ANTIDIABETIC ACTIVITY OF ALCOHOLIC FRUIT EXTRACT OF *MALLOTUS PHILIPPENSIS* MUELL.ARG. IN STREPTOZOTOCIN INDUCED DIBETIC RATS

Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 32.

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

MASTER OF PHARMACY

IN

BRANCH – IV- PHARMACOLOGY

Submitted by

MUHAMMED SHABEER.A

REGISTER NO: 261425506

Under the guidance of

Mrs. G.SUMITHIRA, M. Pharm.,

Assistant Professor,
Dept. of Pharmacology

THE ERODE COLLEGE OF PHARMACY AND RESEARCH INSTITUTE,

ERODE- 638112.

OCTOBER - 2016

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Antidiabetic activity of alcoholic fruit extract of *Mallotus Philippensis* Muell.Arg. in Streptozotocin induced diabetic rats"** submitted by **Register No: 261425506** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy** in **Pharmacology** is the bonafide work carried out under guidance and direct supervision of **Mrs. G.SUMITHIRA, M. Pharm., Assistant Professor** at the Department of Pharmacology, **The Erode College of Pharmacy and Research Institute**, **Erode-638112** and was evaluated by us during the academic year 2015-2016.

1. INTERNAL EXAMINERS **2.EXTERNAL EXAMINERS**

3. CONVENER OF EXAMINATION

Examination Centre: The Erode College of Pharmacy and Research Institute.

Date:

The Erode College of Pharmacy and Research Institute

Dr. V. Ganesan, M.Pharm., Ph.D.,
Principal,
Professor and Head, Department of Pharmaceutics,
The Erode College of Pharmacy and Research Institute,
Erode - 638112.

CERTIFICATE

This is to certify that the dissertation work entitled **"Antidiabetic activity of *alcoholic fruit extract of Mallotus Philippensis* Muell.Arg. in Streptozotocin induced diabetic rats"** submitted by **Register No: 261425506** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy in Pharmacology** is the bonafide work carried out under the guidance and direct supervision of **Mrs. G.SUMITHIRA, M. Pharm., Assistant Professor** at the Department of Pharmacology, The Erode College of Pharmacy and Research Institute, Erode- 638112, during the academic year 2015-2016.

Dr. V. Ganesan, M.Pharm., Ph.D.,
Principal

Place : Erode
Date :

The Erode College of Pharmacy and Research Institute

Prof. Dr. M. Periasamy, M.Pharm., Ph.D.,
Professor and Head,
Department of Pharmacology,
The Erode College of Pharmacy and Research Institute,
Erode - 638112.

CERTIFICATE

This is to certify that the dissertation work entitled **"Antidiabetic activity of *alcoholic fruit extract of Mallotus Philippensis* Muell.Arg. in Streptozotocin induced diabetic rats"** submitted by **Register No: 261425506** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy in Pharmacology** is the bonafide work carried out under the guidance and direct supervision of **Mrs. G. Sumithira, M.Pharm., Assistant Professor** at the Department of Pharmacology, The Erode College of Pharmacy and Research Institute, Erode- 638112, during the academic year 2015-2016.

Prof. Dr. M. Periasamy, M.Pharm., Ph.D.,
Prof. & HOD

Place : Erode
Date :

The Erode College of Pharmacy and Research Institute

Mrs. G.SUMITHIRA, M. Pharm.,
Assistant Professor,
Department of Pharmacology,
The Erode College of Pharmacy and Research Institute,
Erode - 638112.

CERTIFICATE

This is to certify that the dissertation work entitled **"Antidiabetic activity of alcoholic fruit extract of *Mallotus Philippensis* Muell.Arg. in Streptozotocin induced diabetic rats"** submitted by **Register No: 261425506** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy in Pharmacology** is the bonafide work carried out under my guidance and direct supervision at the Department of Pharmacology, The Erode College of Pharmacy and Research Institute, Erode-638112, during the academic year 2015-2016.

Mrs. G.Sumithira, M.Pharm.,
Assistant professor

Place: Erode

Date:

DECLARATION

I do hereby declare that the dissertation work entitled **"Antidiabetic activity of alcoholic fruit extract of *Mallotus Philippensis* Muell.Arg. in Streptozotocin induced diabetic rats"** submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in the partial fulfilment for the Degree of **Master of Pharmacy in Pharmacology**, was carried out by myself under the guidance and direct supervision of **Mrs. G.SUMITHIRA, M. Pharm., Assistant Professor,** at the Department of Pharmacology, The Erode College of Pharmacy and Research Institute, Erode-638112, during the academic year 2015-2016.

This work is original and has not been submitted in part or full for the award of any other Degree or Diploma of this or any other University.

Place: Erode **Register No: 261425506**

Date:

ACKNOWLEDGEMENTS

The secret of success is undaunted ardor, motivation, dedication, confidence on self and above all the blessing of god. I bow in reverence to the almighty for bestowing upon me all his kindness that has helped me throughout the journey of my life. Success is an outcome of collaborated efforts aimed that achieving different goals. I hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

It gives me an immense pleasure to express my deepest than heartfelt, indebtedness and regards to my respected guide **Mrs.G.Sumithira, M.Pharm., Asst. Professor, Department of Pharmacology** for her inspiring nature, constant encouragement, valuable guidance and support to me throughout the course of this work.

I express my sincere thank and respectful regards to the **President Dr .K.R. Paramasivam M.sc., Ph.D.**, and the **Secretary & Correspondant Mr. A. Natarajan, B.A., H.D.C.,** for providing necessary facilities to carry out this dissertation work successfully.I express my deep sense of gratitude to honourable **Principal & Prof. Dr. V. Ganesan, M.Pharm., Ph.D., and HOD, Dept of Pharmaceutics,** The Erode college of Pharmacy and Research Institute, for providing necessary facilities to carry out this dissertation work successfully.

I now take this opportunity to express my sincere thanks to **Prof.Dr. M. Periasamy M.Pharm.,Ph.D., HOD, Dept of Pharmacology** for giving his valuable guidance and constant encouragement throughout the project work.

I express my heartful thank to **Vice- Principal & Prof. Dr. V.S. Saravanan, M.Pharm., Ph.D., and HOD, Dept of Pharmaceutical Analysis,** for providing necessary facilities to carry out this dissertation work successfully.

I express my sincere thanks to **Mr. P. Royal Frank M.Pharm., Mrs. Rajamathanky M.Pharm., and Mrs.Rajeswari M.Pharm.,** Dept of Pharmacology, for their support and encouragement throughout the study.

I express my sincere thanks to **Prof. Dr. P Muralidharan, M.Pharm., Ph.D., Dept of Pharmacology**, **C.L.BAID METHA COLLEGE OF PHARMACY** for providing necessary facilities to carry out this dissertation work successfully.

I express my great thanks to **Mrs. Uma Maheswari, M.Com, Lab attender, (**Department of Pharmacology), for her sincere help and technical support during the extraction process.

I express my heartful thanks to **Mrs. Chithra, D.pharm, (Store keeper), Mr. Velmurugan, D.Pharm, Mr. Kannan, D.Pharm and Mrs. Kanimozhi** for their help during plant extraction process and phytochemical analysis.

I express my sincere thanks to **Mr. Varatharajan Librarian** who helped me to take reference for carryout my project work.

I also thank to my friends **Mr. Danish T.K, Mr.Tamilarasan K, Mr. Muhammed Anas K.P, Ms. Meera Nadhini, Mr. Akhilan, Ms. Ashma, Ms. Kavya, Mr. Subash, Mr. Ragupathi, Mr. Azharudheen T.P, Mr. Haneesh V, Mr. Mohamed Fajir K, Mr. Mohamed Anees V.T, Mr. Shuaib M.V, Mr. Ansar T.P, Mr. Muhammed Shafeeq, Mr. Muhammed Nashad K, Mr. Savya Sai K.P, Mr. Safruq J.B** and all others from the Department of Pharmacology for spending their valuable time during various stages of my project work.

Last but not least I express my warmest and warm and most important acknowledgement to my grand father **Mr.Ayamu** my parents **Mr.Muhammedali A, Mrs. Nafeesa** and My small father **Mr.Yousaf** small mother **Mrs.Sajitha** my loving sister **Ms.Kamarunneesa** and brothers **Mr.Muhammed Kabeer, Mr.Muhammed Muneer, Mr. Shihabudheen, Mr. Ramees** and my friends **Mr. Muhammed Rafi K.V** with deep appreciation and moral support encouragement and everlasting love that served as a source of my inspiration, strength, determination and enthusiasm at each and every front of my life, to transfer my dreams in to reality.

With Thanks

Reg.No: 261425506

LIST OF ABBREVATIONS

ADA	:	American Diabetes Association
AEGs	:	Advanced Glycosylation products
AI	:	Atherogenic index
ANOVA	:	Analysis of variance
ATP	:	Adenosine Triphosphate
CVD	:	Cardiovascular Disease
CNS	:	Central nervous system
DM	:	Diabetes Mellitus
DNA	:	Deoxyribonucleic Acid
EEMP	:	Ethanolic extract of Mallotus Philippensis
FBG	:	Fasting Blood Glucose
GAD	:	Glutamic acid Decarboxylase
GLP	:	Glucogon like peptide
GLIBEN	:	Glibenclamide
GIT	:	Gastro intestinal tract
GTP	:	Guanosine Triphosphate
GLUT	:	Glucose transporter
GDM	:	Gestational diabetes mellitus
HNF	:	Hepatic Nuclear Factor
HDL	:	High Density Lipoprotein
HbA1c	:	Glycosylated Haemoglobin
HLA	:	Human Leukocyte Antigen
IDDM	:	Insulin Dependent Diabetes Mellitus
IGT	:	Impaired Glucose Tolerance
IL	:	Interleukin
IFN	:	Interferon
IAPP	:	Islet Amyloid polypeptide
ICA	:	Islet Cell Antibodies
IGF	:	Insulin like Growth Factor
IAA	:	Insulin Antibodies
LADA	:	Latent Autoimmune Diabetes in Adults
LD50	:	Median Lethal Dose
LDL	:	Low Density Lipoprotein

MODY	:	Maturity onset of Diabetes in young
MAPK	:	Mitogen Activated Protein Kinase
MHC	:	Major Histocompatibility Complex
NIDDM	:	Non Insulin Dependent Diabetes mellitus
NADH	:	Nicotinamide Adenine Di nucleotide
NADPH	:	Nicotinamide Adenine Di nucleotide Phosphate
OGTT	:	Oral Glucose Tolerance Test
OECD	:	Organisation of Economic Co-operation and Development
PP	:	Pancreatic polypeptide
SEM	:	standard error mean
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
TG	:	Triglycerides
TNF	:	Tumour Necrosis Factor
VIP	:	Vasoactive Intestinal peptide
VLDL	:	Very Low Density Lipoprotein
WHO	:	World Health Organisation
Fig	:	Figure
Cm	:	Centimetre
dL	:	Decilitre
i.p.	:	intra peritoneal
Kg	:	Kilogram
Min	:	Minute
Mg	:	Milligram
Ml	:	Millilitre
mmol/L	:	millimoles per litre
Nm	:	nano meter
p.o.	:	per oral
b.w.	:	body weight
qs	:	quantity sufficient
Sec	:	Seconds
◦C	:	degree Celsius
µL	:	micro litre

%PT	:	Percentage protection
GSH	:	Reduced Glutathione
GPx	:	Glutathione Peroxidase
Vit C	:	Vitamin C
Vit E	:	Vtamin E
LPO	:	Lipid Peroxidation
MDA	:	malondialdehyde
SOD	:	superoxide dismutase
CAT	:	Catalase
AST	:	aspartate amino transferace
ALT	:	alanine amino transferace

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Diabetes mellitus commonly referred as a Diabetes, it is a group of metabolic diseases in which there is high blood sugar levels over a prolonged period.[1] Symptoms of high blood sugar include frequent urination, increased thrist, and increased hunger. If left untreated, diabetes can cause many complications.[2] Acute complications can include diabetic ketoacidosis, non ketotic hyperosmolar coma, or death.[3] Serious long term complications include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to eyes.[2]

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced.[4] There are four types of diabetes mellitus:

- Type 1 diabetes results from the pancreas‘s failure to produce enough insulin. This form referred as “insulin dependent diabetes mellitus” (IDDM) or “juvenile diabetes”. The cause is unknown.[2]
- Type 2 diabetes begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progress a lack of insulin may also develop. This form referred as “non insulin dependent diabetes mellitus” (NIDDM) or “adult onset diabetes”. The primary cause is excessive body weight and not enough exercise.[2]
- Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels. They prone to diabetes in future.[5]
- **MODY** Maturity Onset Diabetes of the Young is the fourth type of diabetes. Specific monogenetic defects of the beta-cells have been identified and usually give rise to maturity onset diabetes of the young (MODY). MODY is defined as a genetic defect in beta-cell function.[6]

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM

must be managed with insulin injections. Type 2 DM may be treated with medications with or without insulin.[7] Insulin and some oral medications can cause low blood sugar.[8] Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM.[9] gestational diabetes usually resolves after the birth of the baby. Mody type diabetes managed by sufonylureas treatment.[6]

As of 2015, an estimated 415 million people had diabetes worldwide, with type 2 DM making up about 90% of the cases. This represents 8.3% of the adult population, with equal rates in women and men. As of 2014, trends suggested the rate would continue to rise. Diabetes at least doubles a person's risk of early death. From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes.[10]

ROLE OF PHYTOMEDICINE IN THE TREATMENT OF DIABETES

The Ayurvedic concept appeared and developed between 200 and 500 B.C. in India. The literal meaning of ayurveda is "science of life", because ancient Indian system of health care focused views of man and his illness. It is pointed out that the positive health means metabolically well- balanced human beings. Ayurveda remains an important system of medicine and drug therapy in India.[10]

In Ayurveda, diabetes falls under the term madhumeha. Various types of herbal preparations such as decoctions (boiled extracts), swaras (expressed juices) Asav-Arishta (fermented juices) and powders have been mentioned for the treatment of madhumeha. These indigenous medicines may not have adverse effects in therapeutic doses. It is mentioned in ancient texts such as the Charkas Samhinta that a single herb exerts different actions on many diseases and that each herb may have one dominating effect and other comparatively subsidiary effects. It is also mentioned that an herbal drug can also have synergistic and antagonistic effects in combination with other herbs.[11]

Out of an estimated 250000 higher plants, less than 1% have been screened pharmacologically and very few in regard to diabetes mellitus.[13] In India, indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushruta (6th century BC). Plants have always been an exemplary source of drugs and

a many of the currently available drugs have been delivery directly or indirectly from them. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycaemic activity. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine.[14]

The natural detoxification process of the body is effectively enhanced by herbal medicines and also very good in boosting the immune system and also herbal medicines in the treatment of a disease take into account pathogens, whole body balance, body chemistry with scientific proof of them in the treatment of a disease. Because of all these reasons, herbal medicines are preferred. Wide array of plant derived active principles have demonstrated their anti-diabetic activity. The main active constituents of these plants include guanidine, steroids, carbohydrates, glycopeptides, terpenoids, glycosides, flavanoids, alkaloids, amino acids and inorganic ions. These affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body.[15]

PHYTOCONSTITUENTS HAVING ANTI DIABETIC ACTIVITY [16,17]

The constituents that come under the category of polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthenes, flavanoids lipids, phenoics, coumarins, irirods, alkyl disulfides, inorganic ions and guanidine have been reported to have anti-diabetic activity. Specifically the following constituents are reported to have anti-diabetic activity, amino acid like hypoglycine A and hypoglycine B, alkaloids like catharanthine, leurosine, lochnerine, arecoline and vindoline, pinitol, epicatichin, bengalenoside, anemarans (A, B, C,D), atarctans (A,B,C), dioscorin (A,B,C,D,E,F), ephedrans (A,B,C,D,E), glycoprotein (moran A), mucilage, nimbidin, peptides (P insulin), S- methyl cysteine sulphoxide, S- allyl cysteine sulphoxide, andrographollide, allicin (thio-2- propene-1- sulfinic acid S-allyl ester), shamimin, beta vulgarosides I-IV, glycoside of leucopelargonidin and leucodelphindin, magniferin, marsupsin, pterosupin, pterostilbene, pinoline, naringin, salacinol, hesperidin, berberine, chlorgenic acid, charantin, swerchirin, epigallocatechin gallate, trigonelline, harmane, norharmane, lactucaside, beta-sitosterol, lactucain C, kalopanax saponin A, gymnemic acid IV, hederagenin, furfuran lignin, oleanoloc acid, elatosides (E,G,H,I), cryptolepine,

caffeoyl glucoside, momordin Ic, bellidifolin, kolaviron, scoparianosides A, B and C, kaempferol glucosides, bakuchiol, trihydroxyoctadecadienoic acids, escins (Ia,Ia,IIa,IIb and IIIa), thysanolactone, kotalanol, fagomine, 3-O-beta-D- glucopyranosylfagomine, 4-O_beta- d glucopyranosylfagomine, 3-epifagomine.

Myrciacitrins I and II, myrciaphenones A and B, momordin, prunin, tormentic acid, 8- debenzylpaenoniflorin, coutareagin, senticoside, lithosperman, senegin II, Z-senegasaponins a and b and E and Z-senegasapponins, E and Z- senegins (II, III and IV), boussingoside, paenoflorin, pachymaran, saciharan, coixan, oleanolic acid glycosides, ginsenoside, laminaran, masoprocol, senticoside A, abelmosan, ursolic acid, trichosan also exhibit anti-diabetic activity.

Flavones C- glycoside, icarin, neomyrtillin, kakonein, acarbose, voglibose, ferulic acid, brazilin, hyperin, sappanchalone, anisodamine, multiflorine, 3-deoxy sappanone, protosappanin A also have anti-diabetic activity.

Oral hypoglycaemic agents like sulphonylureas and biguanides are still the major players in the management of the disease, but there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents. Herbal medicines have been highly esteemed source of medicine throughout the human history. They are widely used today indicating that herbs are a growing part of modern high-tech medicine. In recent times, there has been a revived interest within the plant remedies. In this review article an attempt has been made to focus on hypoglyceamic plants and may be useful to the health professionals, scientists and scholars working in the field of pharmacology & therapeutics to develop evidence based alternative medicine to cure different kinds of diabetes in man and animals.[17]

In addition, a major effort was directed towards discovery of novel anti-diabetic agents. The interest in herbal drug research continues with an expectation that someday rather the other day, we would be able to bring a safer and more effective compound with all the desired parameters of a drug that could replace the synthetic medicines, which resulted in the discovery of several patented compounds, cryptolepine, maprouneacin, 3β, 30-dihydroxylupen-20 (29)-en-2-one, harunganin,

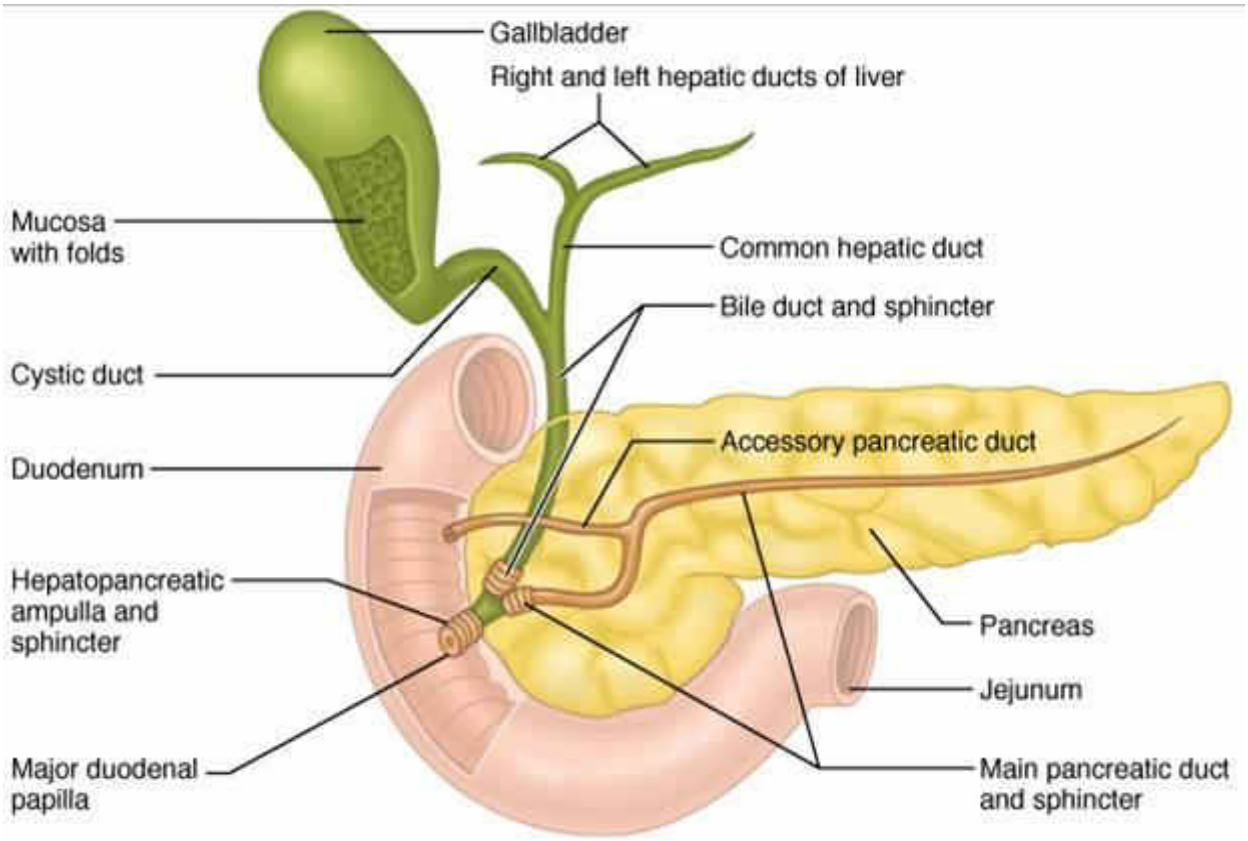
vismin, and quinines SP 18905. The most interesting discovery was nor dihydroguaiaretic acid which besides being active orally in db/db diabetic mice, also lowered cholesterol levels. This is considered as the unique quality of herbs, which was not observed in any synthetic medicine.

2. REVIEW OF LITERATURE

2.1 HUMAN PANCREAS

2.1.1 ANATOMY AND PHYSIOLOGY

The **pancreas** is a glandular organ in the digestive system and endocrine system of vertebrates. In humans, it is located in the abdominal cavity behind the stomach. It is an endocrine gland producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide which circulate in the blood. The pancreas is also a digestive organ, secreting pancreatic juice containing digestive enzymes that assist digestion and absorption of nutrients in the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids in the chyme.[18]



The pancreas is an endocrine organ that lies in the upper left part of the abdomen. It is found behind the stomach, with the head of the pancreas surrounded by the duodenum. The pancreas is about 15 cm (6 in) long.[19]

Anatomically, the pancreas is divided into a head, which rests within the concavity of the duodenum, a body lying behind the base of the stomach, and a tail, which ends abutting the spleen. The neck of the pancreas lies between the body and head, and lies anterior to the superior mesenteric artery and vein. The head of the pancreas surrounds these two vessels, and a small uncinate process emerges from the posterior part of the head, and extends posterior to the superior mesenteric vein, and terminates at the superior mesenteric artery.[20]

The pancreas is a secretory structure with an internal hormonal role (endocrine) and an external digestive role (exocrine). It has two main ducts, the main pancreatic duct, and the accessory pancreatic duct. These drain enzymes through the ampulla of Vater into the duodenum.[21].

2.1.2 FUNCTION OF PANCREAS

2.1.2.1 THE EXOCRINE PANCREAS

The exocrine pancreas consist of acini, which resemble bunches of grapes. Each acinus consists of a single layer of 40-50 pyramidal epithelial cells surrounding a lumen. The epithelial cells produce the secretion (pancreatic juice) containing enzymes, ions and water. The cells become wider during active section. The base of the acinar cells are strongly basophylic owing to the presence of endoplasmic reticulum, where there is a high concentration of RNA. This part of the cells therefore stains darker with haemotoxylin and eosin. The apex of teh cells is abundant with secretory granules containing the zymogen precursors of the pancreatic enzymes. The number of secretory granules increases after fasting, and decreases after a meal.[22]

The lumen of the acini into intercalated duct. Intercalated ducts converg to make larger interlobular ducts, which in turn converge to make interlobular ducts. Interlobular ducts are found in the connective tissue septa between lobules. Interlobular ducts join to

form either the pancreatic or the accessory duct, these ducts drain in to the duodenum. In some cases, the pancreatic duct unites with the bile duct, the bile and pancreatic juice enter the duodenum together.[18]

2.1.2.2 THE ENDOCRINE PANCREAS

In the endocrine pancreas, the islets of langerhans are embedded in the exocrine tissue. Each islet composed of 2-3 thousand epithelial cells. The epithelial cells are arranged in a compact structure that is pervaded by capillary network. A thin layer of reticular fibres separates the islets from the surrounding exocrine tissue. There are four different cell types within the islets of langerhans that each produce different hormones, they include:

α cells- produce glucagon, typically located at the periphery of the islet. They are not present in all islets.

β cells- produce insulin. The predominant cell type, located in the centre of islet and contributing to 70% of all cells.

δ**cells-** produce somatostatin. There are low numbers in all islets.

F cells- produce pancreatic polypeptide and are few in number, they may be present in the exocrine tissues also (table no:1).[23]

Table no 1: Pancreatic Islet Cells and Their Secretory Products[23]

Cell Type	Approximate Percent of Islet Mass	Secretory Products
A cell (α)	20	Glucagon,Proglucagon
B cell (β)	75	Insulin,C-peptide, Proinsulin, Islet amyloid polypeptide (IAPP)
D cell (δ)	3-5	Somatostatin
F cell (PP cell)	<2	Pancreatic polypeptide (PP)

2.2 INSULIN

Insulin is a peptide hormone produced by beta cells of the pancreatic islets, and by the Brockmann body in some teleost fish.[24] It has important effects on the metabolism of carbohydrates, fats and protein by promoting the absorption of, especially, glucose from the blood into fat, liver and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen or fats (triglycerides), or, in the case of the liver, into both.[25] Glucose production (and excretion into the blood) by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues. In high concentrations in the blood it is therefore an anabolic hormone, promoting the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread catabolism.[26]

The pancreatic beta cells (β cells) are known to be sensitive to the glucose concentration in the blood. When the blood glucose levels are high they secrete insulin into the blood; when the levels are low they cease their secretion of this hormone into the general circulation. Their neighboring alpha cells, probably by taking their cues from the beta cells, secrete glucagon into the blood in the opposite manner: high secretion rates when the blood glucose concentrations are low, and low secretion rates when the glucose levels are high. High glucagon concentrations in the blood plasma powerfully stimulate the liver to release glucose into the blood by glycogenolysis and gluconeogenesis, thus having the opposite effect on the blood glucose level to that produced by high insulin concentrations. The secretion of insulin and glucagon into the blood in response to the blood glucose concentration is the primary mechanism responsible for keeping the glucose levels in the extracellular fluids within very narrow limits at rest, after meals, and during exercise and starvation.[25,27]

When the pancreatic beta cells are destroyed by an autoimmune process, insulin can no longer be synthesized or be secreted into the blood. This results in type 1 diabetes mellitus, which is characterized by very high blood sugar levels, and

generalized body wasting, which is fatal if not treated. This can only be corrected by injecting the hormone, either directly into the blood if the patient is very ill and confused or comatosed, or subcutaneously for routine maintenance therapy, which must be continued for the rest of the person's life. The exact details of how much insulin needs to be injected, and when during the day, has to be adjusted according to the patient's daily routine of meals and exercise, in order to mimic the physiological secretion of insulin as closely as is practically possible.[28]

2.2.1 NORMAL INSULIN PHYSIOLOGY

The insulin gene is expressed in the β- cells of the islets of langerhans, where insulin is synthesized and stored in the form granules before secretion. Release from beta cells occurs as a biphasic process involving two pools of insulin.[30]

Like other peptide hormones, insulin is synthesized as a precursor (Preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus, of uncertain function called C-peptide. Insulin and C-peptide are stored as granules in beta-cells, and are normally co-secreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin.[29]

A rise in the blood glucose level calls forth an immediate release of insulin that is stored in the β-cells granules. If the secretory stimulus persists, a delayed and protracted response follows, which involves active synthesis of insulin.[30]

The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration. β-cells respond to both absolute glucose concentration and to the rate of change of blood glucose. Other stimuli to insulin release include amino acids (particularly arginine and leucine), fatty acids and the parasympathetic nervous system, peptide hormones of the gut and drugs that act on sulfonylurea receptors.[29]

Insulin is the major anabolic hormone. It is necessary for [30]

(1) Trans membrane transport of glucose and amino acids

(2) Glycogen formation in the liver and skeletal muscles

(3) Nucleic acid synthesis

(4) Protein synthesis.

(5) Conversion of glucose into triglycerides

The principle metabolic function of insulin is to increase the rate of glucose transport into certain cells of the body which are striated muscle cells, including myocardial cells; fibroblasts and the fat cells, representing collectively about two third of the entire body weight.[30]

Insulin interacts with its target cells by first binding to the insulin receptor; the number and function of these receptors are important in regulating the action of insulin. The insulin receptor is a tyrosine kinase that triggers a number of intracellular responses that effect metabolic pathways. One of the important early responses to insulin involves translocation of glucose transport units (GLUTs, of which there are many tissue- specific types) from the Golgi apparatus to the plasma membrane, which facilitates cellular uptake of glucose. Hence, removal of glucose from the circulation is a primary outcome of insulin action.[30]

2.2.2 BIPHASIC INSULIN RESPONSE TO A CONSTANT GLUCOSE STIMULUS[31]

When the β- cells are stimulated, there will be a rapid first phase insulin response 1-3 minutes after the glucose level is increased; this returns towards baseline in 6-10 minutes later. Thereafter, a gradual second phase insulin response that persists for the duration of the stimulus. Type 2 diabetes mellitus is characterised by loss of the first phase insulin response and a diminished second phase response.

2.2.3 EFFECT OF INSULIN ON ITS TARGETS[23]

Insulin promotes the storage of fat as well as glucose (both are sources of energy) within specialised target cells. (Fig no.2) Insulin promotes synthesis (from circulating nutrients) and storage of glycogen, triglycerides and protein in its major target tissues: liver, fat and muscle. The release of insulin from the pancreas is

stimulated by increased blood glucose, vagal nerve stimulation and other factors (fig no.2).

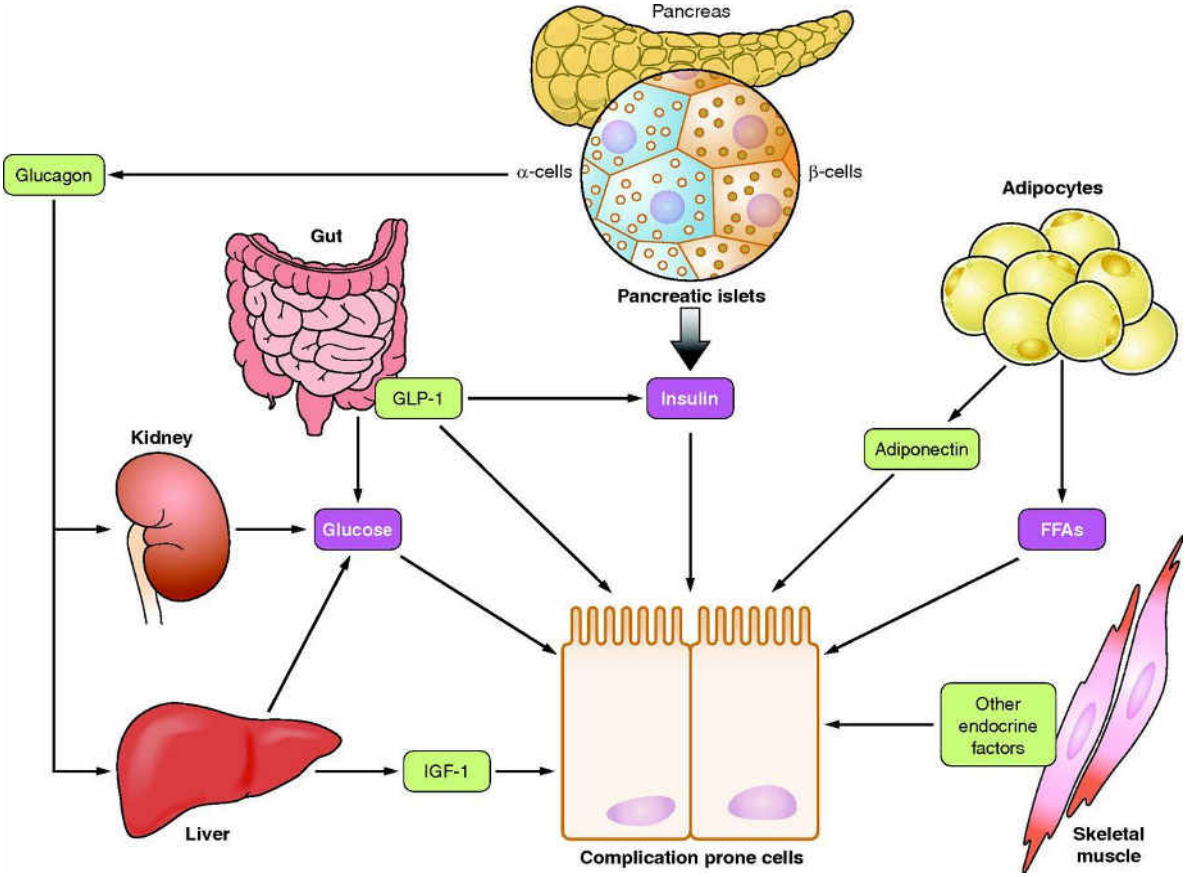


Fig. no- 2: Effect of Insulin on its targets

2.2.4 ENDOCRINE EFFECTS OF INSULIN [23]

EFFECTS ON LIVER

- Reversal of catabolic features of insulin deficiency.
- Inhibits glycogenolysis.
- Inhibits the conversion of fatty acids and amino acids into keto acids.
- Inhibits conversion of amino acids to glucose

- Produces anabolic action
- Promotes glucose storage as glycogen (induces glucokinase and glycogen synthase and inhibits phosphorylase).
- Increases triglycerides synthesis and very low density lipoprotein formation.

EFFECT ON MUSCLE

- Increases protein synthesis
- Increases amino acid transport.
- Increases ribosomal protein synthesis.
- Increases glycogen synthesis.
- Increases glucose transport.
- Induces glycogen synthesis and inhibits phosphorylase.

EFFECT ON ADIPOSE TISSUE

- Increases triglyceride storage.
- Lipoprotein lipase is induced and activated by insulin to hydrolyse triglycerides from lipoproteins.
- Glucose transport into cells provides glycerol phosphate to permit esterification of fatty acids supplied by lipoprotein transport.
- Intracellular lipase is inhibited by insulin.

2.2.5 INSULIN AND CARBOHYDRATE METABOLISM[32]

After eating, various foods are broken into sugars in the stomach. The main sugar is glucose which passes through the blood stream. But to remain healthy, the blood glucose levels should not go too high or too low.

When the blood glucose level starts to rise (after having food), at the same time the level of the hormone called insulin also rises.

Insulin is the hormone that works on the body cells and helps them to take the glucose from the blood stream for producing energy and some is converted into stores of energy (glycogen or fat).

When the blood glucose level begins to fall (between meals), at the same time the level of insulin also falls. When this happens the glycogen or fat is converted back into glucose which is released from the cells into the blood stream.

2.2.6 INSULIN AND PROTEIN METABOLISM[33]

The present status of protein synthesis within cells has been outlined. Protein is formed in the absence of insulin; the net formation of protein is accelerated by insulin. The effects of insulin on protein metabolism take place independently of the transport of glucose or amino acids into the cell; of glycogen synthesis; and of the stimulation of high energy phosphate formation. In the case of protein metabolism, as in certain studies on the pathways of glucose and fat metabolism, these observations reveal striking intracellular effects of insulin in many tissues. Within most tissues the effect of insulin appears to find expression predominantly at the microsomal level. Incidentally, other hormones which affect protein metabolism such as growth or sex hormones appear to act at the microsomes. The fact that insulin exerts effects on protein metabolism at other intracellular sites as well as the above independent effects leads one to agree that its action consists of a stimulation of multiple, seemingly unrelated, metabolic events.

The fact that an immediate effect of insulin on protein synthesis is independent of the immediate need for extracellular glucose or amino acids does not mean that the sustained functioning of cells is likewise independent. The biochemist is fully aware of metabolic defects in diabetes which are not altered by insulin in vitro, but which demand varying periods of pre-treatment of the animal. It is also known that in diabetes some

proteins (enzymes) may be deficient while others may be prodced in excess in the absence of insulin.

2.2.7 INSULIN AND FAT METABOLISM[34]

The triacylglyceroles that comprise the bulk of lipids in the diet are hydrolyzed to free fatty acids, monoacylglyceroles and glycerol in the intestinal tract. During absorption through the intestinal tract mucosa, triacylglyceroles are re-synthesized from free fatty acids, and glycerol-3-phosphate is formed in the intestinal mucose. These globules, called chylomicrons, pass through the liver and adipose tissue, they are reduced in size by an enzyme, lipoprotein lipase (LPL). In the postabsorptive period, free fatty acids and glycerol are released from adipocytes by neural and hormonal stimulation. The free fatty acids can be burned by almost all tissues of the body except the brain. They are burned in the mitochondria by a process of b-oxidation to acetyl-CoA, which can then enter the citrate acid cycle for conversion to CO2, adenosine triphosphate, and water. When excessive quantities of glucose are ingested, the glucose can be converted to a storage form, triacylglycerol. Fatty acids are synthesized by a series of reactions in which acetyl-CoA and malonylo-CoA residues sequentially condense until the fatty acid chain is completed. The fatty acids are then combined with glycerol-3-phosphate, generated in the liver, to form the neutral triacylglyceroles. The insulin has effects on both the synthetic (estrification) and breakdown (lipolysis) pathways. The promotion of triacylglycerol storage in fat is one of the most important of the actions of insulin

DISORDERS OF PANCREAS[35]

- Pancreatitis
- Diabetes mellitus
- Exocrine pancreatic insufficiency
- Cystic fibrosis
- Pseudocysts
- Cysts

2.3 DIABETES MELLITUS

Diabetes mellitus is simply referred to as diabetes which is either because the body does not produce enough insulin or because the cells do not respond to insulin that is produced. The classical symptoms of the diabetes mellitus are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

2.3.1 CLASSIFICATION OF DIABETES[31]

The American diabetes association has proposed four types of diabetes based on their aetiology. They are;

- Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM)
- Type 2 diabetes mellitus or non insulin dependent diabetes mellitus (NIDDM)
- Maturity onset diabetes of the young (MODY)
- Gestational diabetes

2.3.2 TYPE 1 DIABETES MELLITUS[31]

Type 1diabetes (previously insulin dependent diabetes mellitus (IDDM)) is characterised by beta –cell destruction, usually leading to absolute insulin deficiency and associated with usually juvenile onset, a tendency to ketosis and diabetic ketoacidosis and an absolute need for insulin treatment. Most patients have type1A diabetes, which is caused by a cellular mediated autoimmune destruction of the beta-cells of the pancreas.

2.3.3 PATHOGENESIS OF TYPE 1 DIABETES MELLITUS[31]

Type 1 diabetes mellitus (DM) is a disease of multi factorial autoimmune causation. Worldwide, there is a marked geographic variation in prevalence. The overall lifetime risk in Caucasian subjects is approximately 0-4%. Type 1 DM is caused by an interaction between environmental factors and an inherited genetic predeposition.

In twin studies, a significant genetic contribution is suggested by a concordance value for type 1 DM of 30-50%. The risk to a first degree relative is approximately 5%. The high discordance rate supports the notion that type 1 DM is multifactorial in aetiology. Environmental triggers may account for up to two thirds of the disease susceptibility. About 20 different regions of the human genome have been found to have some degree of linkage with type 1 DM. To date, the strongest linkage has been with genes encoded in the human leukocyte antigen (HLA) region located within the major histocompatability (MHC), the contribution of which to disease risk is now designated IDDM 1. This appears to be the most powerful determinant of genetic susceptibility to the disease, accounting for approximately 40% of familial inheritance. More than 90% of patients who develop type 1 DM have either DR3, DQ2 or DR4, DQ haplotypes, whereas fewer than 40% normal controls who have these haplotypes. DR3-DR4 heterozygosis is highest in children who develop diabetes before the age of 5 years (50%) and lowest in adults presenting with type 1 diabetes (20-30%) compared with an overall US population prevalence of 2.4%. specific polymorphism of the DQBI gene encoding the beta- chain of class II DQ molecules predispose to diabetes in Caucasians but not in Japanese. In contrast, the HLS-DQ6 molecule protects against the disease. HLA antigens (class I and II) are cell- surface glycoprotein that play crucial role in presenting auto-antigen peptide fragments to T lymphocytes and thus initiating an immune response. Polymorphism in the genes encoding specific peptide chains of the HLA molecules may therefore modulate the ability of beta cells derived antigens to trigger an autoimmune response against the beta- cells.

Only one non- HLA gene has been identified with certainty and that is the insulin gene (INS) region on chromosomes 11p5.5, now designated as IDDm2. Population studies of Caucasian type 1 subjects and non- diabetic controls initially showed a positive association between alleles within the INS region and disease susceptibility. However, recent genome screens have provided conflicting data regarding the role of the INS gene region (IDDM2). It is thought that INS and HLA act independently in the causation of type-1 diabetes and that the INS gene region (IDDM2) accounts for 10% of familial clustering.

The most likely environmental factor implicated in the causation of type-1 DM is viral infection. Numerous viruses attack the pancreatic beta – cell either directly through a cytolytic effect or by triggering an autoimmune attack against the beta cell. Evidence for a viral factor in aetiology has come from animal models and in humans, from observation of seasonal and geographic variations in the onset of disease. In addition, patients newly presenting with type 1 DM may exhibit serologic evidence of viral infection. Viruses that have been linked to human type 1 DM include mumps, Coxsackie B, retroviruses, rubella, cytomegalovirus and Epstein- Barr virus. Bovine serum albumin, a major constituent of cow's milk has been implicated as a cause of type 1 DM in children exposed at an early age, but definite proof is lacking and this remains controversial. Nitrosamines (found in smoked and cured meats) may be diabetogenic as many chemicals known to be toxic to pancreatic beta- cells, including alloxan, sterptozotocin and the rat poison vector. Recent reports suggested that early ingestion of cereal or gluten increases the risk of type 1 diabetes and remain to be confirmed.

Type 1 DM is associated with autoimmune destruction of the bets cells of the endocrine pancreas. Examination of islet tissue obtained from pancreatic biopsy or that obtained from post-mortem patients with recent onset type 1 DM confirms a mono nuclear cell infiltrate (termed insulitis) with the presence of CD_4 and CD_8 T lymphocytes, B lymphocytes and macrophages suggesting that these cells have a role in the destruction of beta- cells. Although the precise mechanism of such as insulin has not been elucidated, it seems to be that an environmental factor, such as viral infection, in a subject with an inherited predisposition to the disease, triggers the damaging immune response. This results in aberrant expression of class II MHC antigen by pancreatic beta –cells. T lymphocytes recognise antigen presenting cells and are activated, producing cytokines such as interleukin (IL)-2, interferon (INF) and tumour necrosis factor (TNF). This generates a clone of T lymphocytes that carry receptors specific to the presented antigen. Such T-helper cells assist B lymphocytes to produce antibodies directed against the beta- cells. Such antibodies include islet cell antibodies (ICA) directed against cytoplasmic components of the islet cells. ICA presence may precede the development of type 1DM. Some subjects may develop ICA temporarily and not go on to develop the disease, but persistence of ICA leads to progressive beta- cell

destruction associated with the chronic inflammatory cell infiltrate termed 'insulitis'. Type 1 DM ensures. Other antibodies associated with type 1 DM are islet cell- surface antibodies (present in 30-60% of newly diagnosed type 1 DM patients), insulin antibodies (IAA0 and antibodies to an isoform of glutamic acid decarboxylase (GAD).

2.3.4 TYPE 2 DIABETES MELLITUS[31]

Type 2 diabetes (previously non insulin dependent diabetes mellitus (NIDDM)) is associated with obesity and an onset later in life. Patients, at least initially and often throughout their lives, do not have a need for insulin therapy. The disorder manifests as a result of insulin resistance and relative insulin deficiency. A precise cause (or causes) has not been found. This type of diabetes frequently remains undiagnosed for many years despite affected individuals being at risk of developing serious macro vascular or micro vascular complications of the disease. Some patients may masquerade as type 2 diabetic patients, but ultimately are recognised as having a late onset slowly progressing immune mediated type 1 diabetes, so called latent autoimmune diabetes in adults (LADA).

2.3.5 PATHOGENESIS OF TYPE 2 DIABETES MELLITUS[31]

Subjects with type-2 DM exhibit abnormalities in glucose homeostasis owing to impaired insulin secretion, insulin resistance in muscle, liver, adipocytes and abnormalities of splanchnic glucose uptake.

2.3.6 INSULIN SECRETION IN TYPE 2 DIABETES MELLITUS[31]

Impaired insulin secretion is a universal finding in patients with type 2 diabetes. In the early stages of type 2 diabetes mellitus, insulin resistance can be compensated for by an increase in insulin secretion leading to normal glucose tolerance. With increasing insulin resistance, the fasting plasma glucose will rise, accompanied by an increase in fasting plasma insulin levels, until a fasting plasma glucose level is reached when the beta- cell is unable to maintain its elevated rate of insulin secretion at which point the fasting plasma insulin declines sharply. Hepatic glucose production will begin to rise. When fasting plasma glucose reaches high levels,

the plasma insulin response to a glucose challenge is markedly blunted. Although fasting insulin levels remain elevated, postprandial insulin and C-peptide secretory rates are decreased. This natural history of type 2 diabetes starting from normal glucose tolerance followed by insulin resistance, compensatory hyperinsulinemia and then by progression to impaired glucose tolerance and over diabetes has been documented in a variety of populations.

Type 2 diabetes mellitus is characterised by loss of the first phase insulin response to an intravenous glucose load, although this abnormality may be acquired secondary to glucotoxicity. Loss of the first phase insulin response is important as this early quick insulin secretion primes insulin target tissues, especially the liver. There may be multiple possible causes of the impaired insulin secretion in type 2 diabetes mellitus with several abnormalities having been shown to disturb the delicate balance between islet neogenesis and apoptosis. Studies in first degree relatives of patients with type 2 DM and in twins have provided strong evidence for the genetic basis of abnormal beta-cell function. Acquired defects in type 2 diabetes may lead to impairment of insulin secretion. Clinical studies in man and animal studies, have supported the concept of glucotoxicity, where by an elevation in plasma glucose levels, in the presence of a reduced beta- cell mass, can lead to major impairment in insulin secretion.

Lipotoxicity has also been implicated as an acquired cause of impaired beta cell function. Patients with type 2 DM exhibit a reduced response of the incretin glucagon like peptide (GLP)-1 in response to oral glucose, while GLP-1 administration enhances the postprandial insulin secretory response and may restore near normal glycaemia. Amyloid deposits(islet amyloid polypeptide (IAPP) or amylin in the pancreas are frequently observed in patients with type 2 diabetes and have been implicated as a cause of progressive beta-cell failure. However, definitive evidence that amylin contributes to beta-cell dysfunction in humans is lacking.

2.3.7 INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS[31]

Insulin resistance is a characteristic feature of both lean obese individuals with type 2 diabetes. In the fasting state, plasma insulin levels are increased in patients with

type 2 diabetes. Since hyperinsulinemia is a potent inhibitor of hepatic glucose production, an excessive rate of hepatic glucose production is the major abnormality responsible for the elevated fasting plasma glucose in type 2 diabetes. It follows that there must be hepatic resistance to the action of insulin. The liver is also resistant to the inhibitory effect of hyperglycemia on hepatic glucose output. Most of the increase in hepatic glucose production can be accounted by an increase in hepatic gluconeogenesis. Muscle is the major site of insulin-stimulated glucose disposal in humans. Muscle represents the primary site of insulin resistance in type 2 diabetic subjects leading to a marketed blunting of glucose uptake into peripheral muscle. In contrast, splanchnic tissue like the brain is relatively insensitive to insulin with respect to stimulation of glucose uptake. Following glucose ingestion, both impaired suppression of hepatic glucose production and decreased muscle glucose uptake are responsible for the observed glucose intolerance leading to hyperglycemia.

There is a dynamic relationship between insulin resistance and impaired insulin secretions. Insulin resistance is an early and characteristic feature of type 2 diabetes in high risk populations. More over diabetes develops only when the beta – cells are unable to increase sufficiently their insulin output compensate for the defect in insulin action (insulin resistance). Insulin resistance in type 2 diabetes is primarily due to post binding defects in insulin action. Diminished insulin binding is modest and secondary to down-regulation of the insulin receptor by chronic hyperglycemia. Post-binding defects that have been recognised include reduced insulin receptor tyrosine kinase activity, insulin signal transduction abnormalities, decreased glucose transport, diminished glucose phosphorylation and impaired glycogen synthatase activity.

Quantitatively, impaired glycogen synthesis represents the major abnormality responsible for insulin resistance in type 2 diabetic patients.

2.3.8 MATURITY ONSET DIABETS OF THE YOUNG (MODY)[31]

Specific monogenetic defects of the beta-cells has been identified and usually gives rise to maturity onset diabetes of the young (MODY). MODY is defined as a genetic defect in beta-cell function.

2.3.9 PATHOGENESIS OF MODY[31]

Maturity onset diabetes of the young (MODY) is inherited as an autosomal dominant and, upto date, abnormalities at six genetic locations on different chromosomes have been identified. The most common form of MODY is associated with mutations on chromosome 12 in hepatic nuclear factors (HNF)-1α and hence this is referred to as transcription factor MODY. Other mutations affect such transcription factor as HNF-1β, HNF-4α, insulin promoter factor-1 and NEUROD-1. Transcription factor mutations alter insulin secretion in the mature β-cells as well as altering β-cell development, proliferation and cell death. Cell dysfunction ensues until the emergence of frank diabetes. Patients with transcription- factor mutations tend to be lean and insulin sensitive rather than obese and insulin-resistant. Microvascular complications are frequent. The first gene implicated in MODY was the glucokinse gene. Mutations on the glucokinse gene on chromosome 7 result in a defective glucokinase molecule. Glucokinse converts glucose to glucose-6-phosphate, the metabolism of which stimulates insulin secretion by the β-cells tehreby glucokinase serves as a 'glucose sensor'. With defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. Over 100 glucokinase gene mutations have been found in families from several different countries. Fasting hyperglycemia is present from both and worsens very slowly with age. Subjects are usually detected by screening, e.g. in pregnancy or during coincidental illness or by family studies. The mild hyperglycemia of this type of MODY rarely needs any treatment other than diet and microvascular complications are rare.

Other specific genetic defects leading to diabetes include point mutations in mitochondrial DNA, genetic abnormalities which leads to the inability to convert proinsulin to insulin and the production of mutant insulin molecules and also the mutations of the insulin receptor. Diabetes may also result from over disease of exocrine pancreas, secondary to endocrinopathies and also due to some drugs and chemicals. Viruses like cytomegalovirus, coxsackievirus B, adeno virus, mumps and congenital rubella are also associated in the destruction of β-cells. Genetic syndromes

like Down's syndrome, Turner's syndrome, Wolfram's syndrome, Klinefelter's syndrome are accompanied by an increased incidence of diabetes mellitus.

2.3.10 GESTATIONAL DIABETES MELLITUS[31]

Gestational diabetes mellitus (GDM) was first defined as decreased carbohydrate tolerance that develops or may be identified during pregnancy and this definition was changed in 2010 as that GDM is a carbohydrate intolerance that develops during pregnancy or has been discovered for the first time during pregnancy which is not diabetes. Therefore, the GDM definition was not included in overt diabetes in pregnancy.

PATHOGENESIS[31]

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance that develops first time during pregnancy. Type 1 and type2 diabetes also presents in pregnancy occasionally. For GDM there is lack of agreed diagnostic criteria, but this should not detract from the detrimental impact of maternal hyperglycemia on the pregnancy and the future health of the mother and the child. The American Diabetes Association recommends immediate recommendation for those women who are to be at high risk of GDM with marked obesity, previous history of GDM, glycosuria or strong family history of diabetes.

The fasting plasma glucose ≥126 mg/dl (7mmol/1) or a random plasma glucose ≥200 mg/dl (11mmol/1) meets the threshold for diagnosis of GDM and should conform on a subsequent day.

In high-risk and average-risk women of GDM, it will not be found in the initial screening and they should be screened between 24 and 26 weeks of gestation by either a one-step approach using a 100g oral glucose tolerance test (OGTT) or a two-step approach which involves in measuring the plasma glucose level 1 hour after a 50g oral glucose load and performing a 100 g OGTT on those women who exceed the glucose threshold 1 hour after the 50g oral glucose load.

As if the glucose threshold value is ≥140mg/dl (7.8 mg/dl), then around 80% the woman is with GDM. The diagnostic criteria for the 100 g OGTT are as follows; ≥95mg/dl (5.3mmol/1) fasting and ≥180 mg/dl (10mmol/1) at 1 hour, ≥155 mg/dl (8.6mmol/1) at 2 hours and ≥140 mg/dl (7.8mmol/1) at 3 hours. To diagnose the GDM, two or more of the plasma glucose values must meet or exceed. In many countries these testing methods are not used and therefore a 75g IGTT was recommended by the WHO.

Gestational diabetes mellitus is mainly seen in women with obesity, increased maternal age and groups with a high background incidence of type 2 diabetes mellitus. GDM usually occurs after the middle of the second trimester and can be detected by suitable screening tests especially in the persons who are at high risk.

Prenatal morbidity in gestational diabetes mellitus increases with an increase in maternal hyperglycemia. Most of the pregnancy related morbidity of gestational diabetes mellitus is associated with delivering a large–fore gestational- age infant. Caesarean rates have been increased due to increase in gestational diabetes patients but this type of caesarean delivery can be reduced by intensive management of maternal hyperglycemia. In the majority of the mothers with gestational diabetes, it can be managed by diet alone by a dietician. Along with the mother, sequential tests will be done from the foetus to estimate the foetal growth and abdominal circumferences and also to identify features of inappropriate foetal growth and inform the mother the need of maintaining blood glucose level intensively.

The American Diabetes Association recommends that if the dietary management does not maintain fasting plasma glucose level below 5.8 mg/dl (105mmol/1) and the 2 hour post prandial glucose level below 6.7 mg/dl (120mmol/1), then insulin therapy should be considered.

2.3.11 OTHER TYPES OF DIABETES MELLITUS[31]

Diabetes can also result from another process that adversely affects the pancreas and such acquired processes pancreatitis, trauma, pancreatectomy, pancreatic cancer. Hemochromatosis, fibrocalculous pancreatopathy and cystic fibrosis

may also cause diabetes. Diabetes may also caused by the other endocrine diseases especially when there is over-secretion of hormones that antagonize the normal effects of insulin including Cushing's syndrome, acromegaly and pheochromocytoma.

Diabetes may also result from certain rare diseases associated with abnormalities of insulin or the insulin receptor which causes extreme insulin resistance and sometimes found in association with acanthosis nigricans. These disorders are categorized as insulin resistance syndromes. Diabetes may also occur due to a wide array of genetic syndromes like Down's syndrome, Klinefelter's syndrome, Turner's syndrome. Diabetes may also result from the drugs like glucocorticoids, diazoxide, thiazides which have similar effect may also cause diabetes.

2.4 INSULIN DEFICIENCY[31]

Insulin deficiency results in increased hepatic glucose production and hence hyperglycemia by increased gluconeogenesis and glycogenolysis. Insulin deficiency also results in increased proteolysis releasing both glycogenic and ketogenic amino acids. Lipolysis is increased by elevating both glycerol and non-esterified fatty acid levels which further contribute to gluconeogenesis and keto genesis respectively which finally leads to hyperglycemia, breakdown of body fat and protein and academia.

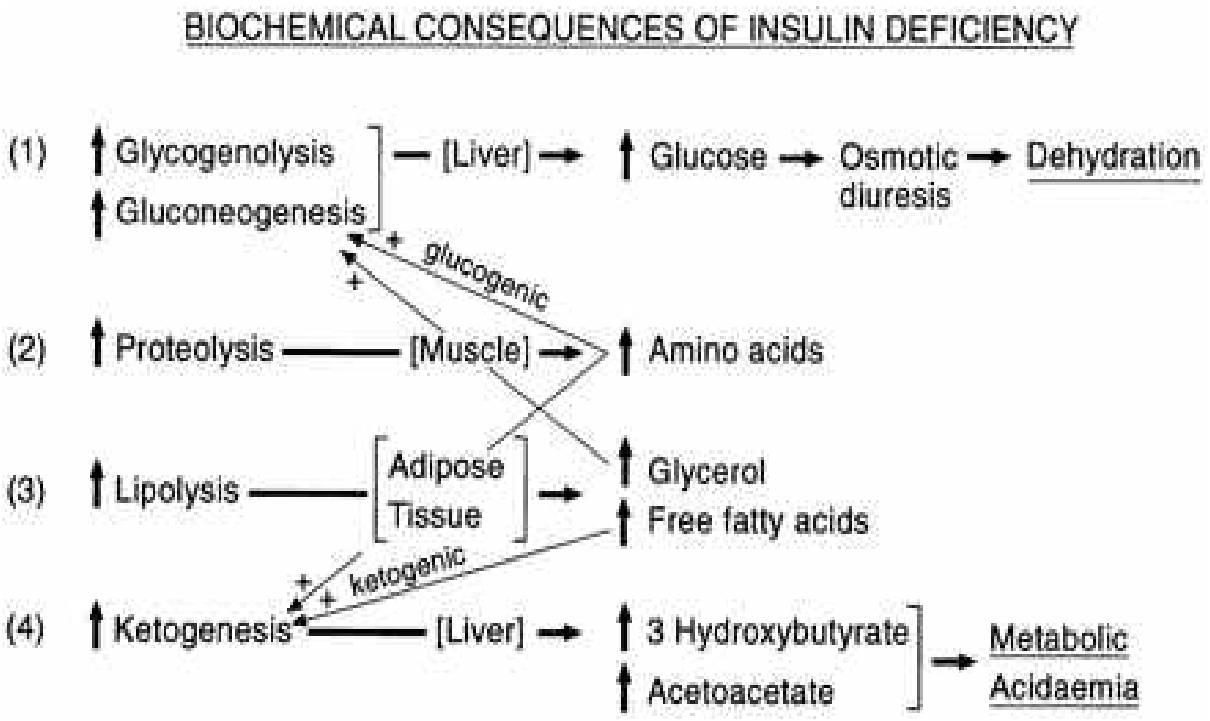


Fig no 3: The biochemical consequence of Insulin deficiency

2.5 COMPLICATIONS OF DIABETES MELLITUS[31]

The morbidity associated with long standing diabetes of either types results in multiple complications mainly like macro vascular, microvascular and neurologic. The basis of these chronic long term complications is the great deal of research. The available experimental and clinical evidence suggests that most of the complications of the diabetes results mainly from the derangements by hyperglycemia. In addition, the

existence of hypertension is common in diabetes which leads to atherosclerosis. It is evident that when a kidney is transplanted to diabetic patient from non diabetic donors develops lesions due to diabetic nephropathy which is due to metabolic abnormalities in diabetic patients. Conversely, the kidneys with lesions due to diabetic nephropathy causes the reversal of the lesions when transplanted into the normal recipients

2.5.1 MACROVASCULAR COMPLICATIONS[36]

Macro vascular refers to the large blood vessels of the heart, brain, and legs. The commonest manifestation of the macro vascular disease is in the coronary arteries and the legs. Atherosclerosis of the coronary arteries is common in most of the people with diabetes which is the most common cause for the death in people with diabetes which may occur at a much younger age than in the general population and even females are not immune in getting the disease. The mechanism for the development of macro vascular disease in people with diabetes is similar to that of people without diabetes which varies in the speed of development between diabetic and non-diabetic patients. The risk of coronary artery disease is enhanced at all levels by the risk factors like cholesterol, smoking, sedentary lifestyle, obesity, hypertension, etc

2.5.2 MICROVASCULAR COMPLICATIONS[36]

A Microvascular disease affects capillaries all over the body and so the manifestations of the disease can be diffused. The eyes and kidneys are the most affected organs. In the eyes, retinopathy which causes blindness, cataracts and glaucoma are the complications. In kidneys, nephropathy accounts for half of the people who go for dialysis and receive kidney transplantation.

2.5.3 PATHOGENESIS OF THE COMPLICATIONS OF DIABETES

3 main mechanisms linking hyperglycemia to the complications of long-standing diabetes have been explored. Currently two such mechanisms are considered important.

First mechanism[30]

Glucose chemically attaches to free amino acid groups of proteins without the aid of enzymes by the process called nonenzymatic glycosylation. The degree of this nonenzymatic glycosylation is directly related to the level of blood glucose. In the management of diabetes mellitus, the measurement of glycosylated haemoglobin (HbA1c) levels in blood is useful, because it provides an index of the average blood glucose levels over the 120 day life span of the erythrocytes. The early glycosylation products of collagen and other long lived proteins undergo a slow series of chemical rearrangements in the interstitial tissues and blood vessel walls to form irreversible advanced glycosylation end products (AGEs) and these products accumulate on the vessel wall over the life time.

AGE have a number of chemical and biological properties which are potentially pathogenic.

The formation of glycosylated end products (AGEs) on the proteins such as collagen causes cross-linkages between polypeptides, which may trap nonglycosylated plasma and interstitial proteins. The circulating low density lipoprotein (LDL) is trapped and retards its efflux from the vessel wall and promotes the deposition of cholesterol and thus causes atherogenesis. AGEs also affects the structure and function of capillaries including those on the glomeruli where the basement membranes become thickened and becomes leaky.

AGEs also bind to receptors of many cell types like monocytes, macrophages, endothelium and mesengial cells. The binding of AGEs on these receptors induces a variety of biological activities, which includes monocytes emigration, release of cytokines and growth factors from macrophages, increased endothelial permeability and enhanced proliferation of fibroblasts and smooth muscle cells and synthesis of extracellular matrix. All these effects can be potentially contributed to diabetic complications.

Second mechanism[30]

The second major mechanism proposed for the complications of intracellular hyperglycemia is the disturbances in the polyol pathways. Some tissues like nerves, lens, kidneys, and blood vessels do not require insulin for the transport of glucose, so hyperglycemia leads to an increase in intracellular glucose, which is then metabolised by aldose reductase into an polyol and eventually to fructose. Thus, the accumulated sorbitol and fructose leads to increase intracellular osmolarity and influx of water and then causes osmotic cell injury. In the lens, osmotically accumulated water causes swelling and opacity. The accumulation of sorbitol also impairs ion pumps and promotes the injury of Schwann cells and pericytes of retinal capillaries, with resultant peripheral neuropathy and retinal micro aneurysms. Inhibition of aldose reductase is capable of ameliorating the development of cataracts and neuropathy.

2.5.4 DIABETIC DYSLIPIDEMIA[31]

Dyslipidemia is the major macro vascular risk factor for the macro vascular complications which leads to the cardiovascular diseases (CVD) in type 2 diabetes mellitus. Along with this, endothelial dysfunction, platelet hyperactivity, impaired fibrinolytic balance and abnormal blood flow which causes atherosclerosis and increases the risk of thrombotic vascular events. In type 2 diabetes mellitus, the most common lipoprotein abnormality is the elevation of triglycerides and very low density lipoprotein (VLDL) which is caused by the overproduction of VLDL triglycerides.

The alteration in the distribution of lipids increases the risk of atherosclerosis in diabetic patients. So, the condition with insulin deficiency and insulin resistance was identified as phenotype of dyslipidemia in diabetes mellitus which is characterised with high plasma triglyceride level, low HDL cholesterol level and increased level of small dense LDL cholesterol. In addition to this, in diabetic patients, there will be an increment of free fatty- acid release which is due to insulin resistance. So, due presence of sufficient glycogen stores in the liver will promote triglyceride production which stimulates the secretion of apolipoprotein B (Apo B) and VLDL cholesterol. This

production of VLDL cholesterol by liver is enhanced due to the disability of insulin to inhibit the release of free fatty-acids. There are many associations between dyslipidemia and increased risk of cardiovascular disease in type2 diabetes mellitus patients due to increased triglyceride levels and low HDL cholesterol

The management of dyslipidemia in diabetes mellitus includes changes in the lifestyle of the patients such as increased physical activity and dietary modifications. Besides this, antihyperlipidemic agents have been utilised for the management of dyslipidemia. For the prevention of primary and secondary cardiovascular disease in type 2 diabetes mellitus, anti platelet agents were recommended in contrast. Dyslipidemia is categorised as one of the cardiovascular risk factors beside to the family history of hypertension, CHD, smoking. Patients with type 2 diabetes mellitus having dyslipidemia are eligible for the prevention of cardiovascular disease with anti-platelet agents.

2.6 SIGNS AND SYMPTOMS OF DIABETES MELLITUS[37]

Polyuria (frequent urination)

The insulin is ineffective, kidneys cannot filter the glucose back into the blood and the kidneys will take water from the blood to dilute the glucose which in turn fills up the bladder and causes frequent urination in diabetic patients.

Polydipsia

High thirst due to osmosis of water from cells into the blood in an attempt to dilute the high blood glucose concentration.

Intense hunger

Tendency to take food frequently more hunger and causes weight gaining.

Unusual weight loss

Weight loss is more common in patients with diabetes. As the body is not making enough insulin then the body will seek out for the other energy source where the cells

are not getting glucose. Muscle tissues and fat will be broken down for the energy to the cells. So, when these muscle tissues and fat is broken down automatically there will be loss in body weight.

Blurred vision

This can be caused by tissue being pulled from the eye lenses and effects the eye's ability to focus and can be treated. If it is severe prolonged vision problems and blindness can occur.

Other symptoms

Cuts, bruises, skin or yeast infections do not heal properly or quickly because if there is more sugar in the body, its ability to recover from infections is affected.

Specially, it is difficult to cure bladder and vaginal infections in women with diabetes.

Red and swollen gums

If the gums are red, tender and swollen this could be the sign of diabetes. The gums will pull away the teeth and the teeth will become loose. Numbness / tingling in feet and palm

2.6.1 Long-term complications of Diabetes:[38,39]

- Microangiopathy: Ischemic heart disease (IHD), stroke, peripheral vascular disease.
- Microangiopathy: retinopathy, nephropathy.
- Neuropathy: peripheral neuropathy, autonomic neuropathy
- Cataract
- Diabetic foot
- Diabetic heart

2.6.2 RISK FACTORS OF TYPE 1 DIABETES[40]

- Family history
- Genetics
- Age
- Exposure to certain virus, such as the Epstein-Barr virus, Coxsackie virus, mumps virus and cytomegalovirus
- Early exposure to cow's milk
- Low vitamin D levels
- Drinking water that contains nitrates
- Early (before 4 months) or late (after 7 months) introduction of cereal and gluten into a baby's diet
- Having a mother who had preeclampsia during pregnancy
- Being born with jaundice.

2.6.3 RISK FACTORS FOR TYPE 2 DIABETES

- Obesity
- Age
- Family history of diabetes
- History of gestational diabetes (diabetes during pregnancy)
- High blood pressure(>130/80mm/Hg)
- Impaired glucose metabolism
- Physical inactivity

- Race/ethnicity- African Americans, Hispanic/ Latino Americans, American Indians and some Asian Americans and Native Hawaiians/Pacific Islanders are particularly at high risk.

2.7 DIAGNOSIS OF DIABETES MELLITUS[38,39]

The diagnosis of the diabetes is an asymptomatic subject should never be made on the basis of a single abnormal blood glucose value. For the asymptomatic person, at least one additional plasma blood glucose tolerance test result with a value in the diabetic range is essential, either fasting, from a random sample, or from the oral glucose tolerance test (OGTT). If such sample fails to confirm the diagnosis of diabetes mellitus, it will be usually be advisable to maintain surveillance with periodic retesting until the diagnostic situation becomes clear. In these circumstances, the clinician should take into consideration such additional factors as ethnicity, family history, age, adiposity, and concomitant disorders, before deciding on a diagnostic or therapeutic course of action. An alternative to blood glucose estimation, the OGTT has long been sought to simplify the diagnosis of diabetes. Glycated haemoglobin, reflecting average glycaemia over a period of weeks, was thought to provide such a test. Although in certain cases it gives equal or almost equal sensitivity and specificity to glucose measurement, it is not available in many parts of the world and is not well enough standardised for its use to be recommended at this time.

2.7 DIAGNOSTIC CRITERIA[38,39]

The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and in severe cases drowsiness and coma; high levels of glycosuria are usually present. A single blood glucose estimation is excess of the diagnostic values indicated it and also defines levels of blood glucose below which a diagnosis of diabetes is unlikely in non pregnant individuals. For clinical purposes, an OGTT (Oral glucose Tolerance Test) only be considered to establish diagnostic status if casual blood glucose values lie in the uncertain range between the levels that establish or exclude diabetes and fasting blood glucose levels are below those which establish the diagnosis of the diabetes. If an

OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75g oral glucose load. For children, the oral glucose load is related to body weight: 1.75 g per kg. The diagnostic criteria in children are same as for the adults.

Table no-2: Diagnosis of types of diabetes mellitus

Conditions	Impaired glucose tolerance (IGT)	Diabetes mellitus
Fasting plasma glucose level (FPG)	110mg/dL- 126mg/L	≥126mg/dL
Postprandial glucose level (PPG)	140 mg/dL- 199mg/dL	≥200 mg/dl
Glycosylated haemoglobin (HbA1c) level	6 -6.99%	≥7%

2.7.2 DIABETES IN CHILDREN[38,39]

Diabetes in children usually presents with severe symptom such as very high blood glucose levels, marked glycosuria and ketonuria. In most children the, diagnosis is confirmed without delay by blood glucose measurements and treatment is initiated immediately often as life-saving measure. An OGTT is neither necessary nor appropriate for diagnosis in such circumstances. A small proportion of children and adolescents, however present with less severe symptoms and may require fasting blood glucose measurement and/or an OGTT for diagnosis.

2.8 TREATMENT OF DIABETES MELLITUS[41]

The backbone of diabetes management is proper diet and regular exercise, which have to be individualized. Both could be the only management needed for controlling blood glucose in gestational diabetes, IGT and in type 2 diabetes in its early phase. Patients with type 2 diabetes may require oral hypoglycaemic agents and /or insulin, while type 1 patients need insulin therapy to survive.

The treatment plan for diabetes may include:

- Diabetes education

- ❖ Meal planning and nutritional requirements
- ❖ Exercise
- ❖ Anti-diabetic agents
- ❖ Insulin therapy
- ❖ Management of associated conditions and complications.

2.8.1 PHARMACOLOGICAL THERAPY[16,23,29,42]

When the lifestyle modifications fails, therapeutic methods should be used that consists of the following categories:-

Sulphonyl urea (Secretagogues)

Acetohexamide

Chlorpropamide

Tolbutamide

Glibenclamide

Glipizide

Gliclazide

Glimipiride

Biguanides

Metformin

Buformin

Thiazolidinediones

Pioglitazone

Rosiglitazone

Dual agonist (PPAR)

Aleglitazar

Muraglitazar

Tesaglitazar

Alpha glycosidase inhibitors

Acarbose

Voglibose

Miglitol

Meglitinides

Repaglinide

Nateglinide

Mitiglinide

Lixisenatide

Taspoglutide

Dipeptidyl peptidase 4 inhibitors

Saxagliptin

Sitagliptin

Vidagliptin

SGLT2 Inhibitors

Asoartame

Benfluorex

Chronimum picolinate

Epalrestat

Glucomannan

Tolrestat

Oral agents may counteract insulin resistance, improve β- cell glucose sensing and insulin secretion or control the rate of intestinal glucose absorption. Combinations of oral agents, in particular sulfonylurea plus metformin or thiazolidinediones plus metformin, have improved the care of diabetic patients and may be used when monotherapy is in effective.

2.9 PRECLINICAL IN VIVO ANIMAL MODEL OF DIABETES MELLITUS FOR SCREENING OF POTENTIAL ANTI-DIABETIC ACTIVITY

2.9.1 PHARMACOLOGICAL INDUCTION OF DIABETES

❖ **Alloxan structure and Sterptozotocin[43]**

The cytotoxic glucose analogues alloxan and sterptozotocin are the most prominent diabetogenic chemical agents in experimental diabetes research. While the mechanism of the selectivity of pancreatic beta cell toxicity is identical, the mechanism of the cytotoxic action of the two compounds are different. Both are selectively toxic to beta cells because they preferentially accumulate in beta cells as glucose analogues through uptake via the GLUT2 glucose transporter. Both compounds are cytotoxic glucose analogues. While the mechanism of cytotoxic action of the two compounds is different, the mechanism of the selectivity of the beta cell action is identical.

2.9.2 Alloxan induced diabetes[44]

Alloxan (2, 4, 5, 6- tetra oxy pyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. Brugnatelli originally isolated alloxan in 1818 and the name is given by Wohler

and Liebig in 1838. Moreover, the compound was discovered by von Liebig and Wohler in 1828 and has been regarded as one of the oldest named organic compounds that exist. The name alloxan emerged from the merging of two words, i.e., allantoin and oxaluric acid. Allantoin is a product of uric acid excreted by the foetus in the allantoin and oxaluric acid has been derived from oxalic acid and urea that is found in urine. Additionally the alloxan model of diabetes induction was first described in rabbit by Dunn, Sheehan and McLetchie in 1943. Alloxan is originally prepared by the oxidation of uric acid by nitric acid. The monohydrate is simultaneously prepared by oxidation of barbituric acid by chromium trioxide.

Alloxan exerts its diabetogenic action when it is administered parentrally; intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse. The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg b.w. when alloxan is given intraperitoneally or subcutaneously its effective dose must be 2-3 times higher. Fasted animals are more susceptible to alloxan, whereas increased blood glucose provides partial protection.

2.9.4 STREPTOZOTOCIN

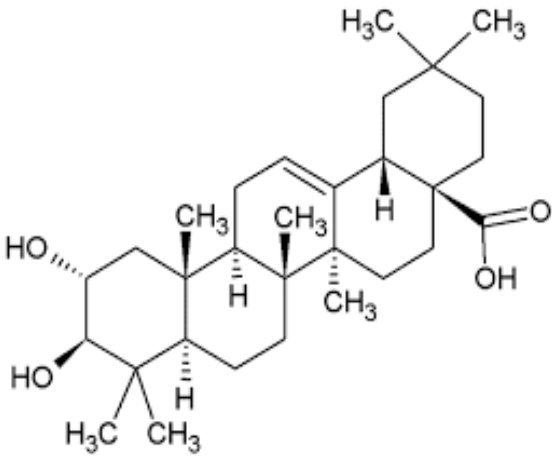


Fig no-4: 2-deoxy-2-({[methyl(nitroso)amino]carbonyl}amino)-β-D-glucopyranose

Sterptozotocin (STZ) (2-deoxy-2-({[methyl(nitroso)amino]carbonyl}amino)-β-D-glucopyranose) is synthesized by Streptomycetes achromogenes and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM respectively).The range of the STZ dose is not as narrow as in the case of alloxan. It is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w. But higher doses are also used[22]. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg b.w. may be ineffective. For instance, when 50 mg/kg b.w. STZ are injected intravenously to fed rats, blood glucose (determined 2 weeks after treatment) can reach about 15mM.). STZ may also be given in multiple low doses. Such treatment is used predominantly in the mouse and the induction of IDDM is mediated by the activation of immune mechanisms. However, that the non- specific activation of the immune system via complete Freund's adjuvant prior to STZ injections allows to reduce its diabetogenic dose even in the rat. NIDDM can easily be induced in rats by intravenous or intraperitoneal treatment with 100 mg/kg b.w.[45]

2.9.5 Usage of STZ for diabetes mellitus

Sterptozotocin is approved by the U.S food and drug administration (FDA) for treating metastatic cancer of the pancreatic islet cells. Since it carries a substantial risk of toxicity and rarely cures the cancer, its use is generally limited to patients whose cancer cannot be removed by surgery. In these patients, sterptozotocin can reduce the tumour size and reduce symptoms (especially hypoglycaemia due to excessive insulin secretion by insulinomas). A typical dose is 500 mg/m²/day by intravenous injection, for 5 days, repeated every 4-6 weeks.

Due to its high toxicity to beta cells, in scientific research, sterptozotocin has also been long used for inducing insulitis and diabetes on experimental animals.

2.9.6 MECHANISM OF STREPTOZOTOCIN[46,47 , 48, 49]

Sterptozotocin is a nitrosamine glucosamine- nitrosourea. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage induces activation of poly ADP- ribosylation, which is likely more important for diabetes induction than DNA damage itself. Sterptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognised by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT 2.

STZ is taken up by pancreatic B cells via glucose transporter GLUT2. A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ observed that STZ itself restricts GLUT2 expression in vivo and in vitro when administered in multiple doses. Intracellular action of STZ results in changes of DNA in pancreatic B cells comprising its fragmentation. Recent experiments have proved that the main reason for the STZ-induced B cell death is alkylation of DNA. The alkylating activity of STZ is related to its nitrosourea moiety, especially at the O6 position of guanine. After STZ injection to rats, different methylated purines were found in tissues of these animals.

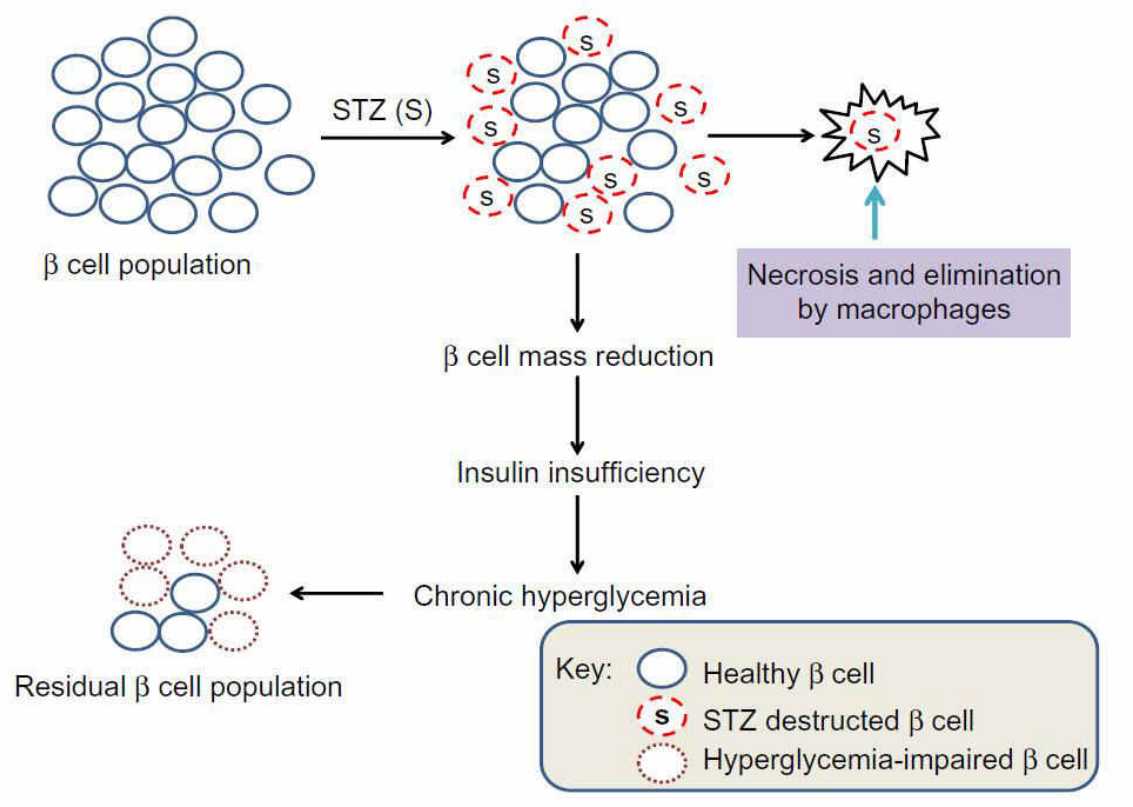


Fig. no-5: Mechanism of action of streptozotocin

2.9.7 OTHER DIABETOGENIC AGENTS

1. Dehydroascorbic acid 650mg/kg for three days in rat
2. Dehydroisoascorbic acid 1.5 mg/kg in rat
3. Dehydroglucoascorbic acid 3.5-3.9 gm/kg in rat
4. Methyl alloxan 53 mg/kg in rat
5. Ethyl alloxan 53-130 mg/kg in rat
6. Oxime and dithizone 53 mg/kg in rabbit
7. Sodium Diethyldithiocarbonate 0.5-1 gm/kg in rabbit
8. Potassium xanthate 200-350 mg/kg in rabbit
9. Uric acid 1 gm/kg in rabbit

Table-3: THE SCIENTIFICALLY DOCUMENTED LIST OF MEDICINAL PLANTS EXHIBITING ANTI-DIABETIC ACTIVITY

S. No.	NAMES OF THE PLANTS AND FAMILY	PARTS USED	TYPES OF PLANT EXTRACT	ANIMAL MODEL	Reference
1	Artemesia sephaerocephala krasch (Asteraceae)	Seeds	Aqueous	Rats (alx)	50
2	Eugenia jambolana (Myrteaceae)	Fruits	Aqueous and ethanolic	Rats (alx)	51
3	Fius microcarpa (Moraceae)	Leaves	Ethanolic	Rats (alx)	52
4	Artanema sesamiodes (Scophuilariaceae)	Aerial parts	Methanolic	Rats (stz)	53

5	Cotu pictus (Zingiberaceae)	Leaves	Aqueous	Rats (stz)	54
6	Phyllanthus rheediii (Euphorbiaceae)	Whole plant	Ethanolic	Rats (stz)	55
7	Justicia beddomei (Acanthaceae)	Leaves	Chloroform, ethanolic	Rats (alx)	56
8	Nymphaea stellata (Nymphaeaceae)	Flowers	Hydroethanolic	Rats (alx)	57
9	Ichnocarpus frutescens (Apocynaceae)	Roots	Aqueous	Rats (stz)	58
10	Salvadora oleoides (Salvadoraceae)	Stems, leaves	Ethanolic	Rats (alx)	59
11	Adathoda zeylanica (Acanthaceae)	Leaves	Hexane, methanolc	Rats (alx)	60
12	Berberis aristata (Beriberidaceae)	Roots	Ethanolic,chloroform,petroleum ether	Rats (alx)	61
13	Cassia glauca linn. (Caesalpiniaceae)	Leaves	Petroleum ether, choloroform,acetone, methanolic	Rats (stz)	62
14	Gymnema sylvestre (Asclepiadaceae)	Leaves	Aqueous	Rats (alx)	63
15	Tinospora cordifolia (Menipermacadeae)	Stem	Aqueous and ethanolic	Rats (stz)	64
17	Neolamarckia cadamba (Rubiaceae)	Stem, bark	Ethanolic	Rats (alx)	65

18	Barleria prionitis (Acanthaceae)	Leaves, roots	Ethanolic	Rats (alx)	66
19	Pongamiapinnata (Leguminosea)	Leaves	Ethanolic,chloroform,petroleum ether	Rats (alx)	67
20	Cathranthus roseus (Apocynaceae)	Leaves	Dichloromethane and methanolic	Rats (alx)	68
21	Cathranthus roseus (Apocynaceae)	Whole plant	Ethanolic	Rats (alx)	69
22	Indigofera pulchra (Papilionaceae)	Leaves	Ethyl acetate, n-butanol	Rats (alx)	70
23	Sarcococca saligna (Buxaceae)	Whole plant	Petroleum ether, ethyl acetate	Rats (stz)	71
24	Sphaeranthus indicus (Compositae)	Roots	Ethanolic	Rats (stz)	72
25	Barleria cristata (Acanthaceae)	Seeds	Ethanol	Rats (alx)	73
26	Aniogeissus latifolia (Combertaceae)	Bark	Aqueous	Rats (stz,NIN)	74
27	Tabebuia rosea (Bignoniaceae)	Leaves	Methanol	Rats(alx)	36
28	Kigelia Africana (Bignoniaceae	Leaves	Methanol	Rats (alx)	36
29	Vitex doniana (Verbanaceae)	Leaves	Aqueous	Rats (alx)	75
30	Cinchona calisaya (Rubiaceae)	Bark	Aqueous	Rats (alx)	75

31	Tetracera indica (Dilleniaceae)	Leaves	Aqueous and methanol	Rats (alx)	76
32	Phyllanthus amarus (Pyhllanthaceace	Leaves	Methanol	Mice (alx)	77
33	Rosmarinus officinalis (Lamiaceae)	Leaves	Aqueous	Rats (stz)	78
34	Emblica officinalis (Pyhllanthaceace)	Seeds	Methanol	Rats (stz)	79
35	Pueraria tuberose (Fabaceae)	Tubers	Ethyl aceate	Rats (alx)	80
36	Anogeissus acuminate	Bark	Methanol	Mice (alx)	81
37	Corallocarpus epigaeus (Cucurbitaceae)	Rhizomes	Ethanol	Rats (alx)	82
38	Fumaria parviflora lam (Fumariaceae)	Aerial parts	Methanol	Rats (stz)	83
39	Vernonia cinerea (Asteraceae)	Bark and leaves	Methanol	Rats (alx)	84
40	Calamus erectus (Arecaceae)	Fruit	Methanol	Rats (stz)	85
41	Cassia kleinii (Caesalpiniaceae)	Leaf, roots	Aqueous, ethanolic	Rats (alx)	86
42	Ficus hispida (Moraceae)	bark	ethanolic	Rats (alx)	87
43	Lycium barbarum (Solanaceae)	Fruits	Aqueous	Rabbits (alx)	88

PLANT DESCRIPTION[89]

Botanical Name

Mallotusphilippensis Muel.Arg.

Common Name

Kamala Dye Tree

Fig. No 6: Whole plant of *Mallotus philippensis* Muell.Arg.

Vernacular Names

English	:	Kamala Tree
Hindi	:	Sindur, Rohini
Bengali	:	Kamalagundi
Gujarathi	:	Kapilo
Malayalam	:	KuranguManjal
Marathi	:	Shindur
Punjabi	:	Kumila
Tamil	:	Kungumam

Telugu : Kunkuma

Assami : Gangai

Oriya : Bosonto-gundi

TAXONAMY

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliopsida
Class	:	Rosidae
Order	:	Euphobiales
Family	:	*Euphorbiaceae*
Genus	:	*Mallotus*
Species	:	*Philippensis*

BOTANY

Leaves are alternate and simple, more or less leathery, ovate to lanceolate, cuneate to rounded with two glands at base. Leaves are mostly acute or acuminate at apex, conspicuously 3-nerved, hairy and reddish glandular beneath, petiole size 1–4 cm long, puberulous and reddish-brown in colour. Male flowers in terminal and axillary position, 2–10 cm long, solitary or fascicled paniculates spikes, each flowers are with numerous stamens, small; female flowers have spikes or slender racemes, each flower with a stellate hairy, 3 celled ovary with 3 papillose stigmas. Fruit is a depressed-globose; 3-lobed capsule; 5, 7 mm, and 10 mm; stellate; puberulous; with abundant orange or reddish glandular granules; 3-seeded . Seeds are subglobose and black in colour and 4 mm across.

Distribution

Mallotus philippensis Muell.Arg. (Family: *Euphorbiaceae*) is a branched herb found in hills of southern districts of Tamilnadu and kerala. It is also common in America and occurs in Eastern Africa and East India.

Parts Used

Fruits

Chemical Constituents

Major phytochemicals present in this genus contain different natural compounds, mainly phenols, diterpenoids, steroids, flavonoids, cardenolides, triterpenoids, coumarin, isocoumarins, and many more to discover. Present knowledge about this endangered species of medicinal plant is still limited with respect to its phytochemistry and biological activity.One of the major chemical constituent, that is, rottlerin of *M. philippinensis,* is listed below with its chemical structure and its major biological activities along with other phytochemicals

Cardenolides

(Corotoxigenin,L-rhamnoside, corogl-aucigenin and L-rhamnoside.)

Steroids

(*β*-Sitosterol.)

Phenolic Compounds

(Bergenin, Kamalachalcone A.,Kamalachalcone B.,Mallotophilippen A.,Mallotophilippen B.,Mallotophilippen C.,Mallotophilippen D. and Mallotophilippen E)

phloroglucinol derivatives

(Rottlerin, Isoallorottlerin and Isorottlerin.)

Triterpenoids

(Betulin-3-acetate,Friedelin,Acetylaleuritolic acid'3b-Acetoxy-22b-hydroxyolean-18-ene and *α*-Amyrin.)

Properties and Uses

According to Ayurveda, leaves are bitter, cooling, and appetizer. All parts of plant like glands and hairs from the capsules or fruits are used as heating, purgative, anthelmintic, vulnerary, detergent, maturant, carminative, Anti-diabetic,[90] and alexiteric and are useful in treatment of bronchitis, abdominal diseases, and spleen enlargement, and if taken with milk or curd (yoghurt), it can be quite useful for expelling tapeworms . Kamala or Kampillakah is also used as an oral contraceptive. The powder and a few other parts of Kamala are also used in external applications to promote the healing of ulcers and wounds. They are used to treat parasitic affections of the skin like scabies, ringworm, and herpes.

Earlier Work Done On *Mallotus philippensis* Muell.Arg.

- Cuong NX et al. have studied ''A new lignan dimer from *Mallotus philippensis*''. A new lignan dimer, bilariciresinol (1), was isolated from the leaves of *Mallotus philippensis*, along with platanoside (2), isovitexin (3), dihydromyricetin (4), bergenin (5), 4-O-galloylbergenin (6), and pachysandiol A (7). Their structures were elucidated by spectroscopic experiments including 1D and 2D NMR and FTICR-MS[91].
- Chan TK et al. have studied ''Anti-allergic actions of rottlerin from *Mallotus philippensis* in experimental mast cell-mediated anaphylactic models''. Allergy is an acquired hypersensitivity reaction of the immune system mediated by cross-linking of the allergen-specific IgE-bound high-affinity IgE receptors, leading to immediate mast cell degranulation. Rottlerin is an active molecule isolated from *Mallotus philippensis*, a medicinalplant used in Ayurvedic Medicine System for anti-allergic and anti-helminthic treatments. The present

study investigated potential anti-allergic effects of rottlerin in animal models of IgE-dependent anaphylaxis and the anti-allergic mechanisms of action of rottlerin in mast cells. Anti-allergic actions of rottlerin were evaluated in passive cutaneous anaphylaxis and passive systemic anaphylaxis mouse models, and in anaphylactic contraction of bronchial rings isolated from sensitized guinea pigs. Direct mast cell-stabilizing effect of rottlerin was examined in RBL-2H3 mast cell line. Anti-allergic signaling mechanisms of action of rottlerin in mast cells were also examined. Rottlerin prevented IgE-mediated cutaneous vascular extravasation, hypothermia, elevation in plasma histamine level and tracheal tissue mast cell degranulation in mice in a dose-dependent manner. In addition, rottlerin suppressed ovalbumin-induced guinea pig bronchial smooth muscle contraction. Furthermore, rottlerin concentration-dependently blocked IgE-mediated immediate release of β-hexosaminidase from RBL-2H3 mast cells. Rottlerin was found to inhibit IgE-induced PLCγ1 and Akt phosphorylation production of IP3 and rise in cytosolic Ca^{+2} level in mast cells. We report here for the first time that rottlerin possesses anti-allergic activity by blocking IgE-induced mast cell degranulation, providing a foundation for developing rottlerin for the treatment of allergic asthma and other mast cell-mediated allergic disorders.[92]

- Hong Q et al. have studied ‘’Anti-tuberculosis compounds from *Mallotus philippensis*’’. Bioassay-directed fractionation of the organic extract of *Mallotus philippensis* gave five compounds (1-5), the most active of which against Mycobacterium tuberculosis was a new compound, 8-cinnamoyl-5,7-dihydroxy-2,2-dimethyl-6-geranylchromene (1) for which the name mallotophilippen F is suggested. Compound (2), 8-cinnamoyl-2,2-dimethyl-7-hydroxy-5-methoxychromene, was isolated from a natural source for the first time, while the remaining three compounds, rottlerin (3), isoallorottlerin=isorottlerin (4) and the so-called "red compound," 8-cinnamoyl-5,7-dihydroxy-2,2,6-trimethylchromene (5), had been isolated previously from this plant. All compounds were identified by analysis of their spectra including 2D-NMR, which was used to correct the literature NMR spectral assignments of compounds 2-4. The C-13 NMR of 5 is reported for the first time.[93]

- Kulkarni RR et al. have studied ‘’Antifungal dimericchalcone derivative kamalachalcone E from *Mallotus philippensis*’’. From the red coloured extract (Kamala) prepared through acetone extraction of the fresh whole uncrushed fruits of *Mallotus philippensis*, one new dimericchalcone (1) along with three known compounds 1-(5,7-dihydroxy-2,2,6-trimethyl-2H-1-benzopyran-8-yl)-3-phenyl-2-propen-1-one (2), rottlerin (3) and 4'-hydroxyrottlerin (4) were isolated. The structure of compound 1 was elucidated by 1D and 2D NMR analyses that included HSQC, HMBC, COSY and ROESY experiments along with the literature comparison. Compounds 1-4 were evaluated for antifungal activity against different human pathogenic yeasts and filamentous fungi. The antiproliferative activity of the compounds was evaluated against Thp-1 cell lines. Compounds 1 and 2 both exhibited IC50 of 8, 4 and 16 µg/mL against Cryptococcus neoformans PRL518, C. neoformans ATCC32045 and Aspergillusfumigatus, respectively. Compound 4, at 100 µg/mL, showed 54% growth inhibition of Thp-1 cell lines.[94]

- Khan H et al. have studied ‘’Antioxidant and Antiplasmodial Activities of Bergenin and 11-O-Galloylbergenin Isolated from *Mallotus philippensis*’’. Two important biologically active compounds were isolated from *Mallotus philippensis*. The isolated compounds were characterized using spectroanalytical techniques and found to be bergenin (1) and 11-O-galloylbergenin (2). The in vitro antioxidant and antiplasmodial activities of the isolated compounds were determined. For the antioxidant potential, three standard analytical protocols, namely, DPPH radical scavenging activity (RSA), reducing power assay (RPA), and total antioxidant capacity (TAC) assay, were adopted. The results showed that compound 2 was found to be more potent antioxidant as compared to 1. Fascinatingly, compound 2 displayed better EC50 results as compared to α-tocopherol while being comparable with ascorbic acid. The antiplasmodial assay data showed that both the compound exhibited good activity against chloroquine sensitive strain of Plasmodium falciparum (D10) and IC50 values were found to be less than 8 µM. The in silico molecular docking analyses were also performed for the determination of binding affinity of the isolated compounds using P. falciparum

proteins PfLDH and Pfg27. The results showed that compound 2 has high docking score and binding affinity to both protein receptors as compared to compound 1. The demonstrated biological potentials declared that compound 2 could be the better natural antioxidant and antiplasmodial candidate.[95]

- Gangwar M et al. have studied ‘’Antioxidant capacity and radical scavenging effect of polyphenol rich *Mallotus philippensis* fruit extract on human erythrocytes: an in vitro study’’. *Mallotus philippensis* is an important source of molecules with strong antioxidant activity widely used medicinal plant. Previous studies have highlighted their anticestodal, antibacterial, wound healing activities, and so forth. So, present investigation was designed to evaluate the total antioxidant activity and radical scavenging effect of 50% ethanol fruit glandular hair extract (MPE) and its role on Human Erythrocytes. MPE was tested for phytochemical test followed by its HPLC analysis. Standard antioxidant assays like DPPH, ABTS, hydroxyl, superoxide radical, nitric oxide, and lipid peroxidation assay were determined along with total phenolic and flavonoids content. Results showed that MPE contains the presence of various phytochemicals, with high total phenolic and flavonoid content. HPLC analysis showed the presence of rottlerin, a polyphenolic compound in a very rich quantity. MPE exhibits significant strong scavenging activity on DPPH and ABTS assay. Reducing power showed dose dependent increase in concentration absorption compared to standard, Quercetin. Superoxide, hydroxyl radical, lipid peroxidation, nitric oxide assay showed a comparable scavenging activity compared to its standard. Our finding further provides evidence that Mallotus fruit extract is a potential natural source of antioxidants which have a protective role on human Erythrocytes exhibiting minimum hemolytic activity and this justified its uses in folklore medicines.[96]
- Khan M et al. have studied ‘’Hexane soluble extract of *Mallotus philippensis* Muell.Arg. root possesses anti-leukaemic activity’’. Root extract of *M. philippensis* was initially extracted in organic solvents, hexane, ethyl acetate, and n-butanol. The hexane extract showed highest toxicity against p53-deficient HL-60 cells (IC50 1.5 mg dry roots equivalent/ml medium) after 72 h and

interestingly, inhibition of cell proliferation was preceded by the upregulation of the proto-oncogenes Cdc25A and cyclin D1 within 24 h. The hexane extract induced 18% apoptosis after 48 h of treatment. Chemical composition of the hexane extract was analyzed by GC-MS and the 90% fragments were matched with polyphenolic compounds. The present study confirms that the hexane fraction of *M. philippensis* root extract possesses anti-leukemic activity in HL-60 cells. The polyphenols were the main compounds of the hexane extract that inhibited proliferation and induced apoptosis.[97]

- Gangwar M et al. have studied ‘’In-vitro scolicidal activity of *Mallotus philippensis* Muell.Arg. fruit glandular hair extract against hydatid cyst Echinococcusgranulosus’’. To investigate new scolicidal agent from natural resources to cope with the side effects associated with synthetic drugs in Echinococcosis. The scolicidal potential of methanolic fruit powder extract (10 and 20 mg/mL) of *Mallotus philippensis* was investigated. Viability of protoscoleces was confirmed by trypan blue exclusion method, where mortality was observed at concentration of 10 and 20 mg/mL in 60 min treatment against Echinococcusgranulosus (E. granulosus), under in-vitro conditions with reference to the known standard drug Praziquantel. At concentration 10 and 20 mg/mL, the mortality rate was observed 97% and 99% respectively for 60 min treatment; while up to 93% mortality was observed with 20 mg/mL for only 10 min treatment. The concentration above 20 mg/mL for above 2 h showed 100% mortality, irrespective of further incubation. As compared with the standard anti-parasitic drug Praziquantel our extract has significant scolicidal activity with almost no associated side effects.[98]

- Goel RK et al. have studied ‘’*Mallotus philippensis* Muell.Arg. (Euphorbiaceae): ethnopharmacology and phytochemistry review. *Mallotus philippensis* Muel.Arg”. (Euphorbiaceae) are widely distributed perennial shrub or small tree in tropical and subtropical region in outer Himalayas regions with an altitude below 1,000 m and are reported to have wide range of pharmacological activities. *Mallotus philippensis* species are known to contain different natural compounds, mainly phenols, diterpenoids, steroids, flavonoids, cardenolides,

triterpenoids, coumarins, isocoumarins, and many more especially phenols; that is, bergenin, *Malloto philippensis*, rottlerin, and isorottlerin have been isolated, identified, and reported interesting biological activities such as antimicrobial, antioxidant, antiviral, cytotoxicity, antioxidant, anti-inflammatory, immunoregulatory activity protein inhibition against cancer cell. We have selected all the pharmacological aspects and toxicological and all its biological related studies. The present review reveals that *Mallotus philippensis* is a valuable source of medicinally important natural molecules and provides convincing support for its future use in modern medicine. However, the existing knowledge is very limited about *Mallotus philippensis* and its different parts like steam, leaf, and fruit. Further, more detailed safety data pertaining to the acute and subacute toxicity and cardio- and immunotoxicity also needs to be generated for crude extracts or its pure isolated compounds. This review underlines the interest to continue the study of this genus of the Euphorbiaceae.[99]

- Furumoto T et al. have studied ‘’*Mallotus philippensis* bark extracts promote preferential migration of mesenchymal stem cells and improve wound healing in mice’’. In the present study, we report the effects of the ethanol extract from *Mallotus philippensis* bark (EMPB) on mesenchymal stem cell (MSC) proliferation, migration, and wound healing in vitro and in a mouse model. Chemotaxis assays demonstrated that EMPB acted an MSC chemoattractant and that the main chemotactic activity of EMPB may be due to the effects of cinnamtannin B-1. Flow cytometric analysis of peripheral blood mononuclear cells in EMPB-injected mice indicated that EMPB enhanced the mobilization of endogenous MSCs into blood circulation. Bioluminescent whole-animal imaging of luciferase-expressing MSCs revealed that EMPB augmented the homing of MSCs to wounds. In addition, the efficacy of EMPB on migration of MSCs was higher than that of other skin cell types, and EMPB treatment improved of wound healing in a diabetic mouse model. The histopathological characteristics demonstrated that the effects of EMPB treatment resembled MSC-induced tissue repair. Taken together, these results suggested that EMPB activated the

mobilization and homing of MSCs to wounds and that enhancement of MSC migration may improve wound healing.[100]

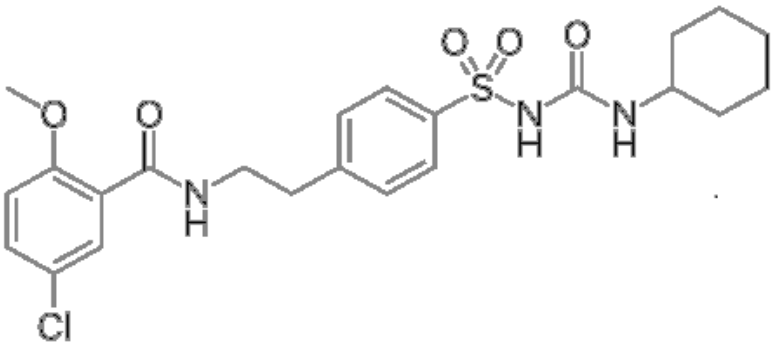
- Gautam MK et al. have studied ''*Mallotus philippensis* Muell.Arg. fruit glandular hairs extract promotes wound healing on different wound model in rats''. The study includes acute toxicity and wound healing potential of 50% ethanol extract of MP fruit glandular hair (MPE). MPE (200 mg/kg) was administered orally, once daily for 10 days (incision and dead space wound) and 22 days (excision wound). MPE was found safe when given to ratsupto 10 times of optimal effective dose. Wound breaking strength (WBS) in Incision wound and rate of contraction, period of epithelization and scar area in Excision wound were evaluated. Granulation tissue free radicals (nitric oxide and lipid peroxidation), antioxidants (catalase, superoxide dismutase, and reduced glutathione), acute inflammatory marker (myeloperoxidase), connective tissue markers (hydroxyproline, hexosamine, and hexuronic acid), and deep connective tissue histology were studied in Dead space wound. MPE significantly increased WBS and enhanced wound contraction, and decreased both epithelization period and scar area compared with control group. MPE was found to decrease free radicals (50.8 to 55.2%, $P<0.001$) and myeloperoxidase (44.0%, $P<0.001$) but enhanced antioxidants (41.1 to 54.5%, $P<0.05$ to $P<0.001$) and connective tissue markers (39.5 to 67.3%, $P<0.05$ to $P<0.01$). Histopathological evaluation revealed more density of collagen formation with minimal inflammatory cells in deeper tissues. Thus, the study revealed *Mallotus philippensis* fruit hair extract, safe and effective in wound healing and the healing effects seemed to be due to decrease in free radical generated tissue damage, promoting effects on antioxidant status and faster collagen deposition as evidenced biochemically and histology.[101]
- Oyedemi BO et al. have studied ''Novel R-plasmid conjugal transfer inhibitory and antibacterial activities of phenolic compounds from *Mallotus philippensis* Mull.Arg''. Antimicrobial resistance severely limits the therapeutic options for many clinically important bacteria. In Gram-negative bacteria, multidrug resistance is commonly facilitated by plasmids that have the ability to accumulate and transfer refractory genes amongst bacterial populations. The aim

of this study was to isolate and identify bioactive compounds from the medicinal plant *Mallotus philippensis* Mull.Arg. with both direct antibacterial properties and the capacity to inhibit plasmid conjugal transfer. A chloroform-soluble extract of *M. philippensis* was subjected to bioassay-guided fractionation using chromatographic and spectrometric techniques that led to the isolation of the known compounds rottlerin [5,7-dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxy-3-methyl-5-acetylbenzyl)-8-cinnamoyl-1,2-chromene] and the red compound (8-cinnamoyl-5,7-dihydroxy-2,2,6-trimethylchromene). Both compounds were characterised and elucidated using one-dimensional and two-dimensional nuclear magnetic resonance (NMR). Rottlerin and the red compound showed potent activities against a panel of clinically relevant Gram-positive bacteria, including meticillin-resistant Staphylococcus aureus (MRSA). No significant direct activities were observed against Gram-negative bacteria. However, both rottlerin and the red compound strongly inhibited conjugal transfer of the plasmids pKM101, TP114, pUB307 and R6K amongst Escherichia coli at a subinhibitory concentration of 100mg/L. Interestingly, despite the planar nature of the compounds, binding to plasmid DNA could not be demonstrated by a DNA electrophoretic mobility shift assay. These results show that rottlerin and the red compound are potential candidates for antibacterial drug lead development. Further studies are needed to elucidate the mode of inhibition of the conjugal transfer of plasmids.[102]

- Chhiber N et al. have studied ‘’Rottlerin, a polyphenolic compound from the fruits of *Mallotus phillipensis* Mull.Arg.’’ impedes oxalate/calcium oxalate induced pathways of oxidative stress in male wistar rats. Oxalate and/or calcium oxalate, is known to induce free radical production, subsequently leading to renal epithelial injury. Oxidative stress and mitochondrial dysfunction have emerged as new targets for managing oxalate induced renal injury. Hyperoxaluria was induced by administering 0.4% ethylene glycol and 1% ammonium chloride in drinking water to male wistar rats for 9 days. Rottlerin was administered intraperitoneally at 1mg/kg/day along with the hyperoxaluric agent. Prophylactic efficacy of rottlerin to diminish hyperoxaluria induced renal dysfunctionality and

crystal load was examined along with its effect on free radicals generating pathways in hyperoxaluric rats. 0.4% ethylene glycol and 1% ammonium chloride led to induction of hyperoxaluria, oxiadtive stress and mitochondrial damage in rats. Rottlerin treatment reduced NADPH oxidase activity, prevented mitochondrial dysfunction and maintained antioxidant environment. It also refurbished renal functioning, tissue integrity and diminished urinary crystal load in hyperoxaluric rats treated with rottlerin. Thus, the present investigation suggests that rottlerin evidently reduced hyperoxaluric consequences and the probable mechanism of action of this drug could be attributed to its ability to quench free radicals by itself and interrupting signaling pathways involved in pathogenesis of stone formation.[103]

GLIBENCLAMIDE



5-chloro-N-[2-[4-(cyclohexylcarbonyl sulfamoyl)phenyl]ethyl]-2-methoxy benzamide

It is an ant diabetic drug in a class of medication known as sulfonyl ureas, closely related to sulfonamide antibiotics.[104]

Mechanism of action

The drug works by binding to and inhibiting the ATP-sensitive potassium channel (KATP) inhibiting regulatory subunit sulfonyl urea receptor 1 in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage dependent calcium channels. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release[105]

Medical use

Used in the treatment of Diabetes[106]

SCOPE OF THE PRESENT STUDY

In the present situation, diabetes is possibly the world's largest growing metabolic disorders and as the knowledge on the heterogenicity of this disorder is advanced, the need for more appropriate therapy increases. A number of allopathic drugs are used for the anti-diabetic effect like Tolbutamide, metformin, phenformin and acarbose which have danger of drug interaction, adverse effects etc. Traditional plant medicines are used throughout the world for a range of diabetic presentations. There are many medicinal plants known to be used in the treatment of diabetes and a number of plants had been screened positively for their anti-diabetic effect. Most of these plants were found to belong to the chemical group glycosides, alkaloids and flavanoids.

Diabetes mellitus has to become one of the world's biggest health problems owing to the projected increase in new cases. In India, the prevalence rate of diabetes is estimated to be 1-5%. Complications are the major cause of morbidity and mortality in diabetes mellitus.

MallotusPhilippensis Muell.Arg. Fruit is having the anti-diabetic activity. As per the literature review, still no anti-diabetic activity has been reported on this plant. Hence, this study has been taken to explore the anti-diabetic activity of *Mallotus Philippensis* Muell.Arg. in sterptozotocin induced diabetes in Wistar albino rats.

AIM AND OBJECTIVES

Aim

The present investigation was aimed to screen the anti-diabetic activity of *Mallotus Philippensis* Muell.Arg. on sterptozotocin induced diabetic *Wistar albino rats.*

Objectives

The objective of the present study to:

a. Find out the phytochemical constituents present in the ethanolic extract of the whole plant of *Mallotus Philippensis* Muell.Arg.
b. Acute toxicity studies
c. Evaluation of anti-diabetic activity
    - Blood glucose level
    - Glycosylated haemoglobin
d. Estimation of Serum parameters
    - Total cholesterol level
    - Serum aspartate amino transferace level (AST)
    - Serum alanine amino transferace level (ALT)
e. Estimation of Oxidative stress parameters
    - Liver malondialdehyde level (MDA)
f. Estimation of enzymic hepatic antioxidant parameters
    - Liver superoxide dismutase level (SOD)
    - Liver catalase level (CAT)
    - Liver glutathione peroxide level (GPx)
g. Estimation of non-enzymic anti-oxidant activity
    - Reduced glutathione (GSH) level
    - Ascorbic acid (Vitamin C) level
    - α –tocopherol (Vitamin E) level
h. Estimation of plasma insulin level
i. Histopathology of pancreas.
j. Statistical analysis

PLAN OF WORK

1. Collection of plant.
2. Authentication of plant, shade drying of the Fruits.
3. Extraction of plant materials with solvents.
4. Preliminary phytochemical screening of ethanolic extract of Fruits *Mallotus Philippensis* Muell.Arg.
5. Evaluation of acute toxicity studies to determine LD_{50} value
6. Evaluation of anti-diabetic activity.
7. Estimation of biochemical parameters.
8. Study of histopathology of pancreas.
9. Statistical analysis

6. MATERIALS AND METHODS

6.1 PLANT MATERIAL

6.1.1 Collection and authentication, shade drying and granulation of plant material

1. The Fruits of *Mallotus Philippensis* Muell.Arg. were collected in the month of December from, Cherpulassery, Palakkad (Dist), Kerala South India. The plant material was taxonomically identified and authenticated by Dr.A.Balasubramanian, Director, ABS Botanical conservation, Research & Training center, Kaaripatti, Salem.

6.1.2 Preparation of Extract

The Fruits of *Mallotus Philippensis* Muell.Arg. were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use.

Purification of Solvent

Ethanol

Rectified spirit was soaked in slaked lime overnight and distilled.

6.1.3 Extraction procedure

The coarse Fruit powder was extracted with ethanol by continuous hot percolation using soxhlet apparatus. After completion of extraction, extract was filtered and the solvent was removed by under reduced pressure. The dried extract was stored in desiccators.

6.1.4 METHOD OF EXTRACTION

Soxhlet extraction

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times. This process involves in the extraction followed by evaporation of the solvent. The vapours of the solvent are

taken to a condenser and the condensed liquid is returned to the drug for continuous extraction. Soxhlet apparatus designed for such continuous extraction consists of a body of extractor attached with a side tube and siphon tube. The lower of the extractor is attached to distillation flask and the mouth of extractor is fixed to a condenser by the standard joints. The powdered crude drug is packed in the soxhlet apparatus directly or in a thimble of filter paper or fine muslin cloth. The diameter of the thimble corresponds to the internal diameter of the soxhlet extractor.

6.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Qualitative Phytochemical Analysis[107]

The ethanolic extract of dried Fruits of crude *Mallotus Philippensis* was analyzed for the presence of various phytoconstituents.

A) CARBOHYDRATE TESTS

1. **Molisch's test**

   To the test solution, few drops of α-naphthol was added, then few drops of concentrated sulphuric acid was added through the sides of test tube, purple to violet colour ring appeared at the junction, indicated the presence of carbohydrates.

2. **Fehling's test**

   To the test solution, equal quantity of Fehling's A and B were added and heated on water bath, brick red precipitate was formed, indicated the presence of carbohydrates.

3. **Benedict's test**

   To the test solution, 5ml of Benedict's reagent was added and heated on water bath, red precipitate was formed, indicated the presence of carbohydrates.

4. Barfoed's test

To 1 ml of the test solution, add 1ml of Barfoed's reagent was added and heated on a water bath, red cupric oxide was formed, presence of monosaccharide.

5. Test for pentoses

To the test solution, equal volume of hydrochloric acid and phloroglucinol was added and heated, no red colour was produced, indicated the absence of pentoses.

6. Selivanoff's test (test for ketones)

To the test solution, crystals of resorcinol and equal volume of concentrated hydrochloric acid were added and heated on a water bath, rose colour was produced, indicated the presence of ketones.

B) ALKALOIDS

1. Dragendroff's test

To 1ml of the extract, Dragendroff's reagent (potassium bismuth iodine solution) was added, no reddish brown precipitate was formed, indicated the absence of alkaloids.

2. Wagner's test

To 1ml of the extract, Wagner's reagent (iodine potassium iodide solution) was added, no reddish brown precipitate was formed, indicated the absence of alkaloids.

3. Mayer's test

To 1 ml of the extract, Mayer's reagent (potassium mercuric iodine solution) was added, no cream colour precipitate was formed, indicated the absence of alkaloids.

4. Hager's test

To 1 ml of the extract, Hager's reagent (saturated aqueous solution of picric acid) was added, no yellow coloured precipitate was formed, indicated the absence of alkaloids.

5. Tannic acid test

To the extract tannic acid solution was added, no buff colour precipitate was produced, indicated the absence of alkaloids.

C) GLYCOSIDE TEST

1. Legal's test

The extract in pyridine and sodium nitroprusside solution was added to make it alkaline, formation of pink to red colour showed the presence of glycosides.

2. Baljet's test

To 1 ml of the extract, 1 ml of sodium picrate solution was added and the change of yellow to orange colour reveals the presence of glycosides.

3. Borntrager's test

Few ml of dilute sulphuric acid was added to 1 ml of the extract solution. Boiled and filtered and then the filtrate was extracted with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of red colour of the ammonical layer showed the presence of anthraquinone glycosides.

4. Keller killani test

1. 1gm of powdered drug was extracted with 10ml of 70% alcohol for 2 minutes, filtered, the filtrate was added to 10 ml of water and 0.5 ml of strong solution of lead acetate and filter, and the filtrate was shaken with 5ml of chloroform.

2. The chloroform layer was separated in a porcelain dish and the solvent was removed by gentle evaporation. The cooled residue was dissolved in 3 ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution.

   The solution was carefully transferred to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids and the upper layer slowly became bluish green, darkening with standing.

D) TEST FOR SAPONINS

Foam test

Small quantity of alcoholic extract was taken and 20ml of distilled water was added and shaked in a graduated cylinder for 15 minutes length wise. No Layer of foam, indicated the absence of saponins.

E) TEST FOR FLAVANOIDS

1. Shinoda's test

To the test solution, few magnesium turnings were added and concentrated hydrochloric acid was added drop wise, pink scarlet, crimson red or occasionally green to blue colour appeared after few minutes indicating the presence of flavanoids.

2. Alkaline reagent test

To the test solution, few drops of sodium hydroxide solution was added, intense yellow colour was formed which turned colourless on addition of few drops of dilute acid indicating presence of flavanoids.

3. **Zinc hydrochloride test**

To the test solution, a mixture of zinc dust and concentrated hydrochloric acid were added. It gave red colour after few minutes showing the presence of flavanoids.

F) TEST FOR TANNINS

1. **Ferric chloride test**

To the test solution, ferric chloride solution was added, green colour appeared showing the presence of condensed tannins.

2. **Phenazone test**

To the test solution, 0.5 grams of sodium phosphate was added, warmed and filtered. To the filtrate 2% phenazone solution was added, bulky precipitate was formed which was often coloured, indicating the presence of tannins.

3. **Gelatin test**

To the test solution, 1% gelatine solution containing 10% sodium chloride was added. Precipitate was formed, indicating the presence of tannins.

4. **Test for Catechin**

Match stick was dipped in the test solution, it was dried and lastly moistened with concentrated hydrochloric acid. Then the stick was warmed near to flame. The colour of the wood changed to pink due to phloroglucinol. (Phloroglucinol was formed when catechins were treated with acids), indicating the presence of tannins.

TEST FOR PROTEINS

1. **Warming test**

   The test solution was heated in a boiling water bath, coagulation was observed, indicating the presence of proteins.

2. **Test with Trichloroacetic acid**

   To the test solution trichloroacetic acid was added, precipitate was formed, indicating the presence of proteins

3. **Biuret test**

   To the test solution (2ml), Biuret (2ml) was added, violet colour was produced, indicating the presence of proteins.

4. **Xanthoproteic test**

   5ml of the test solution, 1ml of concentrated nitric acid was added and boiled, yellow precipitate was formed. After cooling it, 40% sodium hydroxide solution was added orange colour was formed, indicating the presences of proteins.

G) TEST FOR AMINO ACIDS

1. **Million's test**

   To the test solution, about 2ml of Million's reagent was added, white precipitate was obtained indicating the presence of amino acids.

2. **Ninhydrin test**

   To the test solution, ninhydrin solution was added, boiled, violet colour was produced indicating the presence of amino acid.

H) TEST FOR STEROIDS AND TRITERPENOIDS

1. **Libermann-Buchard test**

The extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction of two layers and upper layer turned green which showed the presence of steroids and formation of deep red colour indicating the presence of triterpenoids.

2. **Salkowski Test**

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

3. **Hesses reaction**

The residue dissolved in chloroform and an equal quantity of concentrated sulphuric acid was then added along the side of the tube and observed for the formation of pink coloured ring, which on shaking diffused in both the layers.

4. **Hersch's Sohn's reaction**

To the residue 2-3 ml of trichloroacetic acid was added, heated and observed for the formation of red to violet colour.

❖ **Test for Gums and Mucilage**

Small quantity of the extract was added separately to 25ml of absolute alcohol with constant stirring and filtered. No precipitate was formed, it indicates that absence of gums and mucilage.

6.3 ACUTE TOXICITY STUDY[108]

Whenever an investigator administers a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 (the dose has proved to be lethal (causing death) to 50% of the tested group of animals).

Determination of oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. This article reviews the methods of so far utilized for the determination of median lethal dose (LD50) and the new changes which would be made. This has to go through the entire process of validation with different categories of substances before its final acceptance by regulatory bodies.

Organisation for Economic co-operation and Development (OECD) regulates guidelines for oral acute toxicity study. It is an international organisation which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. To determine the acute oral toxicity OECD frames the following guideline methods.

OECD 401 – Acute Oral Toxicity

OECD 420 – Acute Oral Toxicity: Fixed Dose procedure

OECD 423 –Acute Oral Toxicity: Acute Toxic Classic method

OECD 425 – Acute Oral Toxicity: Up and own Procedure

In the present study the acute oral toxicity of *Mallotus Philippensis* Muell.Arg. was carried out according to OECD 423 guideline (Acute Oral Toxicity: acute Toxic Classic Method).

ACUTE ORAL TOXICITY

Acute oral toxicity refers to those adverse effects that occur following oral administration of a single dose of a substance or multiple doses given within 24 hours.

LD_{50} (median lethal oral dose)

LD_{50} (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

PRINCIPLE

It is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a step wise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound- related mortality of the animals dosed at one step will determine the next step, i.e.

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level.

SELECTION OF ANIMAL SPECIES

The preferred rodent species was the rat. Normally females were used. Females were generally slightly more sensitive. Healthy young adult animals of commonly used laboratory strains were employed. Females were nulliparous and non-pregnant. Each animal, at the commencement of it's dosing, were between 8 to 12 weeks old.

ADMINISTRATION OF DOSES

The test substance was administered in a single dose by gavages using a oral feeding needle. Animals were fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water was withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test

substance administered. After the substance has been administered, food was withheld for a further 3-4 hours in rats.

OBSERVATION

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 21 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation was not fixed rigidly. It was determined by the toxic reactions, time of onset and length of recovery period and extended when considered necessary. The times at which signs of toxicity appeared and disappeared were important, when toxic signs were to be delayed. All observations were systematically recorded with individual records being maintained for each animal.

6.4 PHARMACOLOGICAL SCREENING

ANIMALS

Adult Wistar albino rats of either sex weighing 150-160 gm were procured from the animal house of Kings institute, guindy, Chennai, Tamilnadu, India. used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment.

The animals were housed in polypropylene cages with paddy house bedding under standard laboratory conditions for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow *ad libitum* (H.G.Vogel, 2002).

The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines (**IAEC approval No: IAEC/XLVIII/04/CLBMCP/2016 dated on 04/05/2016**).

6.4.1 STERPTOZOTOCIN INDUCED DIABETES[59]

Experimental induction of diabetes

A freshly prepared solution of STZ (45 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight-fasted rats The rats exhibited hyperglycaemia within 48 h of STZ administration. The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

6.4.2 EXPERIMENTAL DESIGN

The anti diabetic activity was tested on a total of 30 rats (24 diabetic rats and 6 normal rats) and they were divided into five groups and each group consists of 6 animals as follows,

Group I- Served as control, received 0.5% CMC (1ml/kg; p.o) for 21 days.

Group II- Diabetic control received single streptozocin injection (45mg/kg; b.wt; i.p) freshly prepared in citrate buffer on Day 1 and received citrate buffer for 21 days.

Group III- STZ+ plant extract low dose (200mg/kg, b.wt; p.o) suspended in 0.5% CMC for 21days

Group IV- STZ+ plant extract high dose (400mg/kg, b.wt; p.o) suspended in 0.5% CMC for 21days

Group V- STZ+ Standard Glibenclamide (600 µg/kg, b.wt; p.o) dissolved in 5% CMC for 21days

6.4.3 DIABETIC STUDY

The study involved repeated administration of *EEMP* for 21 days at a prefixed times and blood glucose levels were estimated in samples withdrawn after 1st day, 7, 14 and 21st day. The animals had free access to food and water during this period.

Blood samples from the experimental rats were collected from the tail by using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl.

BLOOD SAMPLE AND ORGANS COLLECTION

Fasting blood glucose of all rats was determined before the start of the experiment. On day 21st the blood was collected by retro orbital under mild ether anaesthesia from overnight fasted rats, into tubes containing potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting plasma glucose. Plasma and serum were separated by centrifugation. After centrifugation at 2,000 rpm for 10 minutes, the clear supernatant was used for the analysis of various biochemical parameters. After collection of blood, all the treated animals were sacrificed the pancreas and liver tissues were isolated and rinsed in ice- cold saline and kept in formalin solution (10%) for further histopathological studies.

PREPARATION OF LIVER HOMOGENATE

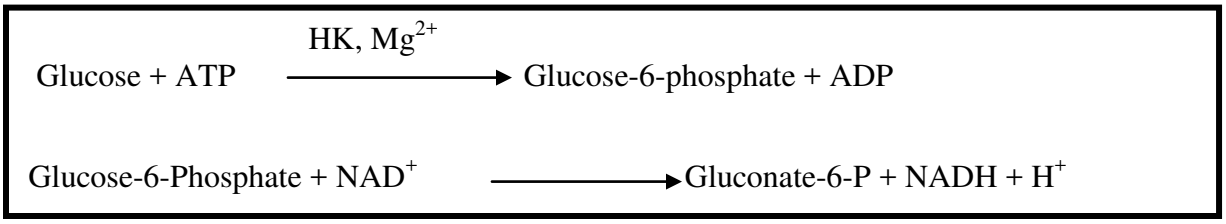
The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, supernatant was collected and used for various biochemical assays.

6.5 EVALUATION OF PARAMETERS

Biochemical Estimation

6.5.1 Estimation of blood glucose level [109]

The Blood glucose levels were estimated by Hexokinase method. Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6- phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidises glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD^+ to NADH. The increase in absorbance at 340nm is proportional to the glucose concentration in the sample.



Reagents

Reagent 1

- ✓ 0.05 M Tris HCl buffer, pH 8.0 with 13.3 mM $MgCl_2$
- ✓ 0.67 M Glucose in Tris-$MgCl_2$ buffer
- ✓ 16.5 mM ATP in Tris-$MgCl_2$ buffer
- ✓ 6.8 mM NAD in Tris-$MgCl_2$ buffer

Reagent 1A

- ✓ 300 IU/ml Glucose-6-phosphate dehydrogenase in Tris-MgCl2 buffer
- ✓ 300 IU/ml Hexokinase in Tris-MgCl2 buffer

Procedure

150 µl of reagent 1 was added with 30 µl of reagent 1A and to this 20 µl of suitable diluents was added and the contents were mixed thoroughly. To this mixture, 2 µl of serum sample was added. Then the contents were mixed and incubated at 37°C for 10 seconds. After zeroing the instrument with blank the absorbance of standard followed by the test sample was measured at 340 nm. The values were expressed as mg/dl.

6.5.1.1 Estimation of Glycosylated Haemoglobin (HbA1C)[110]

Method: “Tina-quant” (turbidimetric inhibition immunoassay”

The concentration A1c (HbA1c) is measured as a percentage of a total hemoglobin in human whole blood (%HbA1c). Consequently, the hemoglobin contained in red blood cells is released by hemolysis of the sample. This method uses the Hemolyzing Reagent containing a detergent (tetradecyltrimethylammonium bromide - TTAB) to specifically lysate red blood cells. HbA1c and Hemoglobin levels in the sample are determined from the obtained hemolysate by two independent reactions. HbA1c During the first stage of the reaction: the HbA1c in the sample reacts with the anti-HbA1c specific antibody (Reagent A1) to form soluble antigen-antibody complexes. Then the polyhapten is added (Reagent A2). The polyhapten reacts with the specific antibody excess from the first reaction, producing insoluble immune complexes which can be measured turbidimetrically at 340 nm.

Reagents

Reagent A1: Monospecific antibodies anti-HbA1c in pH 6.2 buffer.

Reagent A2: Polyhapten-HbA1c in pH 6.2 buffer.

Calculation

%HbA1c = 91.5 x HbA1c (g/dl) / Hb(gm/dl) + 2.15

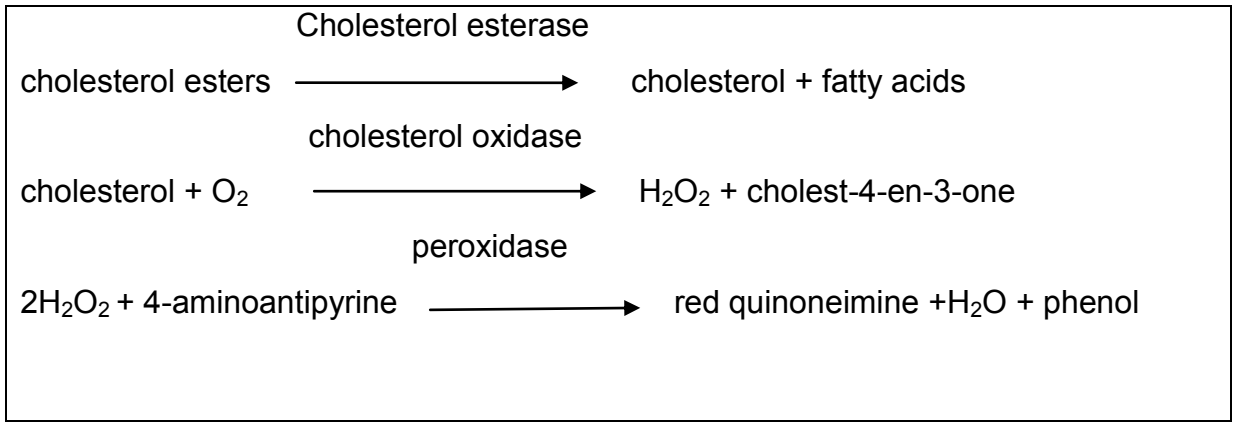
6.5.2 Estimation of serum parameters

6.5.2.1 Estimation of total cholesterol level[111]

Method-cholesterol oxidize- Peroxidase method.

Principle

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest- 4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoeimine dye which has absorbance maximum at 510 nm. The intensity of the red colour is proportional to the amount of total cholesterol in the specimen



Reagents

- ✓ 50 mMol/L Buffer, pH 6.8
- ✓ Cholesterol oxidase
- ✓ Cholesterol esterase
- ✓ Peroxidase
- ✓ 0.mMol/L 4-amino antipyrine
- ✓ Phenol
- ✓ Surfactant

Assay parameters

Reaction type -end point

Reaction time -5 minutes at 37°C/ 10 minutes at 25°-30°C

Wave length -510nm

Procedure

Sample-Serum

The required amount of reagent before use was prewarmed at room temperature, the assay was performed as given below

	Serum	Standard	Blank
	10 µL	10 µL	-
Reagent	1000 µL	1000 µL	1000 µL

Incubation

The assay mixture was incubated for 5 minutes at37°C , for 10 minutes at room temperature (25°-30°C).

After incubation the absorbance of the assay mixture was measured against blank at 510 nm. The final colour was stable for two hours if not exposed to direct light.

Calculation

Total cholesterol (mg/dL)= (absorbance of test/absorbance of standard) X 200.

6.5.2.2 Estimation of Aspartate aminotransferase (AST, Serum Glutamic-Oxaloacetic Transaminase)[112]

The enzyme catalyzes the reaction,

L-aspartate + 2-oxoglutarate → oxaloacetate + L-gluatamate

The enzyme activity was assayed by the method of Reitman and Frankel.

Reagents:

- ✓ Substrate: 1.33 g of L-aspartic acid and 15 mg of 2-oxo glutaric acid were dissolved in 20.5 ml of buffer and 1 N sodium hydroxide to adjust the pH to 7.4 and made up to 100 ml with the phosphate buffer.
- ✓ 0.1N Sodium hydroxide
- ✓ 2,4-dinitro phenyl hydrazine (DNPH) (0.2% in 1 N HCl).
- ✓ Standard pyruvate solution: 11 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer. This contains 1 µmole of pyruvate/ml

Procedure:

1 ml of buffered substrate was incubated at 37°C for 10 minutes. Then 0.2 ml of serum /tissue homogenate was added in the test tubes and incubated at 37°C for 30 minutes. The reaction was arrested by adding 1 ml of DNPH reagent and tubes were kept at room temperature for 20 minutes. Then 10 ml of 0.4N sodium hydroxide solution was added. A set of pyruvic acid was also treated in a similar manner for the standard. The colour developed was read at 520 nm against the reagent blank. The activity of the enzyme was expressed as IU/L / µmoles of pyruvate liberated/min/mg protein.

6.5.2.3 Estimation of Alanine aminotransferase (ALT, Serum Glutamic-Pyruvic Transaminase)[113].

This enzyme c0atalyzes the reaction,

L-alanine + 2-oxoglutarate → oxaloacetate + L-gluatamate

The enzyme activity was assayed by the method of Reitman and Frankel.

Reagents:

- ✓ Phosphate buffer: 0.1 M; pH 7.4.
- ✓ Substrate: 1.78 g of DL-alanine and 30 mg of 2-oxo glutaric acid were dissolved in 20 ml of buffer. About 0.5 ml of 1 N sodium hydroxide was added and made up to 100 ml with buffer.
- ✓ 0.1N Sodium hydroxide.
- ✓ 2, 4-dinitro phenyl hydrazine (DNPH): 0.2% in 1 N HCl.
- ✓ Standard pyruvate solution: 11 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer. This contained 1 µmole of pyruvate/ml.

Procedure:

1 ml of buffered substrate was incubated at 37°C for 10 minutes. Then 0.2ml of serum/tissue homogenate was added in the test tubes and incubated at37°C for 30 minutes. The reaction was arrested by adding 1 ml of DNPH reagent and tubes were kept at room temperature for 20 minutes. Then 10 ml of 0.4N sodium hydroxide solution was added. A set of pyruvic acid was also treated in a similar manner for the standard. The colour developed was read at 520 nm against the reagent blank. The activity of the enzyme was expressed as IU/L / µmoles of pyruvate liberated/min/mg protein.

6.5.3 Estimation of oxidative stress parameters

6.5.3.1 Estimation of Lipid Peroxidation (LPO)[114]

Lipid peroxidation (LPO) was assayed by the method of Ohkawa et al, in which the malondialdehyde (MDA) released served as the index of LPO. The extent of LPO in the hepatic tissue was assayed by measuring one of the end products of this process, the thiobarbituric acid-reactive substances (TBARS). As 99% TBARS is malondialdehyde (MDA), thus this assay is based on the reaction of 1 molecule of MDA with 2 molecules of TBARS at low pH (2- 3) and at a temperature of 95°C for 60 min. The resultant pink chromogen can be detected spectrophonometrically at 532 nm.

Reagents

- ✓ Standard: 1, 1, 3, 3-tetra ethoxypropane (TEP).
- ✓ 8.1% Sodium dodecyl sulphate (SDS)
- ✓ 20%Acetic acid
- ✓ 0.8%Thiobarbituric acid (TBA)
- ✓ 15:1 v/v n-butanol: pyridine mixture

Procedure

To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95.8ºC for 60 min using glass ball as a condenser. After cooling, 1 ml of water and 5 ml of n-butanol: pyridine (15:1 v/v) mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA formed/mg of protein.

6.5.4 Evaluation of Enzymic Hepatic Antioxidants

6.5.4.1 Estimation Superoxide dismutase (SOD)[115]

This enzyme catalyzes the dismutation of superoxide anion ($O2^{-\cdot}$) to hydrogen peroxide and molecular oxygen in the following manner

$$H_2O + 2O_2^{-\cdot} + 2H^+ \rightarrow 2H_2O_2 + O_2$$

The enzyme activity was assayed by the method of Misra and Fridovich.

Reagents

- ✓ 0.1 M Carbonate-bicarbonate buffer; pH 10.2.
- ✓ 0.6 mM EDTA solution
- ✓ 1.8 mM Epinephrine (prepared in situ)
- ✓ Absolute ethanol.
- ✓ Chloroform

Procedure

0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate-bicarbonate (pH 10.2) buffer. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured. One unit of the SOD activity was the amount of protein required to give 50% inhibition of epinephrine autoxidation.

6.5.4.2 Estimation of Catalase (CAT)[116]

This enzyme catalyzes conversion of hydrogen peroxide into water and molecular oxygen.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The enzyme activity was assayed by the method of Sinha .

Reagents

- ✓ Dichromate-acetic acid reagent: 5% potassium dichromate in water was mixed with glacial acetic acid in the ratio of 1:3 (v/v).
- ✓ 0.01 M Phosphate buffer; pH 7.0.
- ✓ 0.2M Hydrogen peroxide

Procedure

0.1 ml of the tissue homogenate was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0) pre-warmed to 37°C, 0.4 ml of distilled water and the mixture was incubated at 37°C. The reaction was initiated by the addition of 0.5 ml of 0.2 M hydrogen peroxide and the reaction mixture was incubated at 37°C for one minute. The reaction was terminated by the addition of 2 ml of dichromate-acetic acid reagent after 15, 30, 45, and 60 seconds. Standard hydrogen peroxide in the range of 4-20 µmoles were taken and treated in the same manner. All the tubes were heated in a boiling water bath for 10 minutes, cooled and the green colour that developed was read at 590 nm against blank containing all components except the enzyme. Catalase activity was expressed in terms of µmoles of H2O2 consumed/min/mg protein.

6.5.4.3 Estimation of Glutathione peroxidase (GPx)[117]

This enzyme catalyzes the reduction of H_2O_2 using glutathione as substrate.

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

The enzyme activity was assayed by the method of Rotruck et al.

Reagents

- ✓ 0.32 M Sodium phosphate buffer; pH 7.0.
- ✓ 0.8 mM EDTA
- ✓ 10 mM Sodium azide.
- ✓ 4mM Reduced glutathione.
- ✓ 2.5 mM Hydrogen peroxide.
- ✓ 10%Trichloro acetic acid (TCA).
- ✓ 0.3M Disodium hydrogen phosphate.
- ✓ 0.04% 5,5'-dithiobis (2-nitro benzoic acid) (DTNB); 40 mg of DTNB in 1% sodium citrate.
- ✓ 10 mM Standard reduced glutathione.

Procedure

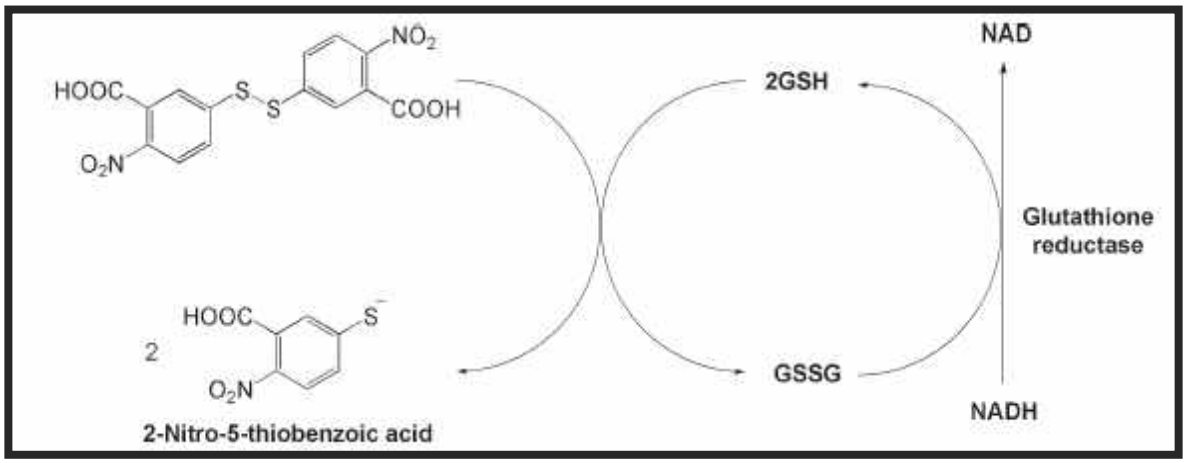
The assay mixture containing 0.5 ml sodium phosphate buffer, 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H_2O_2, and 0.5 ml of 1:10 tissue homogenate was taken and the total volume was made up to 2.0 ml with distilled water. The tubes were incubated at 37 8C for 3 min and the reaction was terminated by the addition of 0.5 ml of 10% TCA. To determine the residual glutathione

content, the supernatant was removed after centrifugation, and to this 4.0 ml disodium hydrogen phosphate (0.3 M) solution and 1 ml DTNB reagent were added. The colour that developed was read at 412 nm against a reagent blank containing only phosphate solution and DTNB reagent in a spectrophotometer. Suitable aliquots of the standard were also treated similarly. The enzyme activity is expressed in terms of µg of GSH utilized/min/mg protein.

6.5.5 Evaluation of Non-Enzymic Hepatic Antioxidants

6.5.5.1 Estimation of Reduced glutathione (GSH)[118]

The total reduced glutathione was determined according to the method of Ellman. The assay procedure is based on the reduction of Ellman´s reagent [5, 5´- dithio bis (2- nitrobenzoic acid)] (DTNB) by SH groups of glutathione to form 2-nitro-S-mercaptobenzoic acid per mole of glutathione. The product is measured spectrophotometically at 412 nm.



Reagents

- ✓ 0.2 M Phosphate buffer; pH 8.0.
- ✓ 0.6 mM DTNB reagent.
- ✓ 5%TCA

Procedure

0.1 ml of tissue homogenate was precipitated with 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged. To 0.1 ml of supernatant, 2 ml of 0.6 mM DTNB reagent and 0.2 M phosphate buffer (pH 8.0) were added to make up to a final volume of 4 ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standards treated in a similar way also run to determine the glutathione content. The amount of glutathione was expressed as nmoles/g tissue.

6.5.5.2 Estimation of Vitamin C (Ascorbic acid)[119]

The level of vitamin C was estimated by the method of Omaye et al. Ascorbic acid is oxidised by copper to form dehydroascorbic acid. The product was treated with 2,4 dinitrophenyl hydrazine to form tris 2,4 dinitrophenyl hydrazone which undergoes rearrangement to form a product with the absorption maximum at 520 nm in spectrophotometer.

Reagents

- ✓ 5% TCA
- ✓ DTC reagent (3 gm of 2,4 dinitrophenyl hydrazine, 0.4 gm thiourea and 0.05 gm of copper sulphate were dissolved in 100 ml of 9 N H_2SO_4).
- ✓ 65 % H_2SO_4 (ice cold)
- ✓ Standard ascorbic acid

Procedure

To 0.5 ml of tissue homogenate, 0.5 ml of water and 1 ml of TCA were added, mixed thoroughly and centrifuged. To 1 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37°C for 3 hours. Then 1.5 ml of H_2SO_4 was added, mixed well and the solution was allowed to stand for 30 minutes at room temperature. The colour developed was read at 520 nm in spectrophotometer. The level of vitamin C was expressed as nmole/ g of wet tissue.

6.5.5.3 Estimation of Vitamin E (α- Tocopherol)[120]

Vitamin E content was estimated by the method of Palan et al. This method involves the conversion of ferric ions to ferrous ions by a-tocopherol and the formation of red colored complex with 2, 2 dipyridyl. Absorbance of chromophore was measured at 520 nm in the spectrophotometer.

Reagents

- ✓ 2% 2,2 dipyridyl solution
- ✓ 5 % $FeCl_3$ solution
- ✓ Standard : 100 mg of α-tocopherol in 0.1% ethanol
- ✓ n- Butanol

Procedure

To 0.5 ml of tissue homogenate, 1.5 ml of ethanol was added, mixed and centrifuged. The supernatant was dried at 80°C for 3 hours. To this 0.2 ml of 2, 2 dipyridyl solution and 0.2 ml of $FeCl_3$ solution were added, mixed well and 4 ml of butanol was added. The colour developed was read at 520 nm in the spectrophotometer. The level of vitamin C was expressed as nmole/ g of wet tissue.

6.5.6 ESTIMATION OF PLASMA INSULIN LEVEL[121]

Insulin was assayed in plasma using a commercial kit by enzyme linked immunosorbant assay (ELISA) technique.

Reagents used for the estimation of plasma insulin level:

1. Monoclonal anti-insulin antibody
2. Enzyme conjugate: Anti-insulin antibodies conjugated to horseradish peroxidise
3. Standard: Human insulin
4. Solution A: Buffer solution containing hydrogen peroxide
5. Solution B: Tetramethylbenzidine
6. Concentrated wash buffer
7. Stock solution: 2 N HCL

Procedure:

25 µl of the plasma taken into micro wells coated with anti-insulin antibody. To this, 100µl of the enzyme conjugate was added to each well, mixed for 5 sec and incubated at 25°C for 30 min. The wells were rinsed for five times with washing buffer. Then, 100µl of solution A and then 100µl of solution B were added to each well. This was incubated for 15 min at room temperature. The reaction was stopped by the addition of 50µl of 2 N HCL to each well and read at 450 nm.

6.7 HISTOPATHOLOGICAL STUDIES[113]

Hematoxylin, a basic bye is oxidized to hematein with a mordant, a metallic ion such as the salts of aluminium. The positively charged aluminium-hematein complex combines with the negatively charged phosphate groups of the nucleic acids (DNA and RNA) forming blue/purple colour, which is characteristic of hematoxylin stains. Eosin is an acidic dye, which is considered to have a selective affinity for the basic parts of the cell, i.e., the cytoplasm. Thus, the hematoxylin and eosin (H & E) stain is used to demonstrate different structures of the tissue.

The various steps involved in the preparation of pancreatic tissues for histological studies are as follows:

- **Fixation**

In order to avoid tissue by the lysosomal enzymes and to preserve its physical and chemical structure, a bit of tissue from each organ was cut and fixed in bouin's fluid immediately after removal from the animal body. The tissues were fixed in bouin's fluid for about 24 hours. The tissues were then taken and washed in glass distilled water for a day to remove excess of picric acid.

- **Dehydration**

The tissues were kept in the following solutions for an hour each; 30%, 50%, 70% and 100% alcohol. Inadequately dehydrated tissues cannot be satisfactorily infiltered with paraffin. At the same time over dehydration results in making the tissues brittle, which would be difficult for sectioning. So, careful precautions were followed while performing the dehydration process

- **Clearing**

Dealcoholization or replacement of alcohol from the tissues with a clearing agent is called as clearing. Xylene was used as the clearing agent for one or two hours, two or three times. Since, the clearing agent is miscible with both dehydration and embedding agents, it permits paraffin to infilterate the tissues. So, the clearing was carried out as the next step after dehydration to permit tissue spaces to be filled with paraffin. The tissues were kept in the clearing agent till they become transparent and impregnated with xylene.

❖ **Impregnation**

In this process the clearing agent xylene was placed by paraffin wax. The tissues were taken out of xylene and were kept in molten paraffin embedding bath, which consists of metal pots filled with molten wax maintained at about 50^{o}C. The tissues were given three changes in the molten wax at half an hour intervals.

❖ **Embedding**

The paraffin wax used for embedding was fresh and heated upto the optimum melting point at about 56–58^{o}C. A clear glass plate was smeared with glycerine. L-shaped mould was placed on it to from a rectangular cavity. The molten paraffin wax was poured and air bubbles were removed by using a hot needle. The tissue was placed in the paraffin and oriented with the surface to be sectioned. Then the tissue was pressed gently towards the glass plate to make settle uniformly with a metal pressing rod and allowed the wax to settle and solidity room temperature. The paraffin block was kept in cold water for cooling.

❖ **Section Cutting**

Section cutting was done with a rotatory microtome. The excess of paraffin around the tissue was removed by trimming, leaving ½ cm around the tissue. Then the block was attached to the gently heated holder. Additional support was given by some extra wax, which was applied along the sides of the block. Before sectioning, all set screws holding the object holder and knife were hand tightened to avoid vibration. To produce uniform sections, the microtome knife was adjusted to the proper angle in the knife holder with only the cutting edge coming in contact with the paraffin block. The tissue was cut in the thickness range of about 7µm.

❖ **Flattening and Mounting of Sections**

The procedure was carried out in tissue flotation warm water bath. The sections were spread on a warm water bath after they were detached from the knife with the help of hair brush. Dust free clean slides were coated with egg albumin over the whole surface. Required sections were spread on clean slide and kept at room temperature

❖ **Staining of Tissue Sections**

The sections were stained as follows; deparaffinization with xylene two times

each for five minutes

Dehydration through descending grades of ethyl alcohol

- ✓ 100% alcohol (absolute) - 2 minute
- ✓ 90% alcohol - 1minute
- ✓ 50% alcohol - 1 minute

Staining with Ehrlich's Haemaoxylin was done for 15-20 minutes. Then the sectioned tissues were thoroughly washed in tap water for 10 minutes. Rinsed with distilled water and stained with Eosin. Dehydration again with ascending grades of alcohol.

- ✓ 70% alcohol - 2 minute
- ✓ 90% alcohol - 2 minute
- ✓ 100%alcohol - 1 minute

Finally the tissues were cleared with xylene two times, each for about 3 minutes interval.

❖ **Mounting**

On the stained slide, DPX mountant was applied uniformly and micro glass cover slides were spread. The slides were observed in Nikon microscope and microphotographs were taken.

STATISTICAL ANALYSIS

The data of all the results were represented as Mean ± S.E.M. on statistically analysed by one-way ANOVA followed by Tukey's multiple comparison test was used for statistical analysis $p<0.05$ was considered significant.

7. RESULTS

7.1 Appearance and percentage yield of *EEMP*

Table no: 4

Drug	*Mallotus Philippensis* Muell.Arg.
Solvent	Ethanol
Colour	Brownish
Consistency	Semi solid
Percentage yield	21.41 % w/w

7.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Table no-5: Results of the Preliminary Phytochemical Constituents present in ethanolic extract of *Mallotus philippensis* Muell.Arg.

Table no: 5 Preliminary phytochemical constituents present in *EEMP*

S. No	**Constituents**	***Mallotus philippensis* Ethanolic extract**
1.	Alkaloids	_
2.	Carbohydrates	+
3.	Protein	+
4.	Steroids	+
5.	Phenols	+
6.	Tannins	+
7.	Flavanoids	+
8.	Gums and Mucilage	–
9.	Glycosides	+
10.	Sterols	_
11.	Saponins	_
12.	Terpenes	+

+ve Indicates the presence -ve Indicates the absence

RESULTS: The phytochemical constituents present in the ethanolic extract of *Mallotus philippensis* Muell.Arg. were carbohydrates, steroids, phenols, tannins, flavanoids, glycosides, proteins and terpenes.

7.3 Estimation of Blood glucose level

The effect of the different doses of ethanolic extract of *Mallotus philippensis* Muell.Arg. on blood glucose level

Table no 6: Results of the effect of *EEMP* on blood glucose level

Blood Glucose level (mg/dl)				
Treatment	**0 day**	**7th day**	**14th day**	**21st day**
Control 0.5% CMC (1ml/kg; p.o)	85.12± 1.87	86.12 ± 2.12	86.54±1.92	87.12±1.24
STZ (45mg/kg; b.wt; i.p)	271.76 ± 4.90	280.45±3.87*	288.78±4.32*	296.56±4.87*
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	276.56±3.65 NS	170.78±2.98**	158.87±3.12 **	153.87±2.87**
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	274.64 ± 3.8NS	150.65±3.72**	135.82±2.12 **	105.32±1.76 **
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	272.24 ± 4.65 NS	104.25±2.34**	98.98±1.65 **	89.21±0.87 **

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

NS-Non significant.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

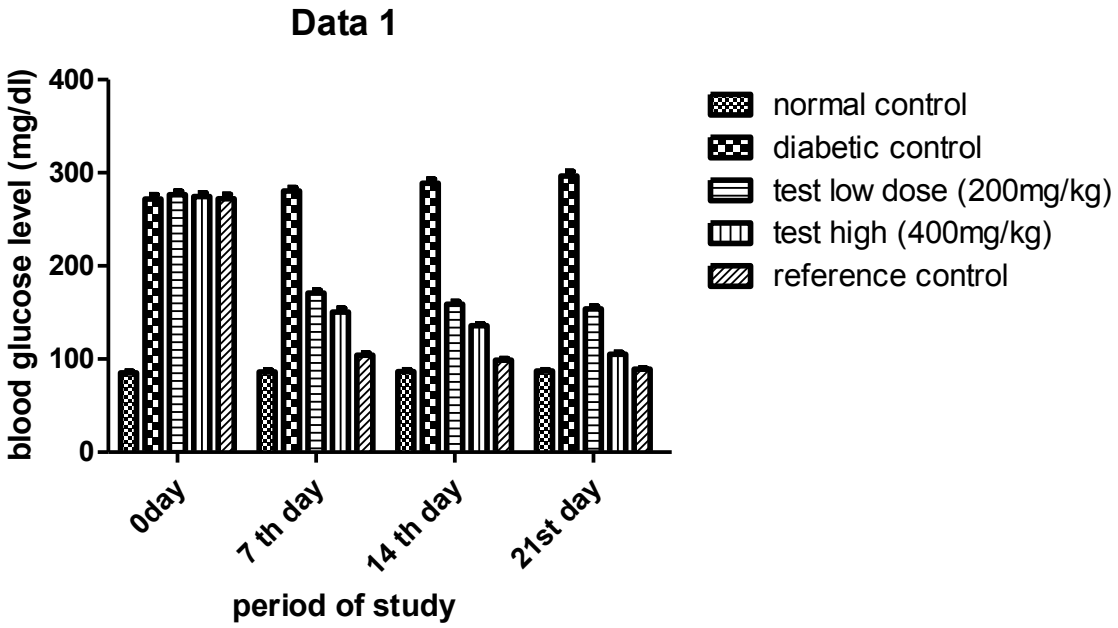


Fig.no-7: Diagrammatic representation of the results of the effects of *EEMP* on blood glucose levels

RESULTS

The blood glucose levels were measured in 0 day, 7th day,14thst day and 21st day as showed in Table no-6 and Fig. no-7.

The diabetic treated (Group 2) on 0 day, 7th day,14thst day and 21st day showed significant increase in blood glucose levels (hyperglycemia) when compared to the normal control (Group 1). Standard (Group 5) in 7thday, 14th day and 21st day showed statistically significant decrease in blood glucose level when compared to diabetic control (Group2). *EEMP* (200 mg/kg) treated (Group 3) showed statistically significant decrease in blood glucose level in 21st day when compared to the diabetic control (Group 2). *EEMP* (400mg/kg) treated (Group 4) showed statistically significant decrease in blood glucose level in 7th day,14th day and 21st day when compared to the diabetic control (Group 2).

7.4 Glycosylated haemoglobin level

The effect of the different dose of Ethanolic extract of *Mallotus philippensis* Muell Arg on Glycosylated Haemoglobin level.

Table no-7: Results of the effect of *EEMP* on Glycosylated haemoglobin level

Groups	**HbA1c**
Control 0.5% CMC (1ml/kg; p.o)	4.61±0.87
STZ (45mg/kg; b.wt; i.p)	8.95±0.76*
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	6.12±0.65**
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	5.87±0.72**
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	5.12±0.93**

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

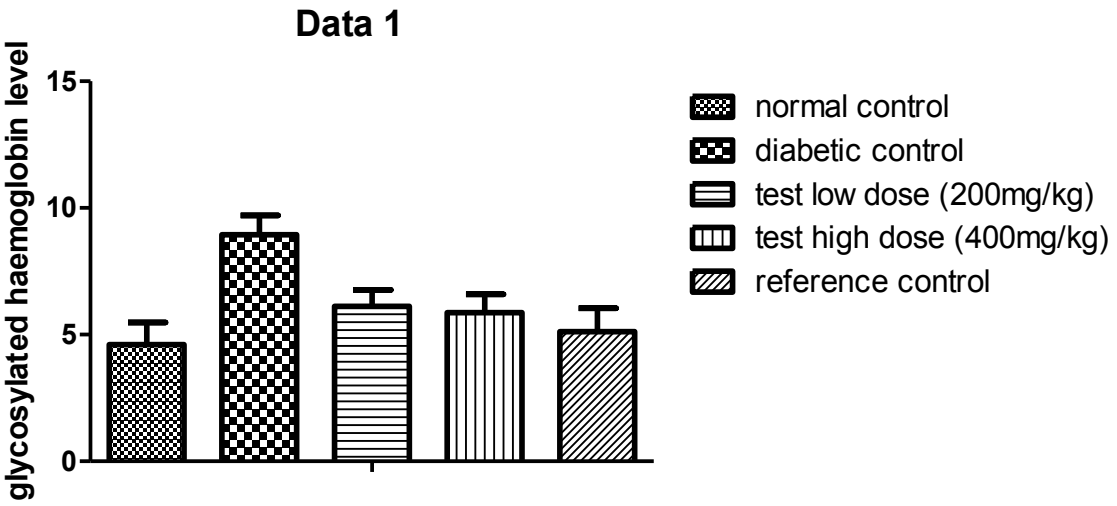


Fig. No-8: Diagrammatic representation of the results of the effects of *EEMP* on Glycosylated Haemoglobin level.

RESULTS

The Glycosylated Haemoglobin levels were measured were showed in Table no-7 and Fig. no-8.

The diabetic control (Group 2) showed significant increase in Glycosylated Haemoglobin level when compared to the normal control (Group1).

Standard (Group 5) showed statistically significant decrease in Glycosylated Haemoglobin level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant decrease in Glycosylated Haemoglobin level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant decrease in Glycosylated Haemoglobin level when compared to the diabetic control (Group 2).

7.5 ESTIMATION OF SERUM PARAMETERS

7.5.1 Estimation of Total cholesterol level

The effect of the different dose of Ethanolic extract of *Mallotus philippensis* Muell.Arg. on Total cholesterol level.

Table no-8: Results of the effect of *EEMP* on Total cholesterol level

Groups	Total cholesterol level
Control 0.5% CMC (1ml/kg; p.o)	65.88±1.203
STZ (45mg/kg; b.wt; i.p)	140.38±1.244*
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	77±2.399**
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	72.06±2.033**
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	69.48±2.333**

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

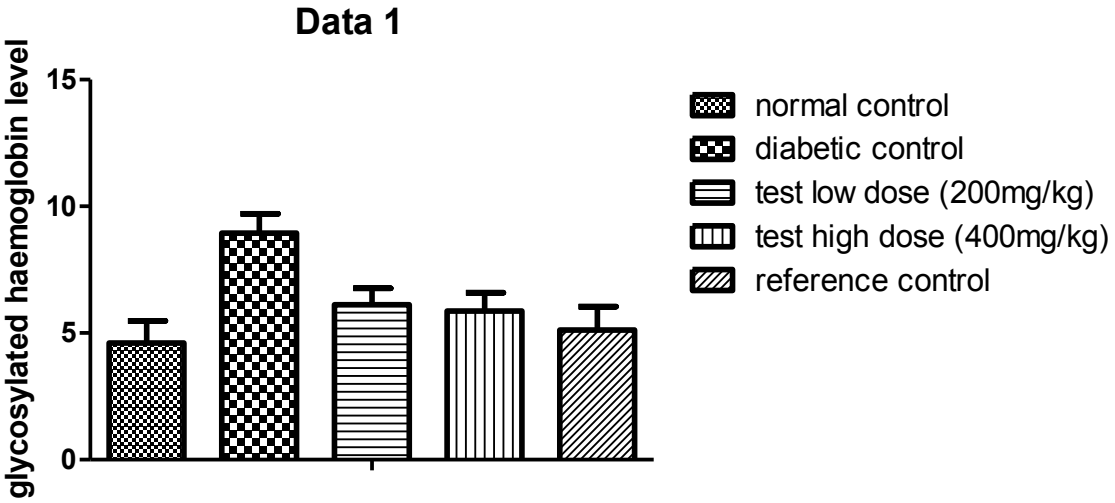


Fig. No-9: Diagrammatic representation of the results of the effects of *EEMP* on Total Cholesterol level.

RESULTS

The Total Cholesterol levels were measured were showed in Table no-8 and Fig. no-9.

The diabetic control (Group 2) showed significant increase in Total Cholesterol level when compared to the normal control (Group1).

Standard (Group 5) showed statistically significant decrease in Total Cholesterol level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant decrease in Total Cholesterol level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant decrease i**n** Total Cholesterol level when compared to the diabetic control (Group 2).

7.5.2 Effect of plant extract on serum aspartate aminotransferase (AST) level and serum alanine aminotransferase (ALT) level

- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on serum aspartate aminotransferase (AST) level.
- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on serum alanine aminotransferase (ALT) level.

Table no 9: Results of the effect of *EEMP* on serum aspartate aminotransferase (AST) level and serum alanine aminotransferase (ALT) level

Groups	AST (IU/L)	ALT(IU/L)
Control 0.5% CMC (1ml/kg; p.o)	52.25±4.26	49.16±3.76
STZ (45mg/kg; b.wt; i.p)	121.76±6.12*	95.76±5.18*
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	67.98±3.86**	58.24±3.89**
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	60.56±3.78**	55.35±2.76**
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	55.24±4.53**	52.68±3.92**

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

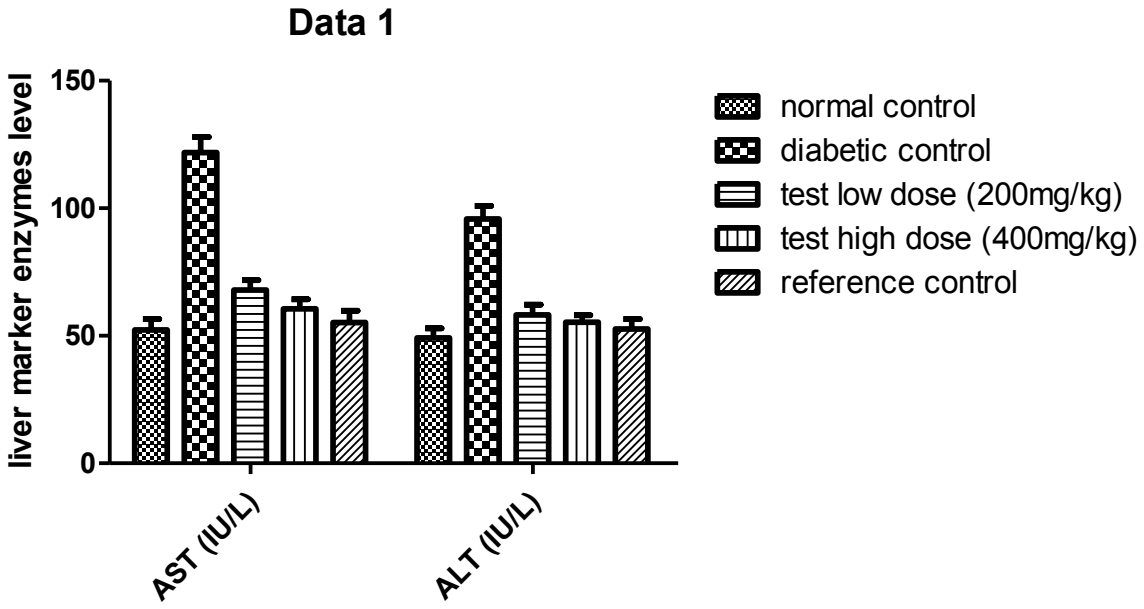


Fig. No-10: Diagrammatic representation of the results of the effects of *EEMP* on Serum aspartate aminotransferase (AST) level and Serum alanine aminotransferase (ALT) level.

RESULTS

- ✓ **Serum aspartate aminotransferase (AST) level**

The serum aspartate aminotransferase (AST) levels were measured were showed in Table no-9 and Fig. no-10.

The diabetic control (Group 2) showed significant increase in serum aspartate aminotransferase (AST) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant decrease in liver serum aspartate aminotransferase (AST) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant decrease in serum aspartate aminotransferase (AST) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant decrease in serum aspartate aminotransferase (AST) level when compared to the diabetic control (Group 2).

- ✓ **Serum alanine aminotransferase (ALT) level**

The serum alanine aminotransferase **(**ALT) levels were measured were showed in Table no-9 and Fig. no-10.

The diabetic control (Group 2) showed significant increase in serum alanine aminotransferase **(**ALT) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant decrease in serum alanine aminotransferase **(**ALT) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant decrease in serum alanine aminotransferase **(**ALT) level when compared to the diabetic control (Group2).

EEMP 400mg/kg treated (Group 4) showed statistically significant decrease in serum alanine aminotransferase **(**ALT) level when compared to the diabetic control (Group 2).

7.6 ESTIMATION OF OXIDATIVE STRESS PARAMETERS

7.6.1 Effect of *EEMP* on liver Malondialdehyde (MDA) level

The effect of the different dose of ethanolic extract of *Mallotus philippensis* Muell Arg on Liver Malondialdehyde (MDA) level.

Table no-10: Results of the effect of EEMP on MDA level

Groups	**MDA**
Control 0.5% CMC (1ml/kg; p.o)	1.78±0.06
STZ (45mg/kg; b.wt; i.p)	3.68±0.03 *
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	2.43±0.03 **
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	1.93±0.04 **
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	1.64±0.02 **

The values were expressed as Mean ± S.E.M. (n=6 animals in each group.

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

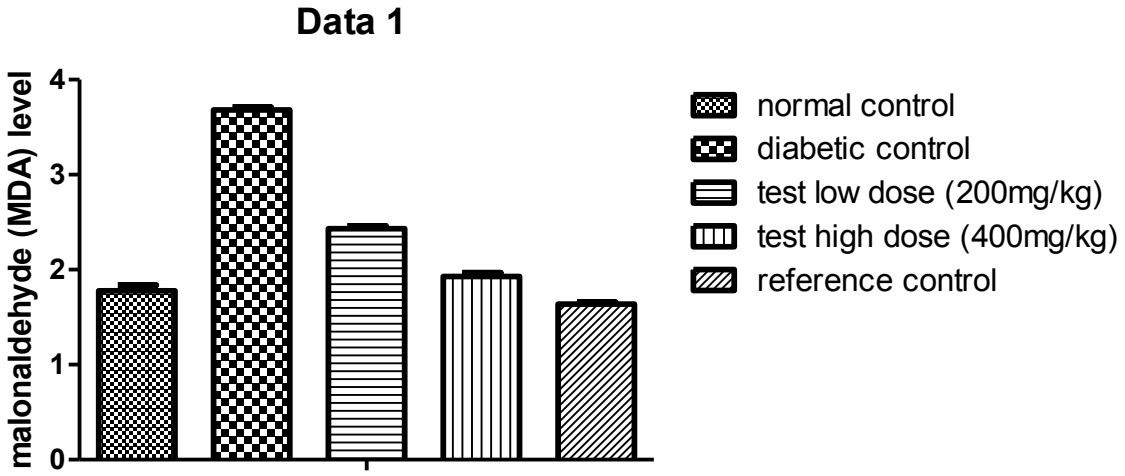


Fig. No-11: Diagrammatic representation of the results of the effects of *EEMP* on liver malondialdehyde (MDA) level.

RESULTS

The Liver malondialdehyde (MDA) levels were measured were showed in Table no-10 and Fig. no-11.

The diabetic control (Group 2) showed significant increase in liver malondialdehyde (MDA) level when compared to the normal control (Group1).

Standard (Group 5) showed statistically significant decrease in liver malondialdehyde (MDA) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant decrease in liver malondialdehyde (MDA) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant decrease in liver malondialdehyde (MDA) level when compared to the diabetic control (Group 2).

7.7 ESTIMATION OF ANTIOXIDANT PARAMETERS

7.7.1 Effect of *EEMP* on Enzymic hepatic antioxidant level (SOD, CAT and GPx.)

- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on Liver superoxide dismutase (SOD) level.
- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on Liver Catalase (CAT) level
- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on glutathione peroxidase (GPx) level

Table no 11: Results of the effect of *EEMP* on enzymic hepatic antioxidant levels (SOD, CAT and GPx.)

Groups	SOD	CAT	GPx
Control 0.5% CMC (1ml/kg; p.o)	2.66±0.01	15.21±0.07	1.71±0.05
STZ (45mg/kg; b.wt; i.p)	0.45±0.02 *	2.76±0.04 *	0.36±0.02 *
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	0.75±0.02**	6.76±0.03 b**	0.44±0.02 NS
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	1.82±0.07 **	12.62±0.11 **	1.14±0.02 **
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	2.13±0.02 **	13.50±0.24 **	1.70±0.09 **

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

NS-Non significant

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

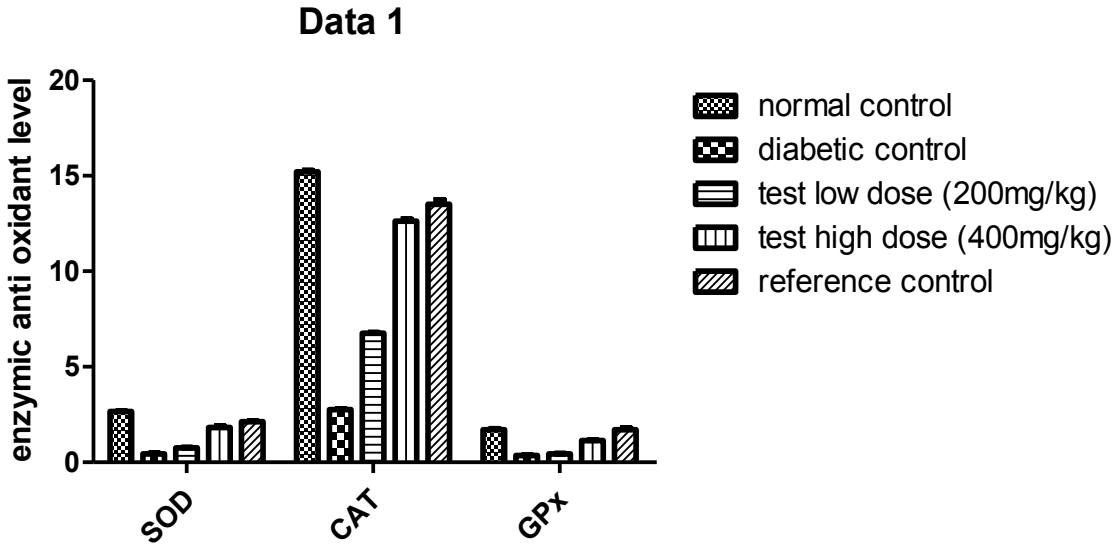


Fig. No-12: Diagrammatic representation of the results of the effects of *EEMP* on Enzymic hepatic antioxidant levels (SOD, CAT and GPx.).

RESULTS

- ✓ **Superoxide dismutase (SOD) levels**

The superoxide dismutase (SOD) levels were measured were showed in Table no-11 and Fig. no-12.

The diabetic control (Group 2) showed significant decrease in liver superoxide dismutase (SOD) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in liver superoxide dismutase (SOD) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in liver superoxide dismutase (SOD) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in liver superoxide dismutase (SOD) level when compared to the diabetic control (Group 2).

- ✓ **Catalase (CAT) level**

The CAT levels were measured were showed in Table no-11 and Fig. no-12.

The diabetic control (Group 2) showed significant decrease in liver catalase (CAT) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in liver catalase (CAT) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in liver catalase (CAT) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in liver catalase (CAT) level when compared to the diabetic group.

- ✓ **Glutathione peroxidise (GPx) level**

The glutathione peroxidase (GPx) levels were measured were showed in Table no-11 and Fig. no-12.

The diabetic control (Group 2) showed significant decrease in glutathione peroxidase (GPx) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in glutathione peroxidase (GPx) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in glutathione peroxidise (GPx) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in glutathione peroxidise (GPx) level when compared to the diabetic group.

7.7.2 Effect of *EEMP* on Non enzymic hepatic antioxidant level (GSH, Vit.C and Vit.E)

- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on Reduced Glutathione (GSH) level.
- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell Arg on Vit. C level
- ✓ The effect of the different doses of Ethanolic extract of *Mallotus philippensis* Muell.Arg. on Vit.E level

Table no 12: Results of the effect of *EEMP* on Non enzymic antioxidant levels (GSH, Vit.C and Vit.E)

Groups	GSH	Vit.C	Vit.E
Control 0.5% CMC (1ml/kg; p.o)	3.68±0.26	0.40 ±0.02	1.80±0.06
STZ (45mg/kg; b.wt; i.p)	1.26±0.09 *	0.18±0.02 *	0.76±0.07 *
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	1.91±0.13 **	0.26±0.03 **	1.45±0.08 **
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	3.05±0.16 **	0.32±0.04 **	1.69±0.09 **
STZ+ Glibenclamide (600 µg/kg, b.wt; p.o)	2.57±0.12 **	0.33±0.03 **	1.75±0.05 **

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

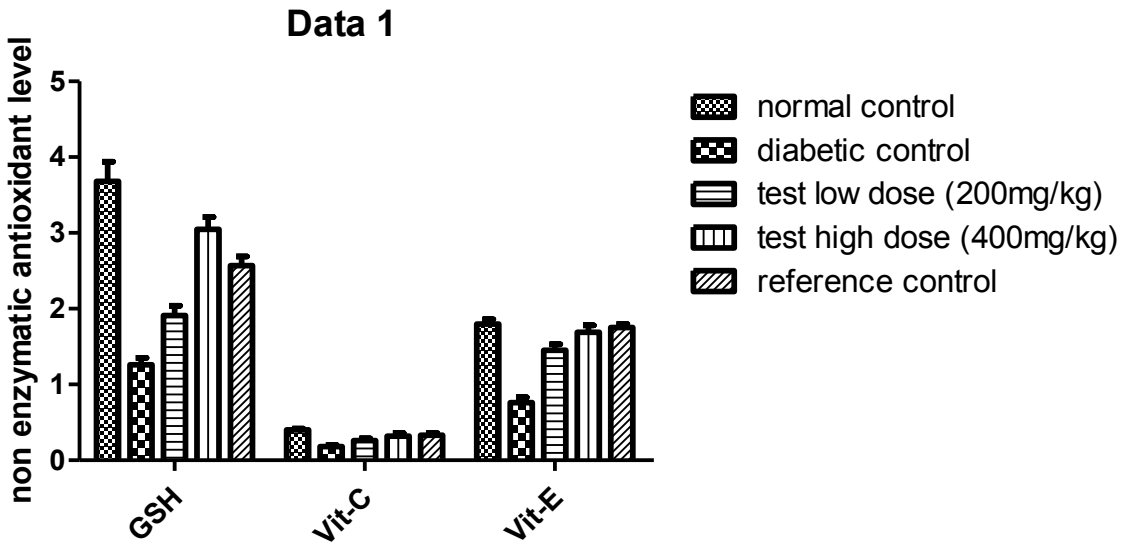


Fig. No-13: Diagrammatic representation of the results of the effects of *EEMP* on Non enzymic antioxidant level (GSH, Vit.C and Vit.E)

RESULTS

- ✓ **Reduced Glutathione (GSH) level.**

The Reduced Glutathione (GSH) levels were measured were showed in Table no-12 and Fig. no-13.

The diabetic control (Group 2) showed significant decrease in Reduced Glutathione (GSH) level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in Reduced Glutathione (GSH) level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in Reduced Glutathione (GSH) level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in Reduced Glutathione (GSH) level when compared to the diabetic control (Group 2).

- ✓ **Vit.C level**

The Vit.C levels were measured were showed in Table no-12 and Fig. no-13.

The diabetic control (Group 2) showed significant decrease in Vit.C level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in Vit.C level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in Vit.C level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in Vit.C level when compared to the diabetic group.

- ✓ **Vit.E level**

The Vit.E levels were measured were showed in Table no-12 and Fig. no-13.

The diabetic control (Group 2) showed significant decrease in Vit.E level when compared to the normal control (Group 1).

Standard (Group 5) showed statistically significant increase in Vit.E level when compared to diabetic control (Group 2).

EEMP 200 mg/kg treated (Group 3) showed statistically significant increase in Vit.E level when compared to the diabetic control (Group 2).

EEMP 400mg/kg treated (Group 4) showed statistically significant increase in Vit.E level when compared to the diabetic group.

7.8 Effect of *EEMP* on plasma Insulin level

The effect of the different doses of Ethanolic extract of *Mallotus Philippensis* Muell Arg on in plasma insulin level.

Table no 13: Results of the effect of *EEMP* on Plasma insulin level

Treatment and Dose	Insulin levels (µIU/ml)
Control 0.5% CMC (1ml/kg; p.o)	7.74 ± 0.41
STZ (60mg/kg; b.wt; i.p)	3.50 ± 0.73*
STZ + Plant extract LD (200mg/kg, b.wt; p.o)	5.01± 0.36**
STZ+ Plant extract HD (400mg/kg, b.wt; p.o)	6.75±0.98**
STZ+ Glibinclamide (600 µg/kg, b.wt; p.o)	7.58 ± 0.72**

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

*= when compared to the control group.

** = when compared to the STZ treated group.

Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

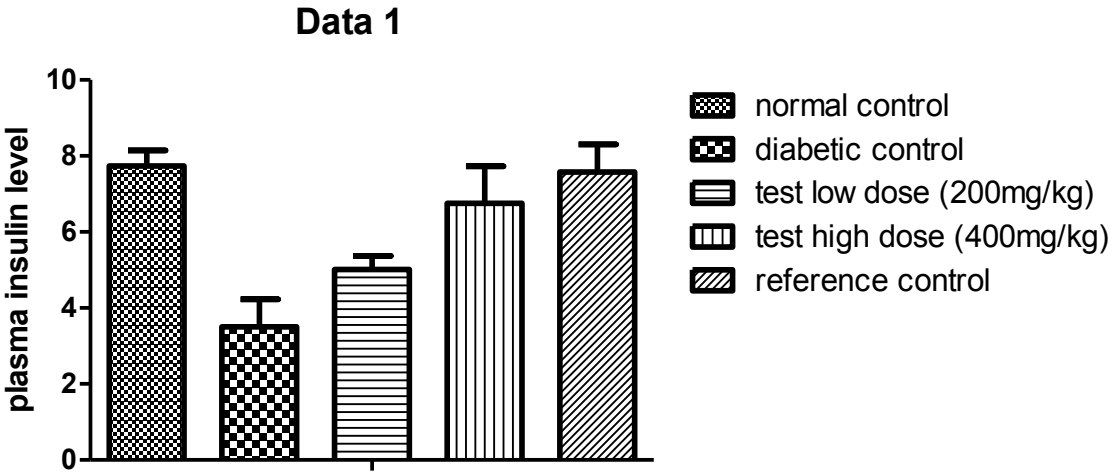


Fig. No-14: Diagrammatic representation of the results of the effects of *EEMP* on Plasma insulin level

RESULTS

The plasma insulin levels were measured and showed in Table no-13 and Fig. no-14.

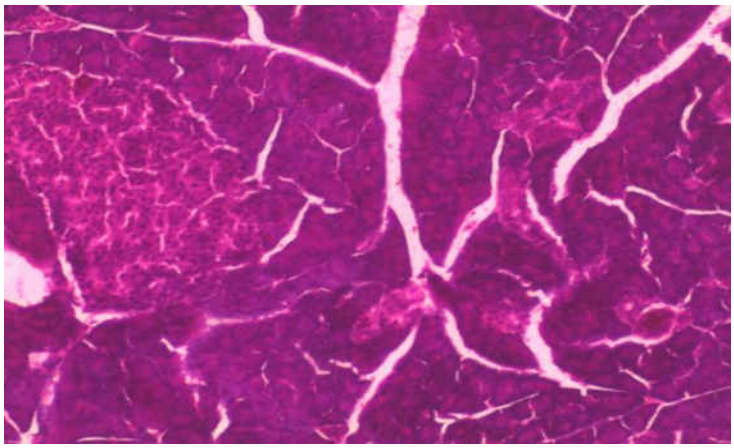
The diabetic control (Group 2) showed significant decrease in the plasma insulin levels when compared to the normal control (Group 1).

Standard (Group 5) showed significant statistically increases in liver the plasma insulin levels when compared to diabetic control (Group 2).

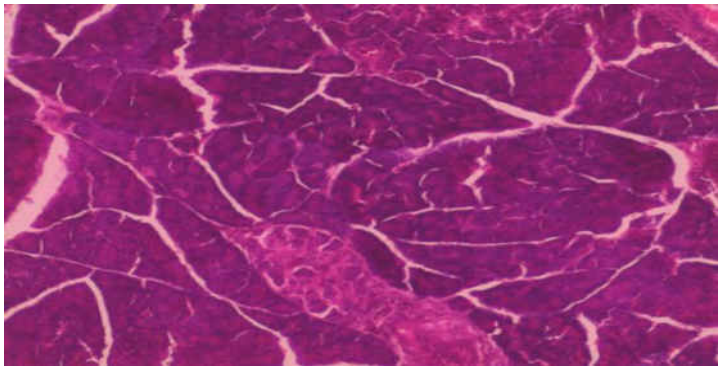
EESO 200 mg/kg treated (Group 3) showed significant statistically increase in the plasma insulin levels when compared to the diabetic (Group 2).

EESO 400mg/kg treated (Group 4) showed significant statistically increase in the plasma insulin levels when compared to the diabetic (Group 2).

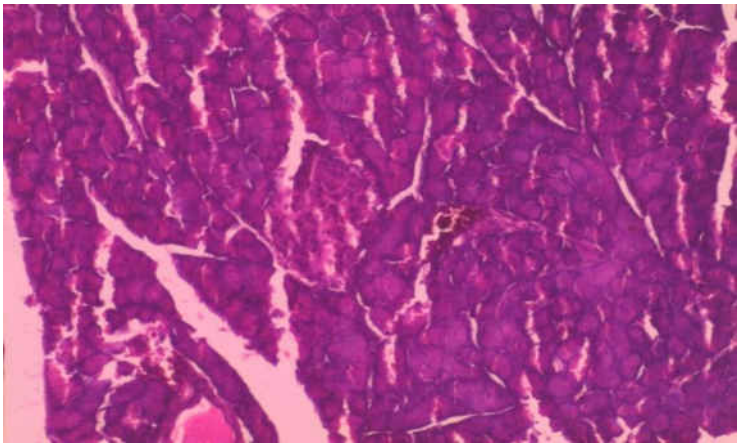
7.9 HISTOPATHOLOGICAL STUDIES



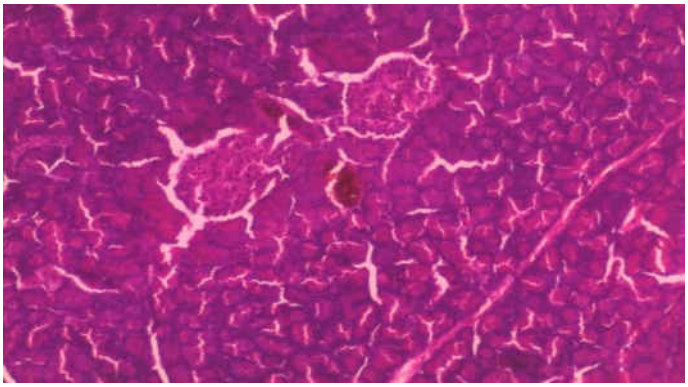
Group I normal control: showed the normal architecture of pancreas



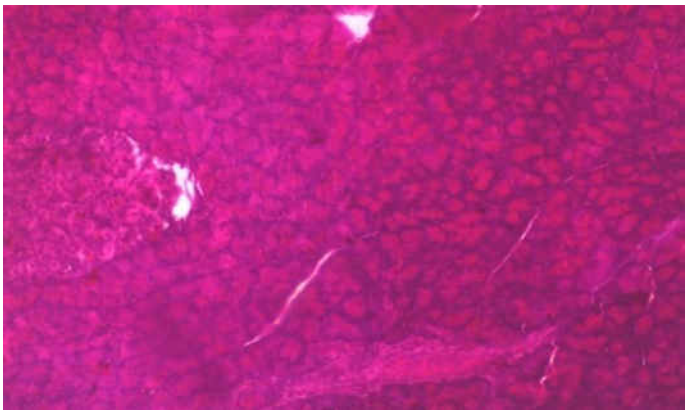
Group II STZ induced diabetes: Displayed extensive reduction in the number of islets, with a reduced number of betacells. Further atrophic islets cells where visualized.



Group III STZ + *M.philippensis* (200 mg/kg): Showed the moderate restoration of pancreatic histology with a slight elevation of islets and beta cells .



Group IV STZ + *M. Philippensis* (400 mg/kg): Effective restoration of pancreatic architecture with an increase in the population of islets and beta cells.



Group V Standard Glibenclamide (600 µg/kg): Restored the normal architecture of pancreas with an increase in the number islets and beta cells

8. DISCUSSION

Diabetes mellitus, a chronic metabolic disease characterized by a deficiency in the pancreas insulin production and/or by peripheral insulin resistance.

The management of diabetes without any side effects is still a challenge to the medical system. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. Wide array of plant derived active principles have demonstrated anti-diabetic activity.

The adverse effects of hypoglycaemic drugs and insulin and the excessive cost of these medications can be mentioned as some disadvantages regarding the diabetes treatment, which stimulate the search for new therapeutic agents that present safety, effectiveness and low cost. Nowadays there is growing trend towards using herbal preparations and/or derivatives in traditional and complementary medicines to treat symptoms.[122] In this way, it has been cresent the interest of current ethnopharmacological research to investigate the plants species with antihyperglycemic effect, focusing in the evaluation of the efficacy and safety of plant preparations for diabetic treatment.

The fundamental mechanism underlying hyperglycemia in diabetes involves over production (excessive glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. The metabolism of glucose, proteins and lipids is abnormal in diabetes due to insulin secretion defect, leading to various metabolic disorders. Herbal drugs may act on blood glucose through different mechanism, some of them may have insulin like substances. Stimulation of beta cells to produce more insulin and others may increase cells in the pancreas by activating regeneration of pancreatic cell

The *Mallotus Phililppensis* Muell.Arg. Fruit had been claimed for its anti-diabetic activity and there is no degree of research work which has not been done but, claiming *Mallotus philippensis* Fruits have therapeutic use on blood glucose levels.[90] Hence, project on *Mallotus Phililppensis* Muell. Arg. Fruit was carried out to provide scientific validation on anti-diabetic activity.

The preliminary phytochemical analysis of *EEMP* revealed the presence of carbohydrates, flavanoids, terpenoids, glycosides, proteins, tannins, steroids and phenols. Mainly flavanoids which may be responsible for its anti-diabetic properties.[113]

Acute toxicity studies revealed the non-toxic nature of the *EEMP* There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an LD_{50} dose of 2000 mg/kg and above was considered as unclassified so the *EEMP* was found to be safe.

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptazotocin (STZ-45mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight.[113] The diabetic group showed marked increase of glucose level as compared to the normal group. The oral administration of *EEMP* reversed the blood glucose level in which the action was through potentiation of pancreatic secretion of insulin from islets beta cells or due to enhanced transport of blood glucose to the peripheral tissue. There was a significant decrease in blood glucose level in the extract treated groups.

Glycosylated haemoglobin concentrations are helpful and solid tool for the appraisal of glycemic control in diabetics as suggested by the international diabetes federation.[123] Treatment groups (*EEMP* test low dose, *EEMP* test high dose and Glibenclamide) of diabetic rats unquestionably decrease the level of glycosylated haemoglobin. A noteworthy decrease of glycosylated haemoglobin showed the ability of the extract in the control of diabetes.[124]

Hypercholesteremia are primary factor involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes.[125] EEMP significantly reduced total cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that EEMP, could modulate blood lipid abnormalities.

The injection of STZ induces a hepatocellular damage, which is indicated by significant increase in AST and ALT in diabetic group as compare to control group. Furthermore STZ induces hepatocellular damage, which results in leakage of AST and ALT from liver

systole to the blood stream and/or may change the permeability of liver cell membrane.[126] in the present study, *EEMP* significantly decreased AST and ALT enzyme activities in diabetic rats. The improvements in the levels of the enzyme are a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of AST and ALT after treatment also indicates a revival of insulin secretion.[127]The results from present study indicates that EEMP may reduce the level of serum cholesterol, SGOT and SGPT. It confirms that functions are on the protection of vital tissues Pancreas, thereby reducing the causation of diabetis in experimental animals.

Lipid peroxidation eventually leads to extensive membrane damage and disfunction.[128] Decreased lipid peroxidation and improved antioxidant status may be one of the mechanism by which drug treatment could contribute to the prevention of diabetic complications.[129] In our study, *EEMP* significantly attenuated the increased lipid peroxidation which could be due to the antioxidant effect of flavanoids, detected in the preliminary phytochemical screening of the extract.

Oxidative stress is a condition of reduction in antioxidative enzymes like SOD, CAT, GPx.[130]

Superoxide dismutase (SOD), is an important defence enzyme which catalyses the dismutation of superoxide radicals, which scavenges the superoxide ions by catalyzing its dismutation.

Catalase (CAT), a heme enzyme which removes hydrogen peroxide.[131] The decreased activities of CAT and SOD thereby result in the increased production of hydrogen peroxide and oxygen by auto oxidation of glucose and non-enzymatic glycation,[132] which is known to occur during diabetis.

Glutathione peroxidise (GPx) is an antioxidant enzyme, catalyses the scavenging and inactivation of hydrogen and lipid peroxidise.[133]

The results showed that hepatic activity of catalase, superoxide dismutase and glutathione peroxidase decreased significantly in STZ induced diabetic group (Group II).

The normal control group maintained optimal value for activity of antioxidants. *EEMP* treatment in diabetic rats significantly increased the antioxidant enzyme activities and reversed them to their normal values. The same phenomenon was seen in the results of glibenclamide treated groups.

An array of non-enzymatic antioxidant like GSH, Vitamin C and Vitamin E are involved in scavenging free radicals *in vivo.*

Reduced glutathione (GSH), a tripeptide present in the all cells, is an important antioxidant.[134] It is essential to maintain structural and functional integrity of cells. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione.[135] Decreased levels of GSH in liver of diabetic rats may increase their susceptibility to oxidative injury.

Vitamin C is an excellent hydrophilic, dietary antioxidant and it readily scavenges ROS and peroxyl radicals.[136] it also acts as co-antioxidant by generating Vitamin A, E and GSH from radicals. A decrease in the level of Vitamin C was observed in liver of diabetic rats. Such a fall in level of Vitamin C could be due to the increased utilization of Vitamin C in the deactivation of increased level of ROS or due to decrease in GSH level, since GSH is required in recycling of Vitamin C.[137]Another possibility is that hyperglycemia inhibits ascorbic acid and its cellular transport.

Vitamin E is an antioxidant, a substance that helps prevent damage to the body's cells.[138] Streptozotocin induced diabetic rats were found to have decreased GSH, Vitamin C and Vitamin E levels in liver as compared to control rats. Treatment with *EEMP* and the standard drug, glibenclamide produced significant increase in the levels of these non-enzymatic antioxidants.

The serum insulin level decreased in diabetic rats, whereas *EEMP* extract, brought about a marked increase in serum insulin in streptozotocin-induced diabetic rats. This increase may be a consequence of the stimulation of insulin synthesis and secretion.[139]

The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in treated group *EEMP*. The regenerative effect of the pancreatic cells by

Mallotus philippensis via exocrine cells of pancreas may enlighten the positive effects of these agents on the production of insulin. Reports on histopathological analysis of pancreas of the *Mallotus philippensis* alone treated rats showed results that were very similar as that of the control group.

Based on the above results, it was concluded that *Mallotus Phililppensis* Muell.Arg. exerted statistically significant anti-diabetic activity against STZ induced diabetic rats.

9. SUMMARY AND CONCLUSION

The present study was undertaken to scientifically elucidate the anti-diabetic activity of alcoholic fruit extract of *Mallotus Phililppensis* Muell.Arg.

The phytochemical investigation revealed the presence of carbohydrates, steroids, phenols, tannins, flavanoids, glycosides, and terpenes in the *EEMP*.

The STZ 45 mg/kg effectively induced diabetes, which was similar to diabetes in human. Therefore it is an effective and an ideal model for diabetes research.

The Blood glucose level and Glycosylated haemoglobin level significantly decreased with treatment of *EEMP* proves it having anti-diabetic activity.

Attenuating the increased serum parameters like total cholesterol, AST and ALT with treatment of *EEMP* showed its anti-diabetic activity.

Reduced level of lipid peroxidation in *EEMP* treated rats, showed *EEMP* has protective effect in oxidative stress induced diabetes.

The enzymic antioxidant parameters such as SOD, CAT and GPx and Non-enzymic antioxidant parameters such as GSH, Vitamin C and Vitamin E are increased significantly with treatment of *EEMP*, which proved as it having antioxidant activity.

The plasma insulin level significantly increased with treatment of *EEMP,* showed the *EEMP* in managing hyperglycemia and diabetic complications.

Mallotus Phililppensis Muell.Arg.fruit extract also have favourable effects to inhibit the histopathological change of the pancreas in STZ induced diabetes.

In summary, alcoholic fruit extract of *Mallotus Phililppensis* Muell.Arg showed statistically significant anti-diabetic activity.

In conclusion, the alcoholic fruit extract of *Mallotus Phililppensis* Muell.Arg. showed and offered a promising therapeutic value in prevention of diabetes. These effects was mainly attributed to its antioxidant properties by significant quenching

impact of the extract on lipid peroxidation along with enhancement of enzymatic and non-enzymatic antioxidant defense systems in liver and pancreatic tissue.

Further studies will be needed in future to determine the exact phytoconstituents in the extract, which having anti-diabetic activity.

10. FUTURE PROSPECTIVES

Further study is required.,

1. To isolate and separate the active phytochemicals present in the ethanolic extract of the Fruit of *Mallotus Phililppensis* Muell Arg.
2. Formulation of the isolated lead molecule can be designed.
3. Clinical trial of the formulated molecule in healthy human volunteers or diseased persons.
4. The formulated lead molecule can be subjected to the clinical trials, patented and marketed for the treatment of diabetes.

11. REFERENCE

1. "About diabetes". World Health Organization. Retrieved 4 April 2014.
2. "Diabetes Fact sheet N°312". WHO. October 2013. Archived from the original on 26 Aug 2013. Retrieved 25 March 2014.
3. Kitabchi, AE; Umpierrez, GE; Miles, JM; Fisher, JN (Jul 2009). "Hyperglycemic crises in adult patients with diabetes.". Diabetes Care. 32 (7): 1335–43. doi:10.2337/dc09-9032. PMC 2699725. PMID 19564476.
4. Shoback, edited by David G. Gardner, Dolores (2011). "Chapter 17". Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. ISBN 0-07-162243-8.
5. Maggard MA, Yermilov I, Li Z, et al; Pregnancy and fertility following bariatric surgery: a systematic review. JAMA. 2008 Nov 19;300(19):2286-96
6. RSSDI textbook of diabetes mellitus. (Rev. 2nd ed.). New Delhi: Jaypee Brothers Medical Publishers. 2012. p. 235. ISBN 9789350254899.
7. "The top 10 causes of death Fact sheet N°310". World Health Organization. Oct 2013.
8. Rippe, edited by Richard S. Irwin, James M. (2010). Manual of intensive care medicine (5th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. p. 549. ISBN 9780781799928.
9. Picot, J; Jones, J; Colquitt, JL; Gospodarevskaya, E; Loveman, E; Baxter, L; Clegg, AJ (September 2009). "The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation". Health Technology Assessment (Winchester, England). 13 (41): 1–190, 215–357, iii–iv. doi:10.3310/hta13410. PMID 19726018.
10. Williams textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435. ISBN 978-1-4377-0324-5.
11. Abha Saxena and Naval Kishore Vikram. “Role of Selected Indian Plants in Management of Type 2 Diabetes: A Review”. The journal of alternative and Complementary medicine. 2004, 10 (2): 369-378.
12. Pranav K, Prabhakar and Mukesh Doble. “A target based therapeutic Approach towards Diabetes Mellitus using Medicinal Plants”. Current Diabetes reviews. 2008, 4:291-308.

13. KA wadkar, CS Magdum,SS Patil,NS Naikwade, anti-diabetic potential and Indian medicinal plants, J herbal med toxicol, volume 2, 2008.
14. Gerard j. Tortora, bryan H. Derrickson. 2009. Principles of Anatomy and physiology". Wiley (Asia) private limited. 12 (2); 942-944.
15. H.P. Rang, M.M Dale, J.M. Ritter, R. J. Flower. 2007. Rang and Dale's Pharmacology". Churchill Living stone Elsevier. (6):307-409.
16. Raju. S, Hemamalini "In vivo animal model for screening of Anti-diabetic activity" Asian Journal of pharmaceutical and Clinical Research Vol 5, Suppl 4, 2012 ISSN- 0974-2441.
17. Review ArticleMani Rupeshkumar,Kunchu Kavitha,et al, Role of herbal plants in the diabetes mellitus therapy: an overreview. East Point College of Pharmacy, Bidarahalli, Bangalore-49, Karnataka, India, 2Department of Pharmaceutical Technology, Jadavpur University, Kolkata-32, West Bengal, India.
18. Khan, Ali Nawaz. "Chronic Pancreatitis Imaging". Medscape. Retrieved 5 January 2014.
19. "Cancer of the Pancreas". NHS. Retrieved 5 November 2014.
20. Drake, Richard L.; Vogl, Wayne; Tibbitts, Adam W.M. Mitchell; illustrations by Richard; Richardson, Paul (2005). Gray's anatomy for students. Philadelphia: Elsevier/Churchill Livingstone. pp. 288–290, 297, 303. ISBN 978-0-8089-2306-0.
21. Young, Barbara, ed. (2006). Wheater's functional histology : a text and colour atlas (5th ed.). Churchill Livingstone/Elsevier. pp. 299–301. ISBN 978-0-443-06850-8.
22. Hall, John (2011). Guyton and Hall textbook of medical physiology (12th ed.). Philadelphia, Pa.: Saunders/Elsevier. p. 781. ISBN 978-1-4160-4574-8.
23. World health Organisation Laboratory Diagnosis and Mdonitoring of Diabetes mellitus 2002.
24. Wright JR, Yang H, Hyrtsenko O, Xu BY, Yu W, Pohajdak B (2014). "A review of piscine islet xenotransplantation using wild-type tilapia donors and the production of transgenic tilapia expressing a "humanized" tilapia insulin". Xenotransplantation. 21 (6): 485–95. doi:10.1111/xen.12115. PMC 4283710. PMID 25040337.

25. Stryer, Lubert (1995). Biochemistry. (Fourth ed.). New York: W.H. Freeman and Company. pp. 773–774. ISBN 0 7167 2009 4.
26. Sonksen P, Sonksen J (July 2000). "Insulin: understanding its action in health and disease". British Journal of Anaesthesia. 85 (1): 69–79. doi:10.1093/bja/85.1.69. PMID 10927996.
27. Sonksen P, Sonksen J (July 2000). "Insulin: understanding its action in health and disease". British Journal of Anaesthesia. 85 (1): 69–79. doi:10.1093/bja/85.1.69. PMID 10927996.
28. American Society of Health-System Pharmacists (2009-02-01). "Insulin Injection". PubMed Health. National Center for Biotechnology Information, U.S. National Library of Medicine. Retrieved 2012-10-12.
29. "Definition, Diagnosis and Classification of Diabetes mellitus and its complications' World health organisation department of Noncommunicable Disease Survelillance Geneva WHO/NCD/ncS/99.2.
30. Duncan C. Moore, Caius D Christoe, Andrew Baum. "Diabetes: a 21st Century Epidemic" Morgan Stanley Research Europe Page no: 1-93.
31. Ian N Scobie. 2007. "Atlas of Diabetes mellitus". Informa healthcare, United Kingdom. (3): 1-121.
32. www,ncbi.nlm.nih.gov/pubmed/23683103.
33. American journal of diabetics insulin and protein metabolism
34. Lipid metabolism. Role of insulin in lipid metabolism.Otto-Buczkowska EJarosz-Chobot P.
35. Disorder of pancreas Wikipedia and encyclopedia
36. Goodman &Gilman's 2008. "Manual of Pharmacology and Therapeutics" McGraw Hill 1035-1050.
37. Sigurd lenzen. "Alloxan and Streptozotocin diabetes". 120-138.
38. Szkudelski T. The mechanism of alloxan and sterptozotocin action in beta cells of the pancreas. Physiol Res 2001; 50:536-46.
39. Ganda OP, Rossi AA, Like AA: Studies on streptozotocin diabetes, 1976 25: 595-603.
40. Ziegler M, Ziegler B, Hehmke B: Severe hyperglycaemia caused autoimmunization to beta cells in rats. Diabetologia, 1984 27: 163-165.
41. Oussama MN Khatib. "Guidelines for the prevention, management and care of diabetes mellitus". World Health Organisation, 2006. 10-43.

42. Schnedl WJ, Ferber S, Johnson JH, Newgard CB: STZ transport and cytotoxicity. Specific enhancement in GLUT2- expressing cells. Diabetes, 1994, 43: 1326-1333.
43. Wang Z, Gleichmann H: GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes, 1998 47: 50-56,
44. Delaney CA, Dunger A, Di Matteo M, Cunningham JM, Green MH, Green IC: Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl and ethyl nitrosoureas and methanesulphonates. Lack of correlation with nitric oxide-releasing or O 6 - alkylating ability. Biochem Pharmaco, 1995l 50: 2015-2020.
45. Ji Zhanga, Yulong Huanga, Tiande Houa, Yunpu Wangb. “hyperglycemic effect of *Artemesia sephaerocephala Krasch* seed polysaccharide in alloxan – induced diabetic rats’. Swiss med Wkly.2006, 136; 529-532.
46. Suman Bala Sharma, Afreena Nasir, Krishna Madhava Prabhu, Potharagada Surynaryana Murthy. “Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus”. Journal of Ethnopharmacology. 2006, 104: 367-373.
47. K. Ashok Kumar, M.Uma Maheswari,A.T. Sivashanmugam, V. Subhadradevi, N.V. Prasant and T.K. Ravi. “Hypoglycemic effect of *Ficus microcarpa* leaves on alloxan induced diabetic rats”. Journal of Biological sciences. 2007, 7(2).
48. Selvan VT, Mankandan I, Senthil Kumar GP, Suresh R, Kakoti BB, Gomathi P, Kumar DA, Sha P, Gupta M, Mazumder UK. “ Anti-diabetic and antioxidant effect of *Artenema sesamoides* in sterptozotocin induced diabetic rats”. International Journal of applied research in natural products. 2008, 1(1): 25-33.
49. M.A. Jayasri, S. Gunasekaran, A. Radha and T.L. Mathew. “Anti-diabetic effect of *Coptus pictus* leaves in normal and sterptozotocin induced Diabetic rats”. International Journal of Diabetes and Metabolism. 2008, 16: 117-122.
50. Vaiyapuri Sivajothi, alanka Dey, Balasundarm Jyakar and Balasubramanian rajkapoor. “ Antihypreglycemic, antihyperlipidemic and antioxidant effect of *Phyllanthus rheediii* on sterptozotocin induced diabetic rats”. Iranian journal of pharmaceutical research. 2008,7(1):53-59.

51. U.Srinivasa, J. Venkateshwara rao, A.M. krupanidhi and K. Divakar. “Antidaibetic activity of *Justicia beddomei* leaves in alloxan induced diabetic rats”. Journal of research and education of Indian medicine. 2008:45-47
52. K. Rajagopal and K. Sasikala. “Anti-diabetic activity of hydroethanolic extracts of *Nymphaea stellata* flowers in normal African Journal of Pharmacy and Pharmacology 2008,2(8): 173-178.and alloxan induced diabetic rats”.
53. Rakesh Barik, Sanjay Jain, Deep Qwatra, Amit Joshi, Girraj Sharan Tripathi, Ravi Goyal. “Anti-diabetic activity of aqueous root of *Ichnocarpus frutescens* in sterptozotocin-nicotinamide induced type-II diabetes in rats”. Indian Journal of Pharmacology. 2008, 40(1): 19-22.
54. A.N.Kalia, J.P.Yadav, Sushila Saini, A.S.Dangi. “Hypoglycaemic and Hypolipidemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan induced diabetic rats”. Indain Journal of Pharmacology 2008,40(1): 23-27.
55. K. Illango, V.Chitra, P.Kanimozhi and G. Balaji. “Anti-diabetic antioxidant and antibacterial activities of leaf extracts of *Adathoda zeylanica.* Medic *(Acanthaceae*)”. Journal of Pharmaceutical Sciences and Research. 2009, 1(2): 57-63.
56. Yogesh Kumar, Bhupesh Chander Semwal, Jitendra Gupta, Sonia Singh, Mahendra Giri. “Antihyperglycemic activity of roots of *Berberis aristata D.C* in alloxan induced diabetic rats” International Journal of Green Pharmacy. 2009: 259-262.
57. Papiya Mitra Mazumder, Mamta Farswan, V. Parcha. “Effects of an isolated active compound (cg-1) of *Cassia glauca Linn.* Leaf on blood glucose, lipid profile, and atherogenic index in diabetic rats”. Indian journal of Pharmacology 2009, 41 (4): 182-186.
58. Pankaj Kishor Mishra, Grijesh Kumar Mall and Veeru Prakash. “Anti-diabetic activity and hypolipidemic activity of *Gymnema sylvestre* in alloxan induced diabetic rats”. Global Journal of Biotechnology & Biochemistry. 2009, 4(1): 37-42.
59. Nagaraj Puranik, Kararashah Fakruddin Kammar, Sheela Devi. “Anti-diabetic activity of *Tinospora cordifolia (Willd.)* in sterptozotocin diabetic rats; does it act like sulfonylureas?”. Turk Journal of Medical Sciences. 2010, 40(2):265-270.

60. Sravan Kumar Bussa, Jyothi Pinnapareddy. “Anti-diabetic activity of stem bark of *Neolamarckia cadamba* in alloxan induced diabetic rats”. International Journal of Pharmacy technology. 2010(2): 314-324.
61. Reema Dheer, Pradeep Bhatnagar. “A study of the Anti-diabetic activity of *Barleria prionitis Linn’*. Indian Journal of Pharmacology. 2010, 42(2): 70-73.
62. Mekesh S Sikarwan, M B Patil. “Anti-diabetic activity of *Pongamia pinnata* leaf extracts in alloxan induced diabetic rats”. International Journal of Ayurveda Research 2010, 1(4):199-204.
63. V Sivakumar, M Jayanth, N Sowbala, G Rajalakshmi, U Kanagavalli. “Study of antihyperglycemic effect of *Cathranthus roseus* in alloxan induced diabetic rats”. International Journal of Pharmacy and Pharmaceutical Sciences. 2010, 2(4): 114-116.
64. Syed Mohammed Kazim, Mohammed Fazil, Syed Safiullah Ghori, Syeda Sughra Mehjabeen, Shaik Rasheed Ahmed, Shaik Mehboob Ali, Mohammed Ibrahim. “Anti-diabetic activity of *Vinca roseus* extract in alloxan induced diabetic ats”. International Journal of Endocrionology 2010; 1-6.
65. Y. Tanko, M.A. Mabrouk, A.B. Adelaiye, M.Y. Fatihu and K.Y. Musa. “Anti-diabetic and some haematological effects of ethylacetate and n-butanolfractions of *Indigofera pulchra* extract on alloxan induced diabetic Wistra rats”. Journal of Diabetes and Endocrinology, 2011,2(1):1-7.
66. T. Harikant,A. Zabeer, Pooja, K.C. Samanta. “Anti hyperglycemic activity of petroleum ether extract &ethyl acetate extract of *Sarcococca saligna*”. International Journal of Current Pharmaceutical Research 2011, 3 (1): 26-29.
67. Rajeev Kumar Jha, Mangilal, Anil Bhandari, Rajesh Kumar Nema. “Anti-diabetic activity of flower head petroleum ether extracts of *Sphaeranthus indicus Linn*”. Asian Journal of Pharmaceutical and Clinical Research. 2011. 3(1): 16-19.
68. Ranjit Singh, P. H. Rajasree and C. Sankar, “Screening for Anti-diabetic activity of the ethanolic extract of *Barleria cristata* seeds”. International Journal of Pharmacy Life Sciences 3(10):Oct., 2012.
69. Subramaniam Ramachandran,Koikaramparambil Robert Naveen, Baskaran Rajinikanth, Mohammad Akbar, Aiyalu Rajasekaran. “Anti-diabetic, Antihyperlipidemic and in vivo Antioxidant potential of aqueous exyract of

Aniogeissus latifolia bark in type 2 diabetic rats". Asian Pacific Journal of Tropical Diseases 2012,S596-S602.

70. C.N. Ezekwesili, H.A. Ogbunugafor and J.O. Ezekwsili-Ofili. "Anti-diabetic activity of aqueous extract of *Vitex doniana* leaves and chincina calisaya bark in alloxan induced diabetic rats". International Journal of Tropical Diseases &Health, 2012 2(4):290-300.
71. Qamar Uddin Ahmed, Bashar B.S. Dogarai, Mohamed Zaffar Ali M. Amiroydine, Muhammed Taher, Jalifah Latip, Abdulrashid Umar and Bala Y. Muhammed. "Anti-diabetic activity of the leaves of *tetracera indica merr.* (*Dilleniaceae*) in vivo and in vitro". Journal of Medicinal Plants Research. 25 December, 2012. vol. 6(49), pp. 5912-5922.
72. Shetti, R. D. Sanakal and B. Kaliwal. "anti-diabetic effect of Ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice". Asian journal of plant Science and Research, 2012,2(1):11-15.
73. Olfat A. Khalil, Kholoud S. Ramadan, Enas N. Danial, Hanan S, Alnahdi and NAjla O. Ayaz. "Anti-diabetic activity of *Rosmarinus officinalis* and its relationship with antioxidant property". African Journal of Pharmacy and Pharmacology, 15 April 2012, vol. 6(14),pp. 1031-1036.
74. Parminder Nain, Vipin Saini, and Sunil Sharma. "Anti-diabetic activity of *Emblica officinalis* seed extraction sterptozotocin induced type-2 diabetesmellitus in rat" International Journal of Natural Products Science, 2012; Spl Issue 1:135.
75. Rashmi Raghuwanshi and Bharati Jain. "Hypoglycaemic effect of *Pueraria tuberose* tubers in healthy and alloxan diabetic rats " Journal of Chemical, Biological and Physical Sciences Nov.2011-Jan2012, vol.2.no.1, 270-272 E-ISSN:2249-1929.
76. Moses Z Zaruwa, Jiredej Manosroi, Toshihiro Akihisa, Worapaka Manosroi, Aranya Manosroi "Anti-diabetic acivity of *Anogeissus acuminate* a medicinal plant selected from the Thai medicinal plant recepie database MANOSROI II" Wudpecker Journal of Medicinal Plants, October 2012Vol.1(20, pp.O11-018.
77. Kattamnachi Gnanath. Kontham Ramakanth reddy, Gudur Pavan Kumar, Bheemanapally Krishna, Karka Srinivasa Reddy, Avvuri Sanjeeva Kumarn "Evaluation of Anti-diabetic activity in *Corallocarpus epigaeus* rhizomes" International Current Pharamceutical Journal, February 2013, 2(3);53-56.

78. Fatemeh Fathiazad, Sanaz Hamedeyazdan, Mohamad Karim Khsropanah, Arash Khaki "Hypoglycaemic activity of *Fumaria parviflora LSam* in sterptozotocin induced diabetic rats," Advanced Pharmaceutical Bulletin, 2013,3(1), 207-210.
79. Md. Ahsanul haque, Chowdhury Sayef Abdullah, Bilquis Romana, Md. Bodrudoza rafiquel, Gazi Md. Zia-ul-Huda, Sarder Fahim Hossain, Bilkis Begum "Evaluation of Anti diarrhoeal and Anti-diabetic activities of the stem, barks and leaves of the plant *Vernonia cinerea* (Family: *Asteraceae)*" Journal of Applied Pharmaceutical Science, October 2012 vol.3 (1),pp. 069-072.
80. Mitali Ghosal and Palash Mandal "Evaluation of Anti-diabetic activity of *Calamus erectus* in sterptozotocin induced diabetic rats' Asian Journal of Plant Science and Research, 2013, 3 (1): 47-53.
81. R. Sundhararajan and V. Ravichandiran. 'Antiinflammatory activity of *Spermacoe ocymoides Burm F"*. Journal of Pharmacy Research 2012,5(1), 719-721.
82. C.K. Kokate. A.P.Purohit, S.B. Gokhale. 2008. "Pharmacology". Nirali Prakasan, Pune, India. A.1-1.6
83. Anonymous. WWW.OECD.com.
84. Mrs. P.H. Agarkar, Dr.J.S. Kulkarni, Dr. V.L. Maheshwari, Dr. R.A. Fursule. 2008. "Practical Biochemistry". Nirali Prakashan, Pune, India.
85. Misra HP,Fridovich I,1972.The role of superoxide anion in the autooxidation of epinephrine anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase.J Biol Chem 247,3170-3184.
86. Indian pharmacopoeia, Government of India, Ministry of Health & welfare, controller of publications, New Delhi, 1996,Vol 2, A53-54.
87. Jayakumari S, Malarkodi Velraj, Vijayalakshmi A and Arthanarieswaran P. Pharmacognostical studies on the leaves of Pisonia grandis R. Br. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(2),(2011).
88. Johnsen DA. Plant microtechnique; New Work, Mc Graw Hill co, 1940,PP-523.
89. Mayank Gangwar, R. K. Goel, and Gopal Nath *Mallotus philippinensis* Muell. Arg (Euphorbiaceae): Ethnopharmacology and Phytochemistry Review.
90. Indian medicinal plants an illustrated dictionary page no:76
91. Cuong NX et al. A new lignan dimer from Mallotusphilippensis.pubmed ncbi.

92. Chan TK et al. Anti-allergic actions of rottlerin from Mallotus philippinensis in experimental mast cell-mediated anaphylactic models. pubmed ncbi.
93. Hong Q et al. Anti-tuberculosis compounds from Mallotus philippinensis. pubmed ncbi.
94. Kulkarni RR et al. Antifungal dimericchalcone derivative kamalachalcone E from Mallotus philippinensis. pubmed ncbi.
95. Khan H et al. Antioxidant and Antiplasmodial Activities of Bergenin and 11-O-Galloylbergenin Isolated from Mallotusphilippensis. pubmed ncbi.
96. Gangwar M et al. Antioxidant capacity and radical scavenging effect of polyphenol rich Mallotus philippenensis fruit extract on human erythrocytes. pubmed ncbi.
97. Khan M et al. Hexane soluble extract of Mallotusphilippensis (Lam.) Muell. Arg. root possesses anti-leukaemic activity. pubmed ncbi.
98. Gangwar M et al. in-vitro scolicidal activity of Mallotus philippinensis (Lam.) Muell Arg. fruit glandular hair extract against hydatid cyst Echinococcusgranulosus. pubmed ncbi.
99. Goel RK et al. Mallotus philippinensisMuell. Arg (Euphorbiaceae): ethnopharmacology and phytochemistry review. pubmed ncbi.
100. Furumoto T et al. Mallotus philippinensis bark extracts promote preferential migration of mesenchymal stem cells and improve wound healing in mice. pubmed ncbi.
101. Gautam MK et al. Mallotus philippinensisMuell. Arg fruit glandular hairs extract promotes wound healing on different wound model in rats. pubmed ncbi.
102. Oyedemi BO et al. Novel R-plasmid conjugal transfer inhibitory and antibacterial activities of phenolic compounds from Mallotus philippensis (Lam.) Mull. Arg. pubmed ncbi.
103. Chhiber N et al. Rottlerin, a polyphenolic compound from the fruits of Mallotus phillipensis (Lam.) Müll.Arg. pubmed ncbi.
104. Marble A (1971) ‘’Glibenclamide, a new sulfonylureas whither oral hypoglycemic agents, drugs 1 (2) 109-25.
105. A b Serrano-martin x, prayins G, Mendoza Leona (December 2006)

106. Riddle mc (February 2003) ‘’Editorial sulfonylureas differ in effects on ischemic pres conditioning- is it time to retire glyburde J cin endocrine, metab 88 (2): 528-30

107. Okhawa H,Ohigni N,Yagi K,1979.Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction.Anal Biochem95,351-359

108. Reitman, S., Frankel, S.A., 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63.

109. Mrs. P.H. Agarkar, Dr.J.S. Kulkarni, Dr. V.L. Maheswari, Dr. R.A. Fursule. 2008. ‘’Practical Biochemistry’’. Nirali Prakashan, Pune, India.

110. B.Jayaprasad, P.S. Sharavanan et al, Anti-diabetic effect of Chloroxylon swietenia bark extracts on streptozotocin induced diabetic rats, Dept of Botany Annamalai university.

111. Rajaram K et al, Antioxidant and anti-diabetic activity of tectona grandis linnn.in alloxan induced albino rats, Dept of Biotechnology Bharathidasan Institute of Technology Anna university.

112. Reitman, S., Frankel, S.A., 1957. Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol.28, 56-63.

113. Anupama V, Narmadha R, et al, Enzymatic alteration in the vital organs of streptozotocin diabetic rats treated with aqueous extract of erythrina variegate bark, Dept of Biochemistry Karpagam university Coimbatore.

114. Okhawa H, Ohigni N, Yagi K, 1979. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 351-359.

115. Misra HP, Fridovich I, 1972. The role of superoxide anion in the auto oxidation of epinephrine and simple assay of superoxide dismutase.J Biol Chem 247,3170-3184

116. Sinha AK, Colorimetric assay of catalase, Analytical Biochemistry, 1972, 47:2:389-394.

117. Halim Eshrat M et al, Effect of ocimum sanctum (Tulasi) and vitamin E in biochemical parameters and retinopathy in streptozotocin induced diabetic rats, Dept of laboratory medicine, All India Institute of Medical Science New Delhi.

118. Bhesh Raj Sharma, et al, Nelumbo nucifera leaf extract attenuated pancreatic β-cell toxicity induced by interleukin-1β and interferon- ý, and increased insulin secretion of pancreatic β-cell in streptozotocin-induced diabetic rats.

119. Stephen O. Owa PhD, Adeolu A. Taiwo, BSc, et al, The biochemical effect of lime concentrate Aporo and mucuana puriens seed extract on alloxan- induced diabetic rats, Biochemistry university Dept of Biological Science Landmark University Omu-Aran,Nigeria.

120. Lachin T, Reza H. Anti-diabetic effect of cheries in alloxan induced dibetes rats. Resent patent on endocrine. Metab Immune Drug Discov 2012; 6: 67-72.

121. Ellaman GL. Tissue sulfhydryl groups. Arch Biochem Biophy 1959; 179(4073): 70-77.

122. Yeh GY et al., ‘’systemic review of herbs and dietary supplements for glycemic control in diabetes’’ diabetes care 26:1277-1294.

123. Consensus committee. Consensus statement on the world wide standardization of the hemoglobin measurement:the American diabetes association, European association for the study of diabetes, international federation of clinical chemistry and laboratory medicine , and the international diabetes federation diab care 2007;30:2399-400.

124. Shirwaikar A et al., ‘’Anti diabetic activity of alcoholic stem extract of coscinium fenestaratum in STZ induced type 2 diabetic rats ethnopharmacol 2005;97:369-74

125. Ananthan R et al., ‘’Effect of gymnema montanum leaves on serum and tissue lipids in alloxan induced diabetes exp diabetesres 2003:4:183-189.

126. Carlos Roberto porto dechandt et al, ‘’Combretum lanceolatum flowers extract shows anti-diabetic activity through activation of AMPK by quercetin’’ 23 (2). 291-300 Apr,2013.

127. Karuppusamy Arunachalam et al, ‘’Anti-diabetic activity of aqueous root extract of merremia tridentate in streptozocin induced diabetics rats’’ (2012) 175-179.

128. P. Daisy et al, ‘’ A novel steroid from elephantopus scaber an etnomedicinal plant with anti-diabetic activity 16 (2009) 252-257.

129. Snehal S et al., ‘’Antihyperglycemic, antihyperlipidemic and antioxidant effects on dihar, a polyherbal formulation, in STZ induced diabetes Indian journal of experimental biology 2009;47:41:1-15.

130. Lee SE et al., ‘’Free radical scavenging and antioxidant enzyme fortifying activities of extract from smilax china roots’’ expt.mol.med 2001;34:263-268.

131. Ali SS et al., “Indian medicinal herbs as sources of antioxidant.” Food research international 2008; 41: 1-15.

132. Lubec B et al., ”Brain lipid peroxidation and the hydroxyl radical attack following the IV infusion of hydrogen peroxide in an infant.” Free rad biol.Med 1996; 21:219-233.

133. Lu SC. “Regulation hepatic glutathione synthesis: current concept and controversies”. Faseb J 1999;6:69-83.

134. Reshmi CR et al., “Antioxidant activity of albizzia lebbeck benth inn alloxan diabetic rats”. Indian J physiol pharmacol 2006;50:297-302.

135. Irshad M et al., “Oxidant-antioxidant system: roll and significant in human body”. Indian J exp biol 2002;40:1233-1239.

136. Madhu CG et al., ”Antioxidant status of STZ induced diabetuic rats”. Indian J biol 1996;34:264-266.

137. Ragavan B et al., ” Effect of terminalia arjuna stem bark on antioxidant status in liver and kidney of alloxan diabetic rats”. Indian J physiol pharmacol 2006;50:133-142.

138. Chatergy IB et al., “Ascorbic acid- a scavenger of oxy radicals”. Indian J physiol pharmacol 1991;28:233-236.

139. Venkateswaran S et al., “Effect of coccinia indica leaves on antioxidant status in streptozotocin induced diabetic rats”. J ethnopharmacol 2003;84:163-168.