.4	990.6	2.88
8	0.42	27.6	0.46	218.4	19.58	0.5	1.9	6546.7	19.04
9	0.55	2.3	0.59	49.2	4.41	0.63	0.2	1710.2	4.97
10	0.64	0.2	0.68	70.4	6.31	0.71	19.6	2162.8	6.29
11	0.77	33.9	0.81	67.0	6.01	0.86	5.2	3019.5	8.78
12	0.96	4.0	0.99	32.0	2.87	1.00	0.1	352.3	1.02

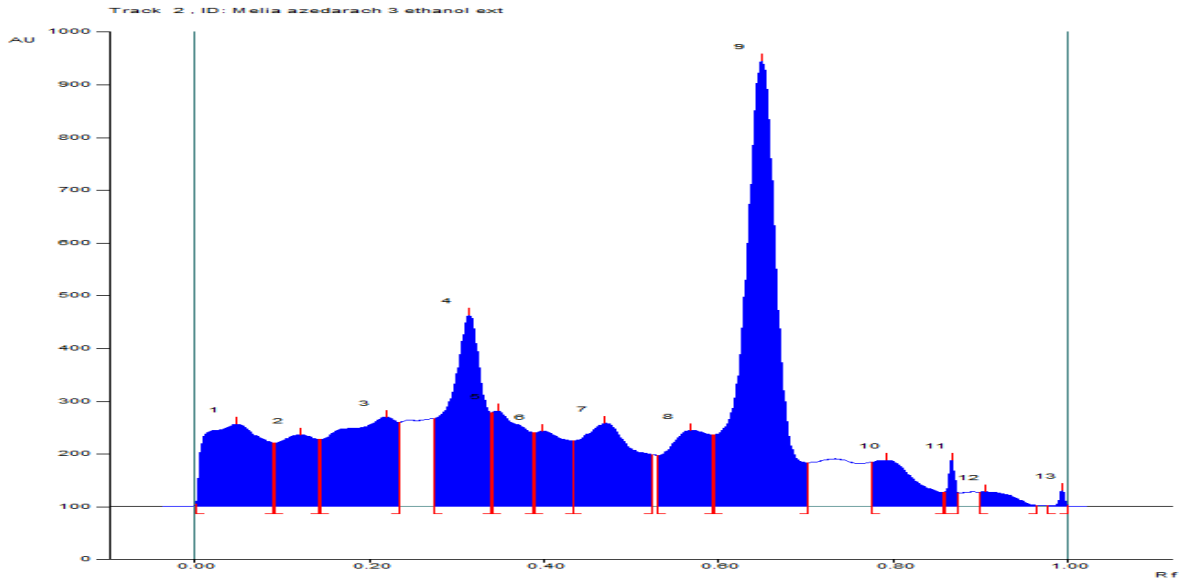


Figure: 44 HPTLC Chromatogram @ 366 nm

Table: 17 HPTLC Chromatogram @ 366 nm

Peak	Start Position Rf	Start Height AU	Max Position Rf	Max Height AU	Max %	End Position Rf	End Height AU	Area AU	Area %
1	0.00	4.5	0.05	155.9	6.16	0.09	19.6	9881.2	9.51
2	0.09	119.7	0.12	135.8	5.37	0.14	26.6	5573.2	5.36
3	0.14	126.8	0.22	169.7	6.71	0.23	59.5	11319.4	10.89
4	0.27	166.1	0.31	363.6	14.37	0.34	77.8	13221.0	12.72
5	0.34	177.9	0.35	181.1	7.16	0.39	39.1	6413.5	6.17
6	0.39	139.3	0.40	142.6	5.64	0.43	24.1	4951.8	4.76
7	0.43	12.1	0.47	157.4	6.22	0.52	97.9	9588.5	9.23
8	0.53	96.7	0.57	144.4	5.71	0.59	35.5	6755.6	6.50
9	0.59	135.7	0.65	845.0	33.4	0.70	82.6	30238.0	29.1
10	0.77	83.2	0.79	87.3	3.45	0.86	26.2	4162.8	4.01
11	0.86	26.0	0.87	88.4	3.49	0.87	25.4	675.3	0.65
12	0.90	27.1	0.90	27.6	1.09	0.96	1.7	977.4	0.94
13	0.98	1.2	0.99	30.8	1.22	1.00	2.0	170.1	0.16

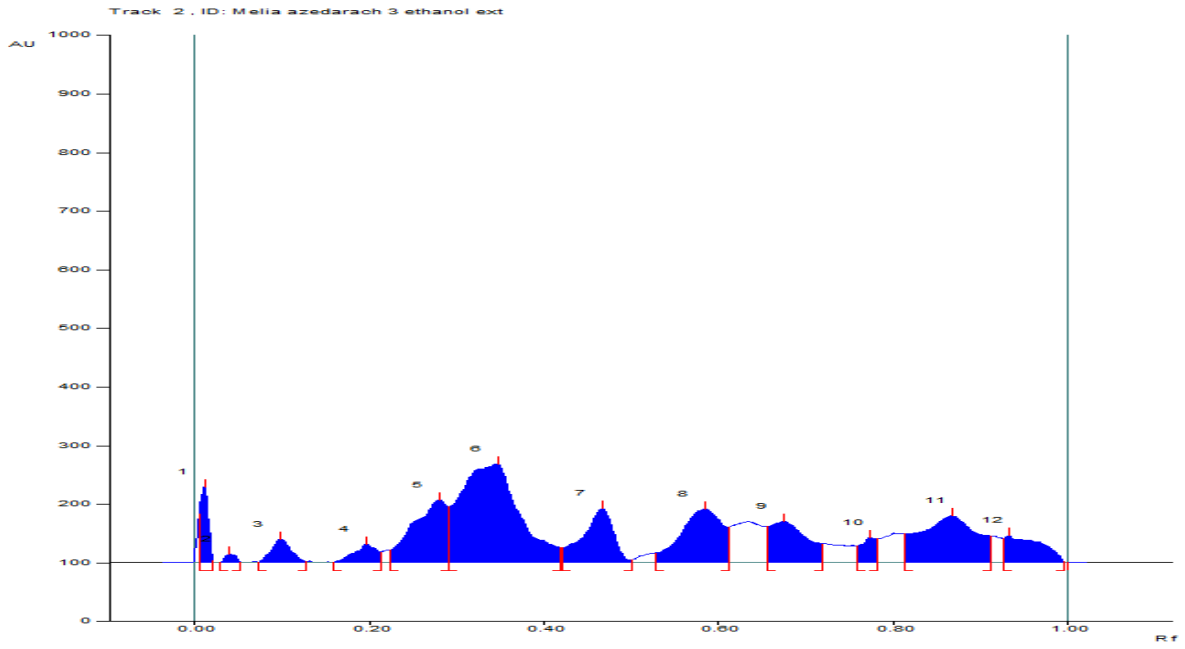


Figure: 45 HPTLC Chromatogram @ 520 nm

Table: 18 HPTLC Chromatogram @ 520 nm

Peak	Start Position Rf	Start Height AU	Max Position Rf	Max Height AU	Max %	End Position Rf	End Height AU	Area AU	Area %
1	0.00	82.6	0.01	128.4	14.17	0.02	2.4	1116.7	3.23
2	0.03	0.3	0.04	13.6	1.5	0.05	0.3	162.8	0.47
3	0.07	0.4	0.10	39.4	4.34	0.13	1.4	827.3	2.4
4	0.16	0.0	0.20	31.0	3.42	0.21	17.2	728.7	2.11
5	0.22	21.3	0.28	106.4	11.74	0.29	94.7	3886.6	11.26
6	0.29	95.0	0.35	167.6	18.49	0.42	24.5	10667.5	30.89
7	0.42	24.7	0.47	92.4	10.19	0.50	4.0	2981.0	8.63
8	0.53	16.0	0.58	91.0	10.04	0.61	60.4	4050.2	11.73
9	0.66	61.3	0.67	69.8	7.70	0.72	31.4	2760.0	7.99
10	0.76	28.4	0.77	41.8	4.61	0.78	40.7	713.8	2.07
11	0.81	48.6	0.87	79.5	8.77	0.91	45.2	4838.5	14.01
12	0.93	40.7	0.93	45.4	5.01	1.00	1.7	1795.4	5.20

PHARMACOLOGICAL STUDIES

IN VITRO ANTIDIABETIC ACTIVITY

1. α-amylase inhibition assay

Table: 19 α-amylase inhibition potentials of various extracts

S. No	Concentration (µg/ml)	Inhibition (%)					
		Pet.ether	Chloro form	Ethyl acetate	Ethanol	Aqueous	Acarbose
1.	25	35.92	16.89	68.29	**68.41**	8.08	**48.44**
2.	50	59.73	65.39	79.76	79.64	62.31	69.52
3.	100	70.51	78.82	88.49	90.02	81.19	73.96
4.	200	83.48	86.76	90.29	90.89	83.81	88.16
5.	400	88.89	89.45	90.89	90.89	87.33	89.61
6.	800	90.89	90.89	90.89	**90.89**	90.89	**90.89**

Ethanol extract showed more inhibitory activity than standard drug. The percentage inhibition at 25, 50, 100, 200, 400, 800 µg/ml concentration of crude extracts shown concentration dependent reduction in percentage inhibition.

2. Non-enzymatic glycosylation of haemoglobin method

Table: 20 Non-enzymatic glycosylation of haemoglobin method

S. No	Concentration (µg/ml)	Inhibition (%)					
		Pet.ether	Chloro form	Ethyl acetate	Ethanol	Aqueous	Acarbose
1.	50	4.04	9.43	21.74	42.34	34.17	48.49
2.	100	27.52	25.41	30.91	49.89	40.37	52.95
3.	200	48.92	36.19	38.82	61.43	59.94	64.34
4.	400	52.36	48.42	49.26	70.1	68.41	73.16
5.	800	55.14	54.39	56.38	79.84	72.32	79.13
6.	1600	58.65	59.89	65.52	**86.42**	78.16	**82.56**

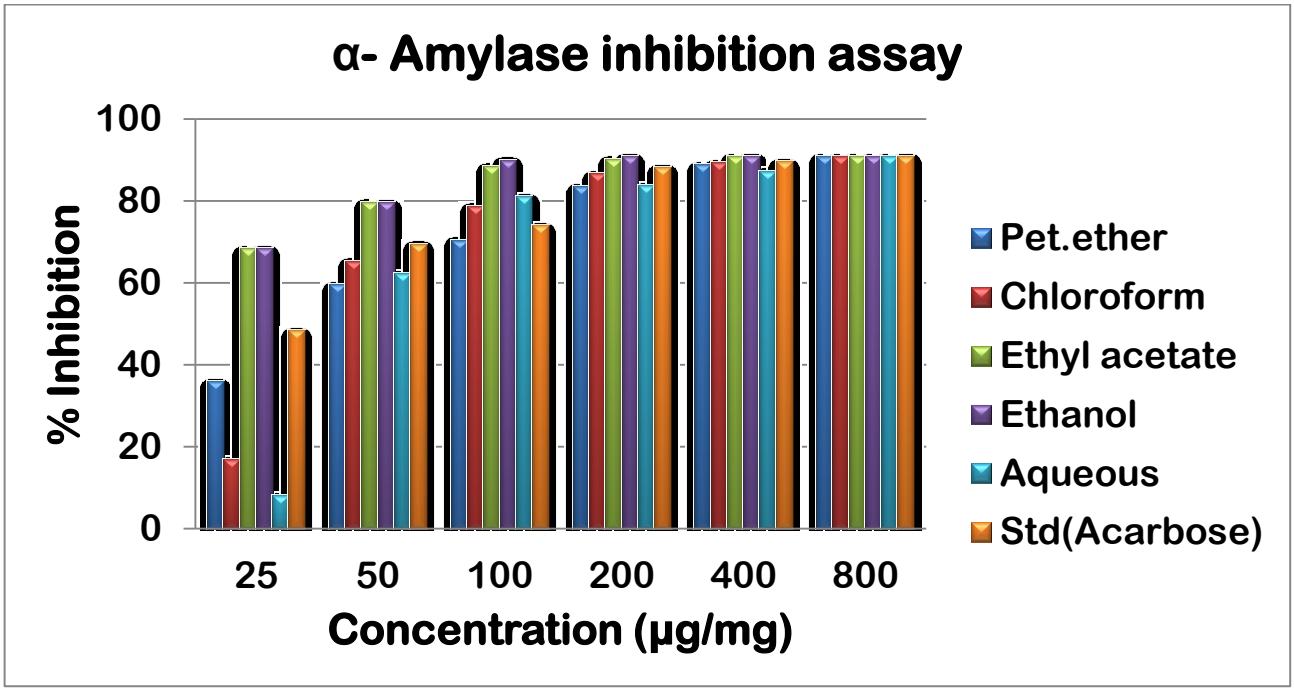


Figure: 46 Graphical representation of α-amylase inhibition

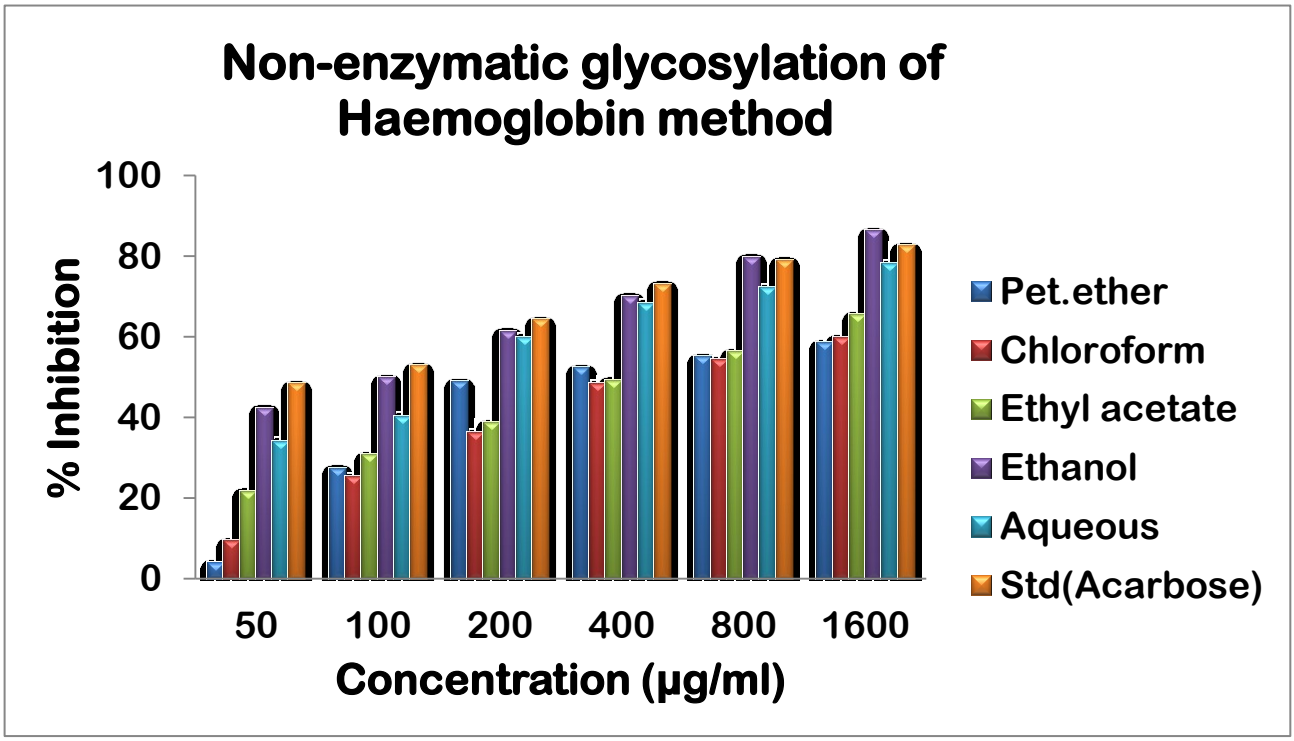


Figure:47 Graphical representation of Non-enzymatic glycosylation of haemoglobin method

***IN VIVO* ANTIDIABETIC ACTIVITY**

STREPTOZOTOCIN (STZ) INDUCED DIABETES MELLITUS IN RATS

Table: 21 Blood glucose level in STZ induced diabetic rats

Day	Normal control	Disease control	Standard	Test Low dose (200mg/kg)	Test High dose (400mg/kg)
Day 0	90±6.64	87±5.23	85±4.74	79±5.96	88±4.21
Day 1	95±6.68	253±5.24	276±15.12	212±16.93	246±24.16
Day 7	96±7.03	257±4.62	138±13.01	155±32.19	178±17.69
Day 14	98±9.66	261±3.97	97±6.19	153±31.75	176±18.40
Day 21	101±8.43	259±7.79	94±5.59	106±12.44	104±13.86
Day 28	92±3.69	272±10.37	91±10.35	107±7.94	93.±6.15

Values are expressed as mean ±SD; n=6: P<0.01.

Table: 22 Body weight in STZ induced diabetic rats

Day	Normal control	Disease control	Standard	Test Low dose (200mg/kg)	Test High dose (400mg/kg)
Day 0	164±15.17	152±14.71	134±10.21	124±8.84	163±16.39
Day 1	165±15.16	152±14.72	134±10.20	125±8.85	163±16.38
Day 7	169±11.86	154±13.76	134±11.04	124±7.63	162±17.90
Day 14	198±11.06	153±13.72	136±11.59	131±10.87	159±19.92
Day 21	207±10.15	148±13.08	136±8.01	132±11.36	163±18.14
Day 28	213±9.004	141±10.999	137±8.57	129±10.63	163±17.99

Values are expressed as mean ±SD; n=6: P<0.0001.

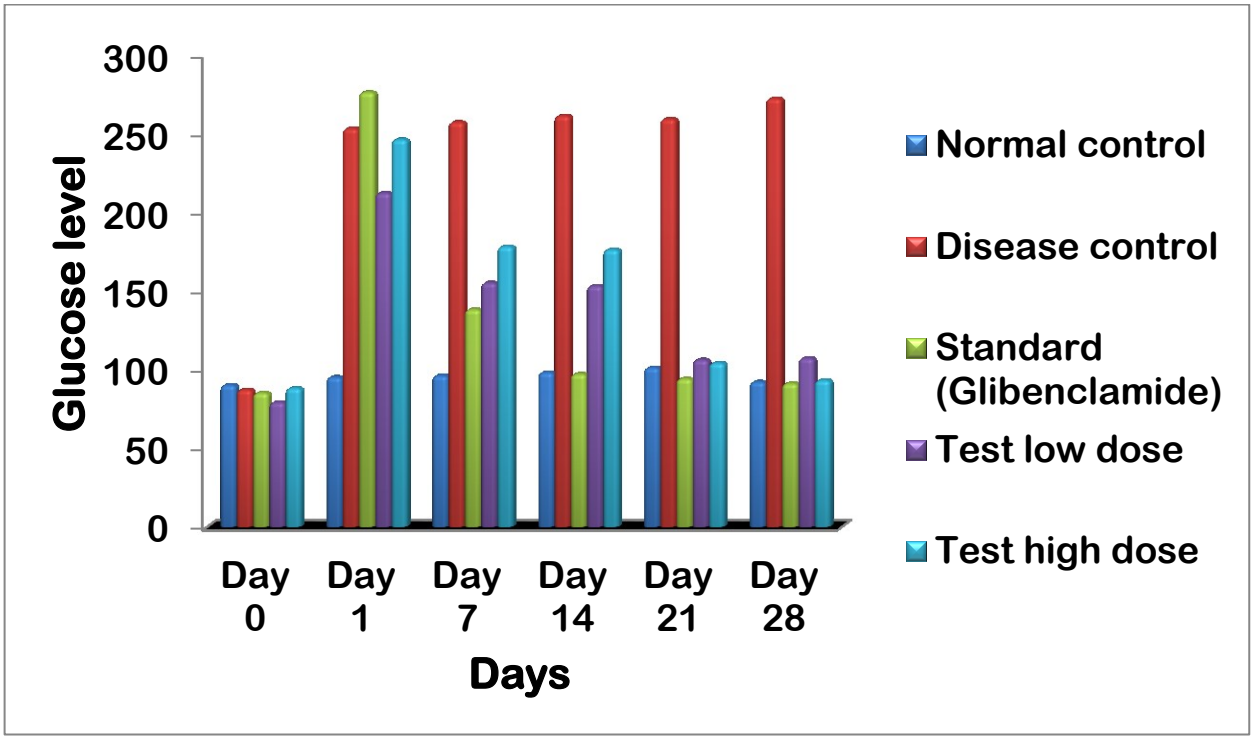


Figure: 48 Blood Glucose Level chart

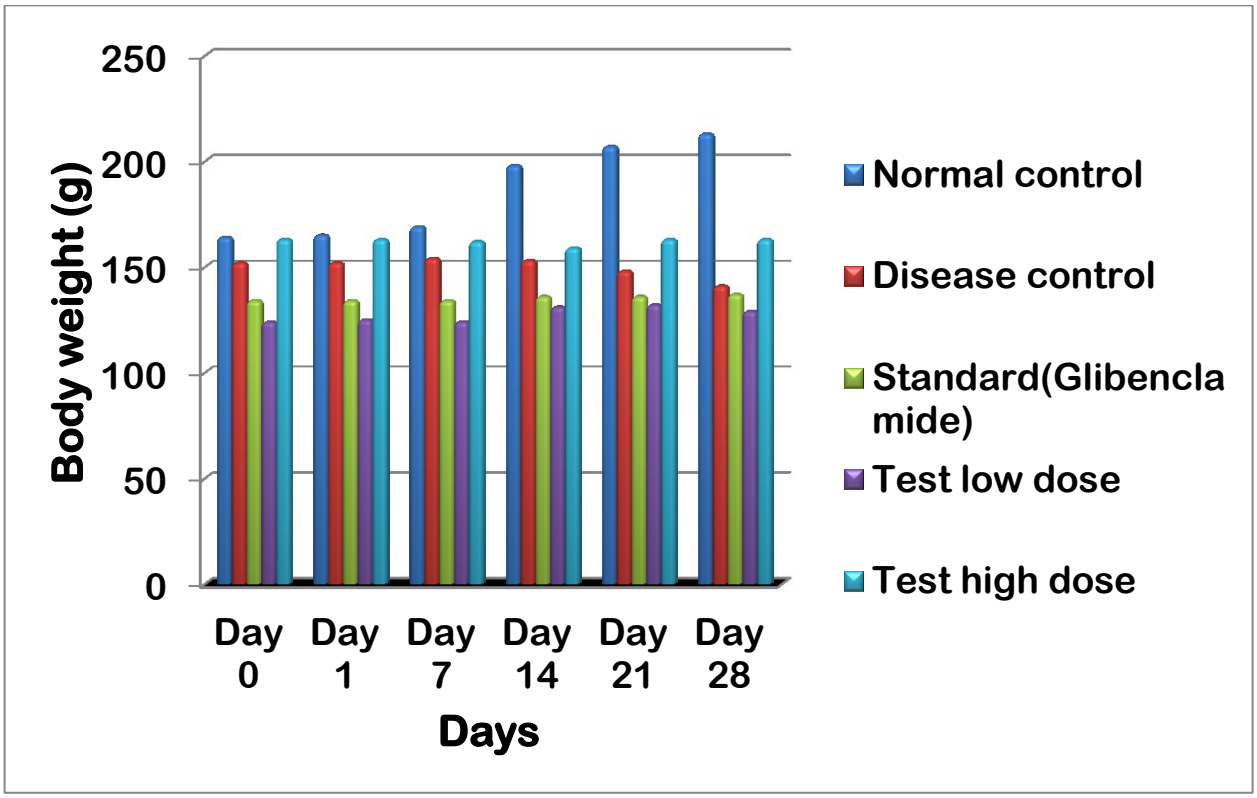


Figure: 49 Body weight chart

Table: 23 Cholesterol and Triglycerides level in STZ induced diabetic rats

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Normal control	70±2.25	76±2.58
Disease control	113±3.31	216±3.16
Standard	76±2.1	165±2.79
Low dose (200mg/kg)	70±1.41	161±1.47
High dose (400mg/kg)	51±2.1	76±2.1

Values are expressed as mean ±SD; n=6: P<0.001

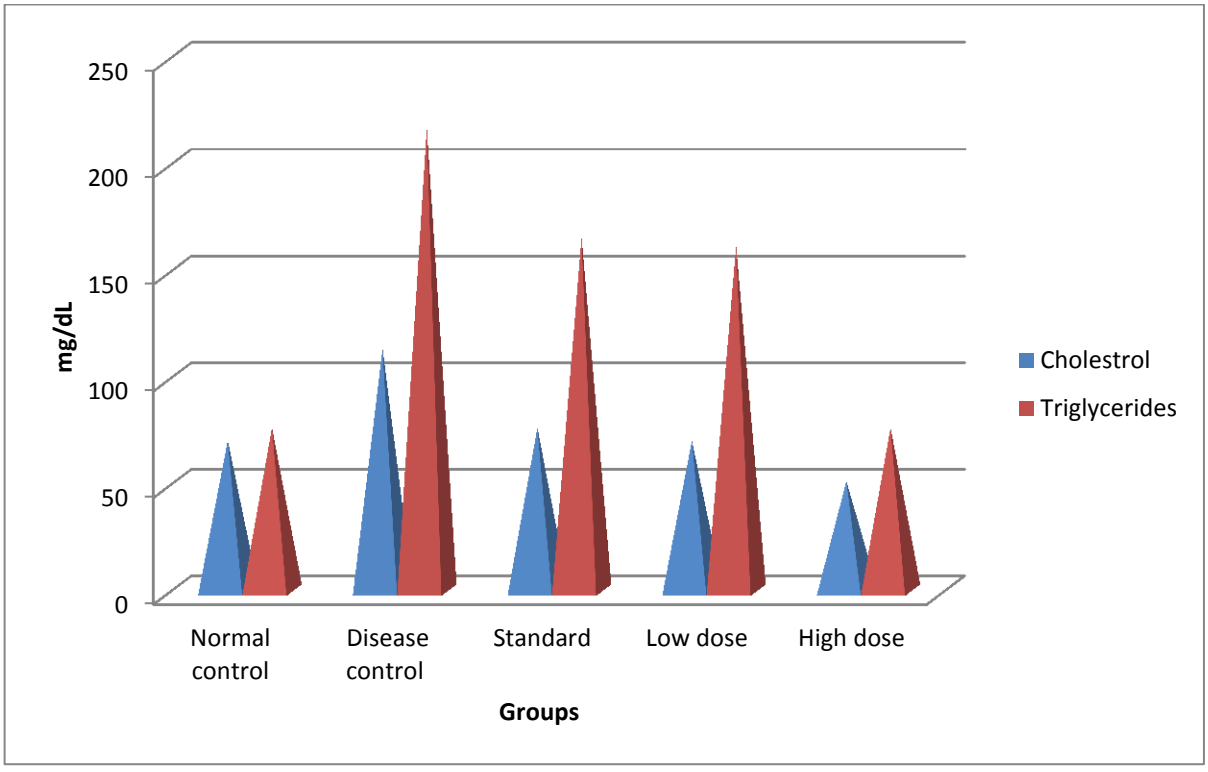


Figure: 50 Lipid profile chart

Histopathological examination of rat pancreas

The Histopathological study of pancreas was performed. Cells in normal, diabetic control and test dose were compared.

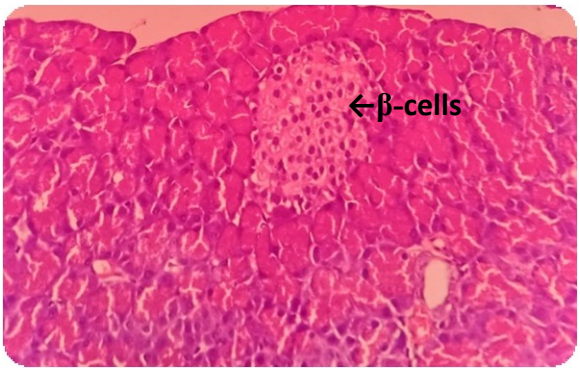


Figure: 51 Normal control

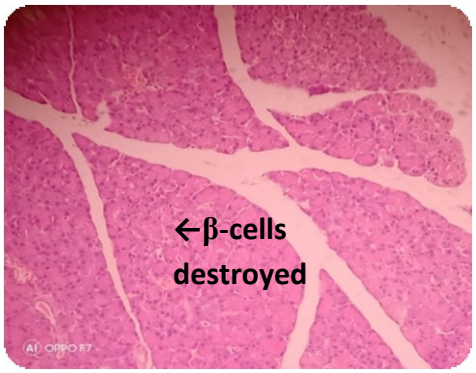


Figure: 52 Disease control

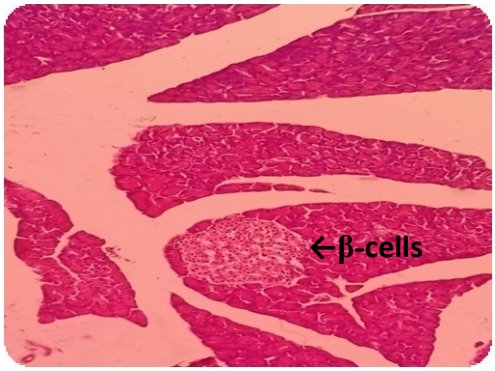


Figure: 53 Standard (Glibenclamide)

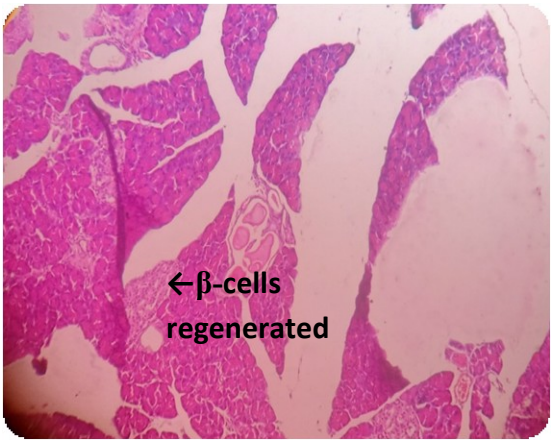


Figure: 54 Low dose (200mg/kg)

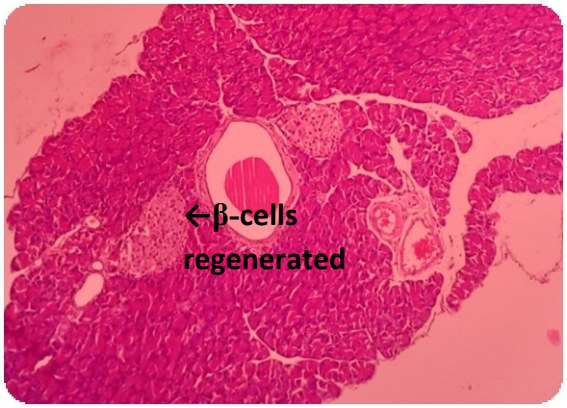


Figure: 55 High dose (400mg/kg)

β-Cells in the normal control group were in normal size (Fig. 51). Where as in the diabetic control group were reduced in size, damaged β-Cells population and extensive necrotic changes, followed by fibrosis and atrophy (Fig. 52). While in the group received that the test dose showed the absence of necrosis, fibrotic changes, increased number and size of the islets and presence of normal pancreatic cells (Fig. 54 & Fig. 55). These were in the levels comparable with the ones that were administered the standard drug Glibenclamide (Fig. 53).

Summary and conclusion

9. SUMMARY AND CONCLUSION

Herbal medicines are found to be effective in treatment of various ailments but the major problem is lack of proper scientific validation. Hence the present study is aimed at investigating the selected plant *Melia azedarach* Linn. for the diabetes problem. The root of plant *Melia azedarach* L. belongs to the family Meliaceae, is claimed to be useful for diabetes, but the claim has not been scientifically validated.

Authentication of the plant material plays a key role in pharmacognostical studies. The roots of *Melia azedarach,* were collected from sunguvarchattram, Kanchipuram. The collected specimens was botanically identified and authenticated by Dr. K.N. Sunil Kumar, R.O. and HOD Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai-106. It was identified as *Melia azedarach* Linn*.,* Meliacea family.

The parameter studied were Macroscopy, microscopy, powder microscopy, histochemical studies and physiochemical constants to establish data for proper authentication and detection of adulterants.

The qualitative analysis and quantitative estimation were carried out to identify inorganic elements present in the plant. The qualitative and quantitative analysis of toxic heavy metals like Cadmium, Arsenic, Lead and Mercury were within the WHO limits and ensure the safety of the drug.

In phytochemical analysis, extraction is the first step involved. The coarse powder was extracted by petroleum ether, chloroform, ethyl acetate, ethanol by successive solvent extraction by hot percolation method and aqueous extract by cold maceration method.

The preliminary phytochemical screenings of various extract of the plant have revealed the presence of phytoconstituents like flavonoids, phenolics, tannins, carbohydrates, triterpenoids and steroids. Quantitative estimation of flavonoids and phenolics compounds done by UV for all Successive extracts.

Fluorescence analysis of both powder and extracts were performed. There is presence of fluorescent compounds.

TLC of successive extracts and HPTLC of ethanolic extract of *Melia azedarach* Linn were carried out to identify phytoconstituents present.

The pharmacological studies include *in vitro* α-amylase inhibition assay, non enzymatic glycosylation of haemoglobin method and *in vivo* antidiabetic activity.

Active extract was selected based on the results of *in vitro* studies carried out for various extracts such as pet. Ether, chloroform, ethyl acetate, ethanol, aqueous extracts. Ethanol extract showed maximum inhibition when compared to others. Hence ethanolic extract selected for *in vivo* studies.

Anti diabetic activity assessed by Streptozotocin induced diabetic model. The parameter examined were blood glucose level, body weight, lipid profile.

The Histopathological study of pancreas was performed. The inference made from it that the β-Cells in the normal control group were in normal size. Where as in the diabetic control group were reduced in size, damaged β-Cells population and extensive necrotic changes, followed by fibrosis and atrophy. While in the group received that the test dose showed the absence of necrosis, fibrotic changes, increased number and size of the islets and presence of normal pancreatic cells. These were in the levels comparable with the ones that were administered the standard drug Glibenclamide.

The present study revealed the ethanolic extract has the significant antidiabetic activity in the both *in vitro* and *in vivo* models.

From the above studies, it can be concluded that the roots of *Melia azedarach* Linn exhibited promising antidiabetic activity and further studies can be directed towards the isolation of active constituents, characterization of individual compounds responsible for the antidiabetic activity and mechanism of action responsible for this activity.

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Annexures

सिद्ध केंद्रीय अनुसन्धान संस्थान

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Certified that the sample submitted by M. Nivedha, M. Pharm Final year, Department of Pharmacognosy, Madras Medical College, Chennai - 03 is identified as

SN	Botanical/Chemical Name	Part	Code
1.	*Melia azedarach* L.	Root	M23081801A

M23081801A

13/11/18

Dr. K.N. Sunil Kumar
Research Officer and HOD Pharmacognosy

13/11/18

Dr. G. Dayanand Reddy
Assistant Director Pharmacology and
In Charge (I/C)

Madras Medical College,Chennai-600 003
Institutional Animal Ethics Committee
Proceedings
Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)
Roc . No. 25 /AEL/IAEC/MMC,Date: 16.11.2018
Sub: MMC-IAEC approval-regarding.
Ref: IAEC meeting held on 09.11.2018

The following order is issued based on the approval of the Institutional Animal Ethics Committee held on 09.11.2018.

Project ID	25/18
CPCSEA registration number	1917/ReBi/S/16/CPCSEA/25.10.2016
Name of the Researcher	M.NIVEDHA, M.Pharm II year, Department of Pharmacognosy.
Name of the Guide	Dr.R.Radha,M.Pharm,Ph.D.,
Title of the Project	Pharmacognostical , Phytochemical studies and evaluation of antidiabetic activity of roots of *Melia azedarach.*
Date of submission of proposal to IAEC	07.08.2018
Date on which IAEC conducted	09.11.2018
Date of submission of modified proposal (if applicable)	09.11.2018
Date on which approved	09.11.2018
Validity of the approved proposal	1 year
Remarks : **Wistar albino rats of either sex -30** numbers approved	

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai-03

To
Dr.R.Radha,M.Pharm,Ph.D.,
Head of the Department, Dept. of Pharmacognosy,
College of Pharmacy,
MMC,Chennai-03.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory,
Madras Medical College,Chennai-03.

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REVIEW ON *MELIA AZEDARACH LINN*

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ABSTRACT

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their world-wide availability and fewer side effects. *Melia azedarach Linn.* (Family: Meliaceae) is a sharp or small evergreen, medium-sized deciduous tree. It grows in temperate and tropical countries like India, China and Japan. The plant requires a wide range of soil, acid to alkaline and it needs moderate moisture level. It knows locally as bakain and drek (Hindi), Persian lilac or China tree (English), Fleurs lilas (French) and also known as pride of India and Persian lilac having a wide spectrum of pharmacological activities. It is used as an Ayurvedic medicine in India and Unani medicine in Arab countries as an Antioxidative, Analgesic, Anti-Inflammatory, Insecticidal, Rodenticidal, Antidiarrhoeal, Deobstruent, Diuretic, Antidiabetic, Cathartic, Emetic, Antirheumatic and Antihypertensive. It is highly nutritious having a calorific value at 5100 kcal/kg. Also, it is used to manufacture agricultural implements, furniture, plywood, boxes, poles, tool handles and fuel wood. This plant is considered as a multipurpose tree because of its multidirectional and widespread uses in medicine, therapeutics and other economic implication. This article provides list of what are all the works done on *Melia azedarach* plant so far and it is more useful for students and research scholers.

KEYWORDS: Melia azedarach, Ayurvedic medicine, Unani medicine and multipurpose tree.

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