# ANTI-HYPERGLYCEMIC AND ANTI-HYPERLIPIDIMIC POTENTIAL OF A POLYHERBAL PREPARATION WITH A TYPE-2 DIABETES

**A Dissertation Submitted to the Sambalpur University in Partial Fulfilment of the Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**IN**

**BIOTECHNOLOGY**

**by**

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**Thesis Submitted for the award of**

**Doctor of Philosophy in Science (Biotechnology)**


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# DECLARATION

*I hereby declare that the matter embodied in the thesis entitled* ***"Anti-hyperglycemic and anti-hyperlipidemic potential of a polyherbal preparation with type-2 diabetes"*** *the result of investigations carried out by me at Department of Biotechnology & Bioinformatics, Sambalpur University, Jyoti Vihar, Odisha under the Joint supervision of Professor Pradeep Kumar Naik, Department of Biotechnology & Bioinformatics, Sambalpur University and Dr. Manas Ranjan Naik, Associate Professor, Dept. of Pharmacology, GMCH Sundargarh, Odisha. The results of the investigation have not been submitted either in part or full for the award of any other degree or diploma in this institute or any other institute or university.*

*In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whatever the work described is based on the findings of other investigators. Any omissions there in may have been occurred by oversight or error in judgment is regretted.*

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# CERTIFICATE

This is to certify that the research work entitled, "Anti- hyperglycemic and anti-hyperlipidemic potential of a polyherbal preparation with type- 2 diabetes" submitted by Deepali Naik bearing registration no: 232/2016/Bio-Tech in partial fulfilment for the degree of Doctor of Science at Sambalpur University, Orissa, India is a bonafide record of her original work carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for any degree or diploma. We recommend this thesis in fulfillment of the award of the degree of **Doctor of Philosophy in Biotechnology.**

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# ABSTRACT OF THE DISSERTATION

The search for an effective drug, alone or in combination for the treatment of diabetes remains elusive. Polyherbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for the treatment and management of Type-2 diabetes. In the present study, a polyherbal formulation was developed consisting of plant materials from 15 medicinal plants collected from the Gandhamardan such as Tinospora cordifolia, Gymnema sylvestre, Mangifera indica, Syzygium cumimi, Terminalia arjuna, Curcuma longa, Alianthu sexcelsa, Andrographis paniculate, Withania somnifera, Caeselpinia bonduc, Swertia chirayita, Holarrhena pubescens, Citrus lemon, Plumeria alba & Murraya koenigi). The methanolic extract of this polyherbal formulation was used for the in vitro and in vivo evaluation of its antidiabetic potential.

The total flavonoids and phenols content of the polyherbal formulation were determined. The total Flavonids content of the polyherbal formulation was found to be 6.545 ± 0.048 mg/g. Whereas, the total phenol content of the polyherbal formulation was found to be 11.071 ± 0.184 mg/g. The results revealed high content of both flavonoids and phenols. The antioxidant activity of the polyherbal formulation was investigated based on DPPH (2,2-diphenyl -1-picryl- hydrazylhydrate) free radical, ferric reducing ability of plasma (FRAP) and ABTS radical scavenging assay. The highest antioxidant activity of 92.22% was found in the polyherbal formulation based on DPPH assay. Similarly, the reducing antioxidant activity of 4.84±0.0487% was noted for the crude extract of polyherbal formulation based on FRAP assay. The high antioxidant activity was due to the presence of high amounts of phenols and flavonoids. The in vitro antidiabetes activity of the extract was investigated based on inhibition of α-amylase & α-glucosidase enzyme. The maximum inhibition of 56.21% for α-amylase was obtained at a concentration of 100 μ g/ml solution of polyherbal formulation. In contrast, the standard drug, ascorbase has an inhibition of 88.92% at a concentration of 100 μ g/ml. The $IC_{50}$ value of α-amylase activity for ascorbase and polyherbal extract was found to be 39.086 μg/ml & 6.195 μg/ml, respectively. Similarly the effective inhibition of α-glucosidase with the treatment of standard and polyherbal extracts were carried out. The maximum percentage of inhibition of α-glucosidase assay was found to be 76.42 % at a concentration of 100 μg/ml of polyherbal extract. The standard drug ascorbase was shown a maximum inhibition of 81.91% of at a concentration of 100 μg/mol. From the

result it was revealed that the polyherbal extract effectively inhibit the action of a α-amylase and α- glucosidase enzyme.

The marker components in the polyherbal formulation methanol extract were identified and confirmed by their respective mass ion, fragmentation pattern, offline and online mass spectral database, and related literature. Data acquisitions were executed under positive (+ve) and negative (−ve) mode of ionization utilizing a full spectrum scan. A total of 17 major compounds such as Cordifolide A, Gymnemagenin, Naringenin 4'-glucoside, 5-hydroxy-2-methyl-9 dihydroxyphenyl)-5,7 -dihydroxy-3,4- dihydro-2h-1-benzopyran-3-yl]oxy}butanedioic acid, Mangiferin, Pterocarpol A, 3,7,4'-Trihydroxy flavonone, -({[(2r,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy} methyl)-8h,11h-oxepino [2,3-h] chromen-4-one, (2r)-2-{[(2r,3r)-2-(3,4- Cernuine, Curcumin-L, 2-O-β-d-glucosyloxy-4-methoxy benzenepropanoic acid, and 14- Deoxy-11,12-didehydroandrographolide were identified from the polyherbal formulation using LC-HRMS.

The efficacy of the polyherbal formulation in reducing the serum blood glucose level was determined using in vivo diabetic animal model. Wistar rats were divided into four different groups, each are having six animals. Diabetes was induced in overnight, fasted rats by administrating a single intraperitoneal (i.p.) injection of freshly prepared alloxan with a single dose of 100mg/kg BW. Diabetes was confirmed in the alloxan treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of alloxan injection, the rats were given 5% w/v of glucose solution to prevent the mortality. The rats were fasted overnight, collection of blood samples and sera glucose determination were drawn from their tail tips. Sera glucose estimation was done by one touch electronic glucometer using glucose test strips, and the glucose level more than 250 mg/dl was used for the study. As per different groups of animal study (**Group I:** Non diabetic normal control rats, **Group II:** Negative control, **Groups III:** Diabetic rats administered with standard drug metformin (50 mg), **Groups IV:** Diabetic rats administered with standard drug voglibose (1 mg), and **Groups V:** Diabetic rats was given the polyherbal extract of 250 mg/kg BW. Blood glucose level of diabetic animals decreased tremendously from Day I to Day 10 from 497 to 101 with the Polyherbal Treated group (Unit mg/dL)) as compared to normal untreated group and Metformin Treated group. At the end of the experiment day- 14, the rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and analysed. The polyherbal formulation also reduced elevated levels of selected boichemical parameters and

prevented other complications of hyperglycemia. These findings provide scientific evidence for antidiabetes use of a traditional formulation and suggest that administration of polyherbal formulation to alloxan/metformin induced diabetes rats, in a dosage used safely by humans, reduces the production of various diabetes causing biochemical parameters and it may prevent the development of type-2 diabetes in established animal models.

The acute toxicity study of the polyherbal formulation was carried out on Winstar rats as per the Organization for Economic Co-operation and Development (OECD) guidelines. Wistar rats were used for the study and fasted for 12 hours prior to dosing. Each animal was given a single dose of the polyherbal extract 5000 mg/kg body weight. After dosing they were observed first 30 mins for any behavioural changes, then were observed for another 24 hours and 72 hours subsequently. the polyherbal formulation was found non-toxic at a concentration >5000mg/kg body weight. The animals were divided into four groups normal control, and three treatment groups, each containing three animals. Group-I was set as control and this group was given normal food and water. The other three groups received doses of 150mg/kg b.w/day, 250mg/kg b.w/day, and 500mg/kg b.w/day, respectively. The weight of the animals was measured daily and their behavioural and morphological changes were observed on the 28th day of treatment. The animals were anesthetized in the anaesthesia chamber containing isoflurane. Cardiac puncture was performed to collect blood sample and was analysed, histopathological study was done for different organs. The histopathology and haemetology study revealed no toxicity to the vital organs and organ function among the treated and control untreated groups.

Therefore, we strongly believe that the polyherbal formulation developed in the study has a greater potential as for the treatment and management of type-2 diabetic without any side effects.

# *"Dedicated To My Parents"*

*My loving husband and my cutest Purbi and Punya*

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My foremost thanks go to my research supervisor and mentor **Prof. Pradeep Ku. Naik**, Professor, Department of BT and BI, Sambalpur University for his constant support, guidance, encouragement, and for allowing me the freedom to pursue my interests. Under his guidance I not only learned how to conduct research, but also how to communicate my ideas. I have learned a great deal from interacting with him.

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I thank all those, whose names I cannot remember at this moment, but have contributed in their own way to the completion of the present work.

**(Deepali Naik)**

**Date:**

# ABBREVIATIONS

ABTS - 2, 2'-azino-bis (3- ethylbenzthiazoline-6-sul-phonic acid

AD - Anno Domini

ADP - Adenosine diphosphate

$AgNO_3$ - Silver nitrate

AgNp - Silver nanoparticle

ALP - Alkaline phosphatase

ALT - Alanine transaminase

AMP - Adenosine monophosphate

AOAC - Association of Official Analytical Chemists

AST - Aspartate transaminase

ATP - Adenosine triphosphate

AYUSH - Ayurvedic, Yoga and Naturopathy, Unani, Siddha and Homeopathy

BC - Before Christ

bw - Body weight

CAT - Catalase

COPD - Chronic obstructive pulmonary disease

COSY - Correlation Spectroscopy

CTC - Circulating tumor cell

CVD - Cardiovascular disease

DAG - Diacyl glycerol

DEPT - Dynamic Excitation of Proton Transitions

DM - Diabetes mellitus

DNA - Deoxy ribonucleic acid

DPP-4 - Dipeptidyl peptidase-4

DPPH - 2,2-diphenyl-1-picrylhydrazyl

D.T - Disintegration time

$EC_{50}$ - Half maximal effective concentration

EDTA - Ethylenediamine tetra acetic acid

EDX/EDAX - Energy Dispersive X-Ray Spectroscopy

FRAP - Ferric reducing ability of plasma

FT-IR - Fourier Transform Infrared Spectroscopy

GAE - Gallic acid equivalent

GC-MS - Gas chromatography – mass spectrometry

GDM - Gestational diabetes mellitus

GLP-1 - Glucagon-like peptide-1

GLUT - Glucose transporter

GLUT1 - Glucose transporter 1

GLUT4 - Glucose transporter type 4

Hb - Hemoglobin

HbA1c - Glycated hemoglobin

HCl - Hydrochloric acid

HDL - high density lipoprotein

HIV - Human immunodeficiency virus

HMP shunt - Hexose monophosphate shunt

HPLC - High Performance Liquid Chromatography

HPTLC - High Performance Thin Layer Chromatography

HRBC - Human red blood cell

HRESIMS - High resolution mass spectra

$IC_{50}$ - Inhibitory concentration

ICH - International Council for Hormonisation

ICP-MS - Inductively Coupled Plasma- Mass Spectrometry

ICP-OES - Inductively Coupled Plasma- Optical Emission Spectrometry

IDDM - Insulin-dependent diabetes mellitus

IFG - Impaired fasting glycemia

IGT - Impaired glucose tolerance

IP - Indian Pharmacopoeia

IR - Infrared Spectroscopy

IRS - Insulin receptor substrate

KBr - Potassium bromide

kg - Kilogram

LC-MS - Liquid chromatography-mass spectrometry

$LD_{50}$ - Lethal dose

LDL - Low density lipoprotein

LOX enzyme - Lipoxygenase enzyme

MAP kinase - Mitogen-activated protein kinase

MIC - Minimum Inhibitory Concentration

MCC - Microcrystalline cellulose

MCH - Mean corpuscular hemoglobin

MCHC - Mean corpuscular hemoglobin concentration

MCV - Mean corpuscular volume

MDR - Multi drug resistance

MIC - Minimum Inhibitory Concentration

mg - Milligram

MODY - Maturity onset diabetes of young

MS - Mass spectrometry

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

$N_2O_3$ - Dinitrogen trioxide

NA - Nicotinamide

NAD - Nicotinamide adenine dinucleotide

NDDG - National Diabetes Data Group

NIDDM - Non-Insulin- dependent diabetes mellitus

NMN - Nicotinamide mono nucleotide 1

DNMR - One Dimensional Nuclear magnetic resonance

NO - Nitric oxide

NOESY - Nuclear Overhauser Effect Spectroscopy

OECD - Organisation for Economic Co-operation and Development

ONOO - Peroxynitrite

PBMC - Peripheral blood mononuclear cells

PC-3 cell line - Human prostate cancer cell line

PCV - Packed cell volume

PHF - Polyherbal formulation

PPAR-ϒ - Peroxisome proliferator-activated receptor gamma

RA - Rheumatoid arthritis

RBC - Red blood cell

RH - Relative humidity

SEM - Scanning Electron Microscopy

SO - Superoxide

SOD - Superoxide dismutase

STZ - Streptomycin

STZ-NA - Streptomycin-Nicotinamide

TEM - Transmission Electron Microscopy

TLC - Thin Layer Chromatography

TRP-1 - Tyrosinase-related protein 1

TRP-2 - Tyrosinase-related protein 2

TINEL - Terminal deoxynucleotidyl transferase dUTP nick and labeling

UPLC-MS - Ultra performance liquid chromatography – mass spectrometry

USP-NF - United States Pharmacopeia – National Pharmacopeia

UV - Ultra Violet UV-VIS - Ultra Violet-visible spectroscopy

VLDL - Very low-density lipoprotein

WBC - White blood cell

WHO - World Health Organization

XRD - X- Ray diffraction

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# ABSTRACT

*The search for an effective drug, alone or in combination for the treatment of diabetes remains elusive. Polyherbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for the treatment and management of Type-2 diabetes. In the present study, a polyherbal formulation was developed consisting of plant materials from 15 medicinal plants collected from the Gandhamardan such as Tinospora cordifolia, Gymnema sylvestre, Mangifera indica, Syzygium cumimi, Terminalia arjuna, Curcuma longa, Alianthu sexcelsa, Andrographis paniculate, Withania somnifera, Caeselpinia bonduc, Swertia chirayita, Holarrhena pubescens, Citrus lemon, Plumeria alba & Murraya koenigi). The methanolic extract of this polyherbal formulation was used for the in vitro and in vivo evaluation of its antidiabetic potential.*

*The total flavonoids and phenols content of the polyherbal formulation were determined.* The total Flavonids content of the polyherbal formulation was found to be 6.545 ± 0.048 mg/g. Whereas, the total phenol content of the polyherbal formulation was found to be 11.071 ± 0.184 mg/g. The results revealed high content of both flavonoids and phenols. *The antioxidant activity of the polyherbal formulation was investigated based on DPPH (2,2-diphenyl -1-picryl-hydrazylhydrate) free radical, ferric reducing ability of plasma (FRAP) and ABTS radical scavenging assay.* The highest antioxidant activity of 92.22% was found in the polyherbal formulation based on DPPH assay. Similarly, the reducing antioxidant activity of 4.84±0.0487% was noted for the crude extract of polyherbal formulation based on FRAP assay. The high antioxidant activity was due to the presence of high amounts of phenols and flavonoids. *The in vitro antidiabetes activity of the extract was investigated based on inhibition of α-amylase & α-*

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*were divided into four different groups, each are having six animals. Diabetes was induced in overnight□fasted rats by administering a single intraperitoneal (i.p.) injection of freshly prepared alloxan with a single dose of 100mg/kg BW. Diabetes was confirmed in the alloxan treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of alloxan injection, the rats were given 5% w/v of glucose solution to prevent the mortality. The rats were fasted overnight, collection of blood samples and sera glucose determination were drawn from their tail tips. Sera glucose estimation was done by one touch electronic glucometer using glucose test strips, and the glucose level more than 250 mg/dl was used for the study. As per different groups of animal study (**Group I:** Non diabetic normal control rats, **Group II:** Negative control, **Groups III:** Diabetic rats administered with standard drug metformin (50 mg), **Groups IV:** Diabetic rats administered with standard drug voglibose (1 mg), and **Groups V:** Diabetic rats was given the polyherbal extract of 250 mg/kg BW. Blood glucose level of diabetic animals decreased tremendously from Day I to Day 10 from 497 to 101 with the Polyherbal Treated group (Unit mg/dL)) as compared to normal untreated group and Metformin Treated group. At the end of the experiment day-14, the rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and analysed. The polyherbal formulation also reduced elevated levels of selected boichemical parameters and prevented other complications of hyperglycemia. These findings provide scientific evidence for antidiabetes use of a traditional formulation and suggest that administration of polyherbal formulation to alloxan/metformin induced diabetes rats, in a dosage used safely by humans, reduces the production of various diabetes causing biochemical parameters and it may prevent the development of type-2 diabetes in established animal models.*

*The acute toxicity study of the polyherbal formulation was carried out on Winstar rats as per the Organization for Economic Co-operation and Development (OECD) guidelines. Wistar rats were used for the study and fasted for 12 hours prior to dosing. Each animal was given a single dose of the*

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*Therefore, we strongly believe that the polyherbal formulation developed in the study has a greater potential as for the treatment and management of type-2 diabetic without any side effects.*

# CHAPTER-1

# INTRODUCTION

# Introduction

Type-2 diabetes mellitus is a major global health problem associated with excess morbidity and mortality. As the prevalence of this metabolic disorder is rapidly increasing and current treatment fails to stabilize the disease in most patients, prevention should be considered as a key objective in the near future (Scheen AJ, 2003). Type-2 diabetes is a progressive disease resulting from defects in the action and/or secretion of insulin (Power A & Alessio D, 2011). People who develop type-2 diabetes pass though a phase of impaired states of glucose regulation. Impaired fasting glucose (IFG) and Impaired glucose tolerance (IGT) represent intermediate states that exist between normal glucose homeostasis and diabetes (Ramachandran A et al., 1986). The progression of impaired glucose tolerance to diabetes is a time-dependent phenomenon. The transition from the early metabolic abnormalities that precede diabetes to frank diabetes may take many years; however, current estimates indicate that most individuals, perhaps up to 70% with these IGT states eventually develop diabetes. In the Indian population, the only longitudinal data showed that in 107 subjects with IGT after 2-8 years of follow-up, 32% of subjects still had IGT, 32% had reverted to normal glucose tolerance (NGT) and 36% had developed diabetes. Any intervention in the impaired glucose tolerance phase that reduces insulin resistance or protects the β cells or both should prevent or delay progression to diabetes.

The anti-hyperglycemic and anti-hyperlipidemic potential of a polyherbal preparation in the context of type-2 diabetes suggests that the herbal formulation may have beneficial effects in managing blood sugar levels and lipid profiles in individuals with diabetes.

**Polyherbal Preparation:** A polyherbal preparation refers to a medicinal formulation that contains a combination of multiple herbs. The use of multiple herbs in a single preparation is often based on the idea that the synergistic effects of different plant compounds may enhance therapeutic outcomes.

**Anti-Hyperglycemic Potential:** "Anti-hyperglycemic" means the ability to lower high blood sugar levels. In the context of type-2 diabetes, maintaining blood glucose levels within a normal range is crucial for managing the condition.

**Anti-Hyperlipidemic Potential:** "Anti-hyperlipidemic" refers to the ability to lower elevated levels of lipids (fats) in the blood. People with type-2 diabetes often have imbalances in lipid metabolism, leading to elevated levels of cholesterol and triglycerides, which can contribute to cardiovascular complications.

**Type-2 Diabetes:** Type-2 diabetes is a metabolic disorder characterized by insulin resistance and elevated blood sugar levels. Managing diabetes involves various approaches, including lifestyle modifications, medications, and, potentially,

complementary and alternative therapies such as herbal preparations.

**Mechanism of Action:** The polyherbal preparation likely contains bioactive compounds with properties that contribute to its anti-hyperglycemic and anti-hyperlipidemic effects. These may include compounds that enhance insulin sensitivity, regulate glucose metabolism, and influence lipid synthesis and metabolism.

There are several classes of drugs used for the treatment of type-2 diabetes. The choice of medication depends on various factors, including the individual's health status, preferences, and the presence of other medical conditions. It's important for individuals with type-2 diabetes to work closely with their healthcare providers to determine the most appropriate treatment plan. Some common classes of drugs used for the treatment of type-2 diabetes:

- **Metformin:** Metformin is often the first-line medication for type-2 diabetes. It works by reducing glucose production in the liver and improving insulin sensitivity in peripheral tissues.
- **Sulfonylureas:** Examples include glipizide, glyburide, and glimepiride. These drugs stimulate the pancreas to release more insulin.
- **Meglitinides:** Repaglinide and nateglinide are examples. They also stimulate insulin release from the pancreas but have a shorter duration of action than sulfonylureas.
- **Dipeptidyl Peptidase-4 (DPP-4) Inhibitors:** Sitagliptin, saxagliptin, and linagliptin are examples. These drugs

increase insulin release and decrease glucagon secretion, helping to control blood sugar levels.

- **Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists:** Exenatide, liraglutide, and dulaglutide are examples. They mimic the effects of GLP-1, a hormone that stimulates insulin release, suppresses glucagon secretion, and slows gastric emptying.
- **Sodium-Glucose Co-Transporter-2 (SGLT2) Inhibitors:** Canagliflozin, dapagliflozin, and empagliflozin are examples. They work by inhibiting glucose reabsorption in the kidneys, leading to increased glucose excretion in urine.
- **Thiazolidinediones (TZDs):** Pioglitazone and rosiglitazone are examples. These drugs improve insulin sensitivity in peripheral tissues.
- **Insulin:** Insulin therapy may be prescribed when other medications are not sufficient to control blood sugar levels. There are various types of insulin, including short-acting, intermediate-acting, and long-acting, and they can be used in different combinations.
- **Combination Medications:** Some medications combine two or more classes of drugs to provide a dual or triple action in controlling blood sugar levels. For example, metformin may be combined with a sulfonylurea or a DPP-4 inhibitor.

The choice of medication depends on factors such as the individual's overall health, the presence of complications, and potential side effects. It's crucial for individuals with type-2 diabetes to adhere to their prescribed treatment plan, monitor blood sugar levels regularly, and work closely with their

healthcare team to adjust their medication regimen as needed. Lifestyle modifications, including a healthy diet and regular exercise, are also essential components of managing type-2 diabetes.

The primary objective of the research is to evaluate the potential of a polyherbal preparation in managing two critical aspects of type-2 diabetes: hyperglycemia (high blood sugar levels) and hyperlipidemia (elevated lipid levels in the blood). The intervention under investigation is a polyherbal preparation, indicating that it contains a combination of multiple herbs. These herbs may have been selected based on traditional medicinal knowledge or previous evidence suggesting their potential anti-diabetic and lipid-lowering properties.

The study likely explores how the polyherbal preparation affects blood glucose levels in individuals with type-2 diabetes. Anti-hyperglycemic effects refer to the ability to lower elevated blood sugar levels, a key concern in diabetes management. In addition to addressing hyperglycemia, the research investigates the impact of polyherbal preparation on hyperlipidemia. This aspect focuses on reducing elevated lipid levels in the blood, such as cholesterol and triglycerides, which are commonly associated with diabetes-related complications. The study likely involves individuals diagnosed with type-2 diabetes, as the title specifically mentions its relevance to this diabetes subtype. This is important as type-2 diabetes is characterized by insulin resistance and often requires a combination of lifestyle modifications and medications for effective management.

# *CHAPTER-2*

# REVIEW OF LITERATURE

## 2.1 Definition and Classification of Diabetes Mellitus:

The World Health Organization (WHO), in consultation with an expert committee of the American Diabetes Association (ADA), has recommended new classification and diagnostic criteria for diabetes mellitus.

According to American Diabetes Association JAN 2014, "Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, heart, and blood vessels (American Diabetes Association, 2014).

Although a number of nomenclature and diagnostic criteria were proposed for diabetes, no generally accepted systemic categorization existed until the National Diabetes Data Group (NDDG) classification system was published in 1979 (National Diabetes Data Group, 1979). The WHO Expert committee on Diabetes in 1980 and later the WHO study group on Diabetes mellitus endorsed the substantive recommendations of the NDDG (World Health Org, 1985). These groups recognize two major form of diabetes but their classification system went on to include evidence that diabetes mellitus was an etiologically and clinically heterogenous group of disorders that share hyperglycemia in common (Diabetes Care, 1997 & 2003; LL Bruton et al., 2011).

## 2.2 Diagnostic Criteria:

For decades the diagnosis of diabetes has been based on glucose criteria either the Fasting Plasma Glucose (FPG) or the 75mg Oral Glucose Tolerance Tests (OGTT). In 1997, the first expert committee on Diagnosis and classification of Diabetes

mellitus revised the diagnosis criteria. The revised criteria for diagnosing DM have been issued by consensus panels of experts from American Diabetes Association and World Health Organiztions. The revised criteria reflect the new epidemiological and metabolic evidences.

Broad categories of glucose homeostasis as defined by the fasting blood glucose level following an oral glucose challenge include (LL Bruton et al., 2011):

**Table-2.1-** American Diabetes Association has recommended the following diagnostic criteria.

| | |
|---|---|
| Normal Glucose homeostatis | Fasting plasma glucose <5.6 mmol/L (100mg/dL) |
| Impaired Fasting Glucose (IFG) | 5.6 – 6.9 mmol/L (100-125 mg/dL) |
| Impaired Glucose Tolerance (IGT) | Glucose level between 7.8 – 11.1 mmol/L (140-199 mg/dL) 120 minutes after ingestion of 75 gm liquid glucose solution |

A person is diagnosed as diabetic if his/her HbA1c $\geq$ 6.5%. The test should be performed in the laboratory using a method that is NGSP certified and standardized to DCCT assay and FPG $\geq$ 126 mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours. Also, another diagnostic criteria for diabetes mellitus is 2hr plasma glucose $\geq$ 200 mg/dl (11.1 mmol/L, (Diabetes Care, 2014) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 mg of anhydrous glucose dissolved in water. Otherwise, diabetes mellitus is diagnosed in a patient with classic symptoms of hyperglycemia or hyperglycemic

crisis, with a random plasma glucose ≥ 200 mg/dl (11.1 mmol/L).

**Diagnosis of Gestational Diabetes Mellitus (GDM) (**Genuth S et al., 2003)

The diagnosis of GDM is made when any of the following plasma glucose values are exceeded:

Fasting ≥ 92 mg/dl (5.1 mmol/L)

1 hr ≥ 180 mg/dl (10.0 mmol/L)

2 hr ≥ 153 mg/dl (8.5 mmol/L)

Perform a 75 OGTT with plasma glucose measurement fasting and at 1 hr and 2 hr at 24-28 weeks of gestation in women not previously diagnosed with overt diabetes. The OGTT should be performed in the morning after an overnight fast of at least 8 hours (World Health Organization, 1980).

**Metabolic abnormalities:**

Type-2 DM individuals are characterized by (1) defects in insulin secretion (2) insulin resistance involving muscle, liver and adipocyte and (3) abnormalities in splanchnic glucose uptake (De Fronzo RA, 1997; Mc Garry JD, 2001).

**2.3 Pathophysiology:**

In the natural history of T2DM, individuals progress from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to overt T2DM and this progression has been demonstrated in populations of diverse ethnic groups (Ferrannini E, 2004). It is widely recognized that both insulin resistance and β cell dysfunction are important in the pathogenesis of glucose intolerance in populations with a high prevalence of T2DM, insulin resistance is well established long before the development of any impairment in glucose homeostatis as long as β cell can secret sufficient amounts of insulin (Figure 2.1) to

offset the severity of insulin resistance, glucose tolerance remains normal (Defronzo RA, 2009). This dynamic interaction between insulin secretion and insulin resistance is essential to the maintenance of NGT (Normal Glucose Tolerance) and interruption of this cross-talk between β cell and peripheral tissues results in the progressive deterioration of glucose homeostatis (Gastaldelli A et al., 2004).

Early in the development of T2DM, the intial burst of insulin release in response to food intake is compromised allowing post prandial hyperglycemia to develop.

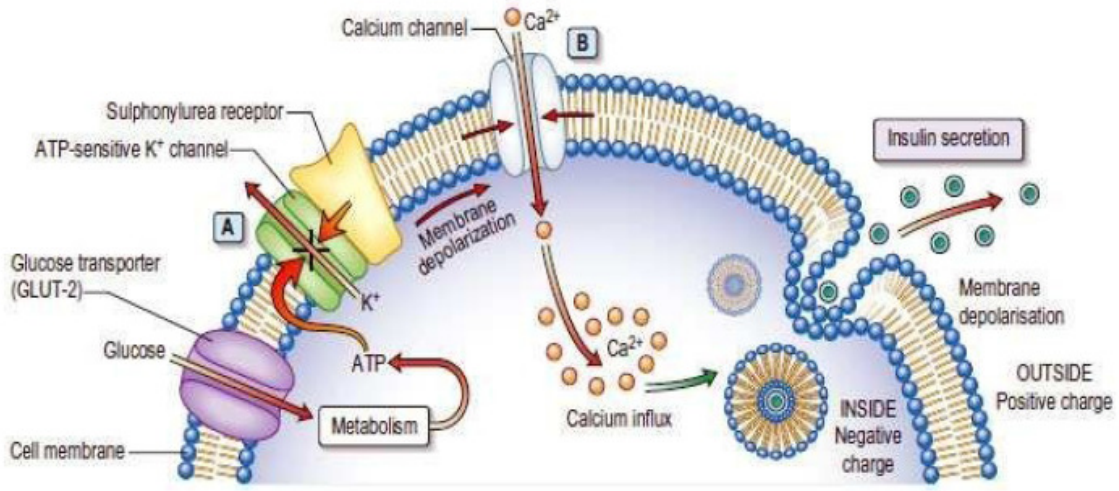



**Figure-2.1 Secretion of Insulin in β cells**

**In the Fasting State** the suppression of insulin and stimulation of glucagon production control the concentration of blood glucose. These processes allow the liver to mobilize glucose from its glycogen stores and synthesize glucose from amino acids and pyruvate (gluconeogenesis). In addition, when insulin levels are low the uptake of glucose by muscle is minimized and adipocytes release free fatty acids (FFA) (Stuart R et al., 1996).

**In the Fed State** insulin is released in two phases a short, small burst released on food intake or an increase in plasma glucose concentration and decreases postprandial glucose elevation. Later a more sustained, second-phase insulin release directly proportional to plasma glucose elevations occurs. In response to this biphasic release of insulin, the liver and muscles take up glucose, converting it to glycogen and adipose tissues also take up glucose, and store it as triglycerides (Gastaldelli A et al., 2004; Stuart R et al., 1996).

In addition to muscle, liver and β cells (triumvirate), adipocytes (Accelerated lipolysis), gastrointestinal tract (incretin deficiency/resistance) (Word WK et al., 1984; Quddusi S et al., 1988; Nauck MA et al., 2011; Stefater MA et al., 2012; Saeidi N et al., 2013), α cells (hyperglucagonemia), kidneys (increased glucose absorption) and brain insulin resistance and neurotransmitters dysregulation plays an important role in the development of glucose intolerance in T2DM individuals (Stuart R et al., 1996; Groop LC et al., 1989; Knop FK et al., 2007; Nauk MA et al., 2011). Collectively, these eight players comprise the ominous octet (Baron AD et al., 1987; Matsuda M et al., 2002; Holst JJ 2005; Abdul Ghani MA et al., 2011).

Individuals destined to develop T2DM inherit genes that make their tissues resistant to insulin (Schern Thaner G et al., 2010). In the liver insulin resistance is manifested by glucose production during the basal state despite fasting hyperinsulinemia and impaired suppression of hepatic glucose production (HGP) by insulin as occurs following a meal (Ferranni E et al., 1988; Groop LC et al., 1989). In muscles insulin resistance is manifest by impaired glucose uptake after

carbohydrate ingestion, resulting in post-prandial hyperglycemia (Pendergrassg M et al., 2007).

In addition to the triumvirate (β cell failure and insulin resistance in muscle and liver) at least five other pathophysiological abnormalities contribute to glucose intolerance in T2DM (Mogensen CE et al., 1971; Matsuda M et al., 1999; Obici S, et al. 2001; Obici S et al., 2002).

**2.3.1. Impaired Pancreatic Insulin Secretion:**

The β cell dysfunction in diabetics falls into two distinct types,

(a) The pulsatile insulin delivery is lost even when the glucose tolerance is normal and

(b) The loss of compensatory mechanisms, which include increased β cell mass, quantitative insulin output and maximum secretory capacity (V. Seshah et al., 2013).

The normal fasting insulin level is between 5 and 15 mu/ml. It may be low (<5 mu/ml) in subjects with high insulin sensitivity and elevated (>15 mu/ml) in insulin resistant subjects (V. Seshah et al., 2013).

The insulin secretion following a glucose load shows biphasic response. The first phase of acute insulin response (AIR) is due to the release of insulin stored in the granules, which suppresses the hepatic glucose output. This occurs within 4-5 minutes and returns to normal within 10 minutes. The second phase is a response to the ambient rise in glucose level, which promotes the disposal of glucose in peripheral tissues (muscle and adipose tissues).

In T2DM subjects, the ultradian oscillations of insulin delivery is no longer present and the first phase of insulin release is lost (Figure 2.2).

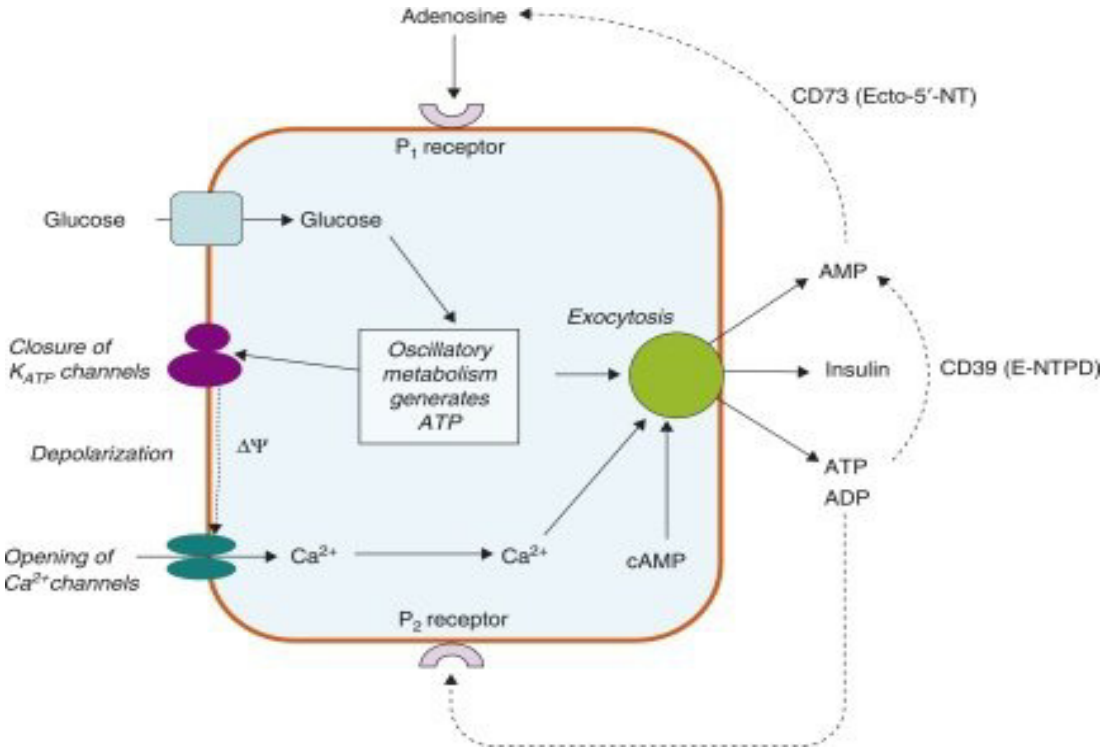



**Figure 2.2.** Oscillation of cytoplasmic ATP generate pulsatile release of insulin from a β-call.

**2.3.2. β cell Dysfunction** (Quddusi S et al., 1984; Rahier J et al., 2008; Jurgens CA et al., 2011; Nauck MA et al., 2011; )

The β cell mass is mildly reduced especially when obesity is taken into account (O Rahilly SP et al., 1986; Dula SB et al., 2010; Nicole LE et al., 2013). Amyloid deposits are frequently observed in the islets. Morphologically islets appear normal and insulinitis is never present. Amylin or islet amyloid peptide (IAPP) is an amino acid protein normally produced by the β cells and co-packaged with insulin in the secretory granules and co-secreted in the sinusoidal space (Clark A et al., 1987; Richard Kahn et al., 2005). For reasons unknown this material tends to get accumulate extracellularly in close contact with β cells and forms fibrils. Amylin has been reported to lower basal and insulin-stimulated glycogen synthesis in the muscles and to

inhibit glucose-stimulated insulin secretion (Westermark P et al., 1989; Parte D Jr et al., 1989). These abnormalities of deficient insulin secretion and insulin action are similar to the pathogenic factors of T2DM (V. Seshah et al., 2013).

**2.3.3. Insulin secretory abnormalities in T2DM (**Miller RE, 1981**)**

- Decreased glucose sensing
- Impaired ability to respond to elevations and reductions during glucose infusion
- Reduced or absence first phase insulin secretion in response to intravenous glucose administration
- Reduced or absence early insulin secretory responses to oral glucose
- Inadequate insulin secretion for the magnitude of hyperglycemia

**2.3.4. Impaired Peripheral Action of Insulin:**

Numerous longitudinal and cross-sectional studies have provided evidence that hyperinsulinemia antedates the development of T2DM. This insulin resistance can occur in various tissues, liver, muscle, splanchnic, etc (Olefsdky JM and Kollerman OG, 1981; Arner P et al., 1987; Keller M et al., 1995). After glucose ingestion, insulin is released into the portal vein and is carried to the liver, where it binds to its specific receptors on the hepatocytes and suppresses the glucose output. Failure of the liver to perceive this signal results in increased hepatic glucose output and is manifested as raised blood glucose levels in T2DM (Pendergrassg M et al., 2007).

In muscles the defects in action are (Arner P et al., 1987; Keller M et al., 1995):

1) Impaired insulin receptor tyrosine kinase activity (Caro JF et al., 1986)
2) Diminished glucose transporters
3) Diminished glycogen synthase and pyruvate dehydrogenase

These defects result in disturbances in major intracellular pathways of glucose disposal, namely glycogen synthesis and glucose oxidation.

In T2DM subjects both receptor and post receptor defects have been shown to contribute to insulin resistance. Post-binding defects are of three types:

a) Impaired generation of insulin's second messenger
b) Diminished glucose transport into the cell
c) Post-glucose transport abnormality in some critical steps involved in glucose utilization (V. Seshah et al., 2013)

In diabetic subjects with moderate to severe hyperglycemia, post-binding defects in insulin action are responsible for insulin resistance. In subjects with impaired glucose tolerance, the defect may be at insulin binding to its receptor.

**2.3.5. Insulin Resistance as a Primary Defect:**

Prospective studies have shown that hyperinsulinemia and insulin resistance preceded the development of IGT (Impaired Glucose Tolerance). IGT represents a transient stage between normal glucose tolerance and the development of type-2 diabetes. Insulin resistance is the inherited defect that initiates the diabetic event. The hyperglycemia to insulin resistance occurs in three phases.

**Development of Insulin Resistance** (Caro JF et al., 1986; V. Seshah et al., 2013; IDF Diabetes Atlas, 2013)

**First Phase:** Plasma glucose remains normal despite demonstrable insulin resistance because the insulin levels are high.

**Second Phase**: Insulin resistance tends to worsen so that post-prandial hyperglycemia develops despite elevated insulin concentration.

**Third Phase**: Insulin resistance does not change but declining insulin secretion causes fasting hyperglycemia.

A number of well-designed and executed clinical trials confirmed that type-2 diabetes could be prevented by life style modifications and by pharmacological interventions. Though life style interventions with diet and physical activity are attractive but are difficult to sustain for longer periods. Various drugs like Metformin Thiazolidinediones and α-glucosidase-inhibitors and efficacious in the prevention of type-2 diabetes in placebo-controlled clinical trials in individuals with IGT. Metformin reduced the relative risks of new-onset diabetes at the end of the US Diabetes Prevention Programme by 31% and a 26% reduction was seen in the Indian Diabetes Prevention Programme (Ramachandran A et al., 2006). Treatment with thiazolidinediones resulted in a greater risk reduction (60% with rosiglitazone in DREAM and 81% with pioglitazone in ACT_NOW trials). Another approach that would not directly affect insulin sensitivity or insulin secretion is to competitively inhibit the brush border α-glucosidase, which are enzymes present in the juxtaluminal epithelium in the intestine. In the STOP-NIDDM trial (Chaisson J-L et al., 2002), done in Canada and various European countries, a 25% reduction was reported in a number of patients with new-onset diabetes and impaired glucose tolerance who were given acarbose compared with those given

placebo. Furthermore, acarbose significantly increased the reversion of impaired glucose tolerance to normal (Chaisson J-L et al., 2002).

The major complications associated with Metformin treatment are gastrointestinal symptoms which are dose-related and transient. However, vitamin B12 malabsorption was found in 35% of the patients with diabetes during long-term treatment with metformin (Harold E et al., 2010). Megaloblastic anemia and lactic acidosis are also rare adverse effects of metformin treatment. The major side effects observed with rosiglitazone and pioglitazone have been fluid retention with peripheral edema, congestive heart failure and weight gain. Idiosyncratic liver toxicity with liver failure developed in few people treated with troglitazone (Harold E et al., 2010). But the side effects of α-glucosidase inhibitors are only gastrointestinal, the most frequent are flatulence, diarrhea and abdominal discomfort. All the symptoms regress with time and following months of therapy, the abdominal side effects are minimal. α-glucosidase inhibitor does not appear to have any systemic effects as these drugs are not absorbed.

Voglibose, another α-glucosidase inhibitor is reported to be 20-30 times more potent than acarbose in inhibiting small intestine dissaccharidases (Vichayanrat A et al., 2014). Once the gastrointestinal side effects of Voglibose is tolerated in the early part of the treatment then it can be used safely for long-term treatment in IGT. Hardly any controlled study of the preventive role of voglibose is there for the Indian subcontinent where rice is a staple food.

In this context, a placebo-controlled open study was planned to be conducted to evaluate the effect of α-glucosidase inhibitor voglibose on glycaemic excursion pattern in alloxan-induced diabetic rabbit model and compared the effect with the polyherbal formulation.

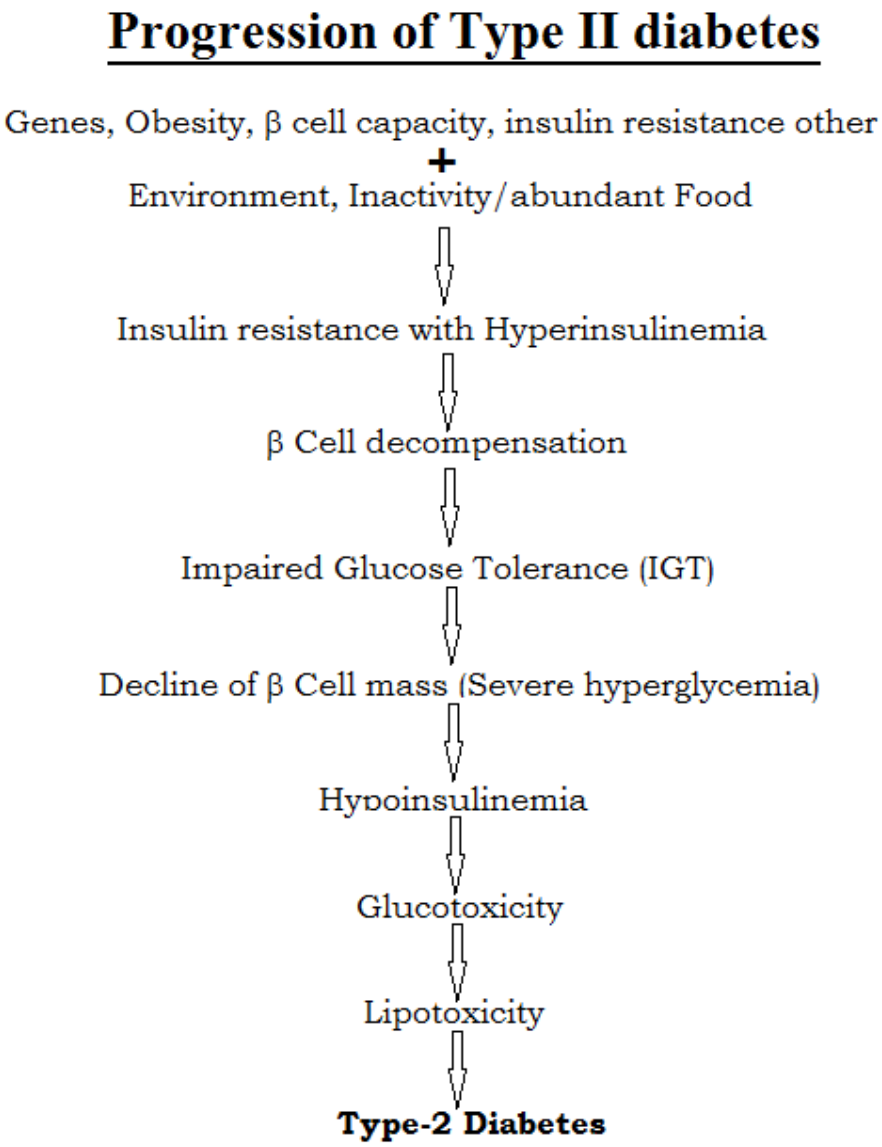



Fig 2.3- Progression of Type II diabetes

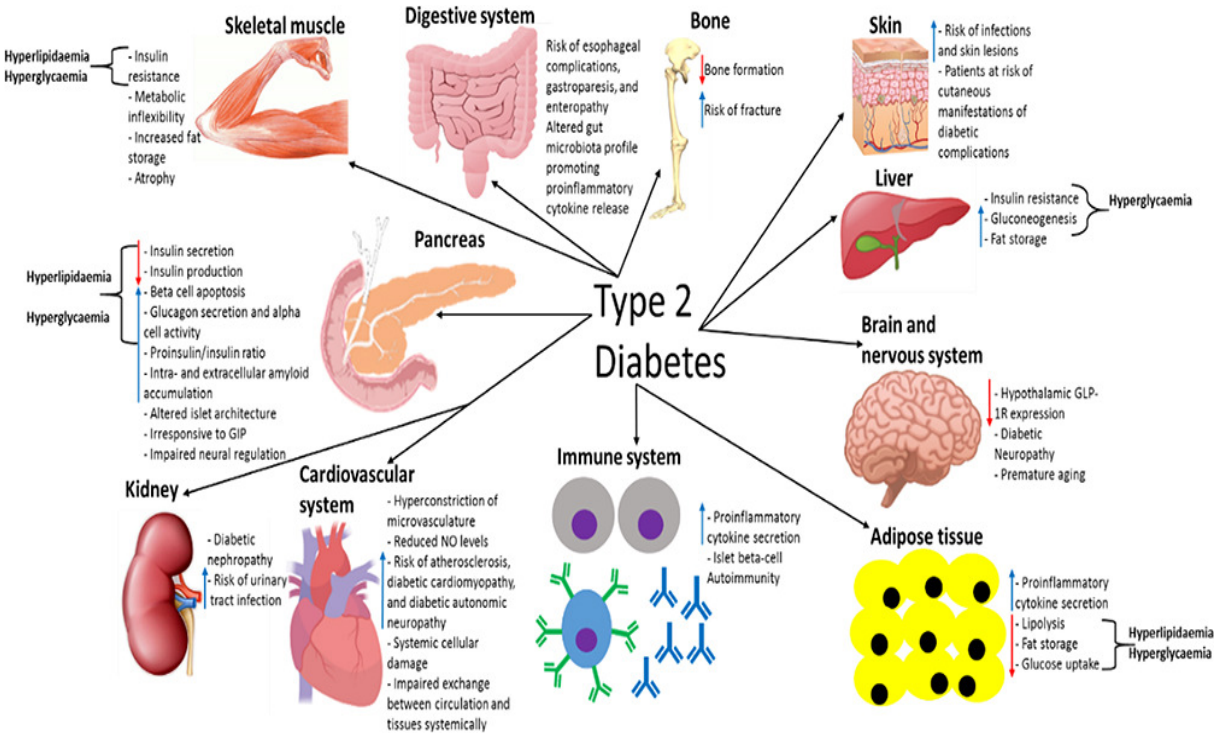



**Fig 2.4.** Pathogenesis of Type-2 Diabetes

## 2.4. Polyherbal formulation

Polyherbal formulation (PHF) is the use of more that one herb in a medicinal preparation. The concept is found in Ayurvedic and other traditional medicinal system where multiple herbs in a particular ratio may be used in the treatment of illness. According to WHO more than 75% of the world population depends on traditional medicines which involve the plant extract on their active constituents. India having its mega biodiversity with vast knowledge of ancient traditional system of ayurveda medicine and local and traditional medicine and provide a forest to livelihood plant in general care and alleviation of common aliment of the people (Thakur GS et al., 2012). Some of the medicinal plants used in the formulations for diabetes are included herewith.

### 2.4.1. ***Tinospora Cordifolia***

*Tinospora cordifolia* commonly named as "Guduchi" or amrita, is one of the important medicinal plants (giloy). It is extensively used herbs in Ayurvedic domain. This plant is also found in Berma and Shrilanka. Tinospora cordifolia widely available in tropical region and it climb upto a great height sticking with trunks of large trees. The stem of this plant is grey and creamy white in nature, deeply screwed spirally and longitudinally, with large rosett like lenticels. The climber woods looks like white and soft but porous in nature. The leaves looks like heart shaped, alternatively placed with long patiolate, chordate in shape with multicoated reticulate variation. Long thread like aenial roots which tends to come out with branches. Flower of this plant are small and unisexual, male tends to be in cluster and females are solitary six petals arranged in two wholes and six sepals arranged in three wholes of three each like other plant this plants also provides fruits which is red in nature, fleshy with so many druplets with a thick stalk, the scanlets are also colours (Lili K et al., 1992).

Gilloy (TC) is an important drug of Indian Ayurvedic system of medicines and it is extensively prescribed for fevers, diabetes, jundice, diarrhoea and dysentery, skin diseases, dyspepsia, urinary problem. Its also useful for the treatment of heart disease and helmenthiasis. The stem are starchy in nature which are highly nutritive and digestive and used for many diseases. The recent scientific studies of TC have emphasised the possible end use applicability in modern medicine.

### 2.4.2. ***Gymnema Sylvestre***

Gymnema Sylvestre is a perennial woody vine native to Asia (including the Arabian Peninsula). Africa and Australia, it

has been used in Ayurvedic medicine common names include gymnema, Australian cowplant and periploca of the woods and the hindi term gurman, which means “Sugar destroyer”. The leores and extracts contain A gymnemic acids, the major lioaotire constituents that interact with tate receptors in the tongue to temporarily suppress the taste of sweetness.

Anti-diabetic molecules of group gymnemic acids-successfully isolated and purified from the leaves of GS. (Lili 1992, Finishmen and Manni 1965). Later (1965) (Manni) and Sinsheimen, 1970, Yoshikawa et al. (1989). Phyto constituents of GS were isolated and their structural analysis were studied. Gymnemia acid is a group of triterpenoid saponins belonging to Oleanane and Dammarene classes. Gymneric acid of GS are highly responsible for anti-diabetic activity. In Gymneric acid (VIII) supposed to be the major component of an extract to stimulate insulin release from the panecrease (Persaud et. al. 1999)

Gymnema saponins I to V group coming under the category of anti-sweet principles with a novel D- glucoside structure which is also present in the extract. Besides this some other plant constituents are present such as flavons, antha quinones, hentry-aconrane, pentatria, contane, L & B Chlorophyll, Tartaric acid, formic acid, bataric acid, B amyrin related glucoside and stigma steral. Gurmarin, which is known to be another anti-sweet agent found in Symnema which has been elucidate which is a polypeptide comprising of 35 amino acids resideus (Fletcher, 1999) and the plant extract also test positive for alkaloids

### 2.4.3. ***Magnifera Indica***

*Magnifera Indica*, commonly known as Mango, is a species of flowering plant in the family Anacardiaceae. Mangoes are believed to have originated from the region between north western Myanmar, Bangladesh and India. It is a juicy fruit belonging to the family of Anacardiseae and is grown in many parts of India as well as all over the world. Particularly in tropical countries as now mango is commercially grown in more than 90 countries (Chua LK et al., 2019). Mango is known to be an important source of vitamins, micronutrients, and other phytochemicals. It is very rich in dietary fiber proteins, carbohydrates and one of the important source of energy with phenolic compounds (Chua LK et al., 2019). Mango helps in human growth, development and health, (Tejan, N. et al., 2018) and for more than (100). The extract of Mangifera (leaves, fruit, seed kernet, fruit pulp, roots, bark) has been extensively used for medicinal purposes in many countries. (Coon, J. T. et al., 2012). The ethnomedical use of mango stem/bark well documented in Cuba (Persaud SJ et al., 1999) and also it is used for cancer, diabetes, asthma, prosthetists, prostatic, hyper plazia, gastric disorder and mouth pain (Tejan, N. et al., 2018). (PHCOG Rev. Pharmacognosy reviews Jan to June 2010 Vol. 4 Issue 7)

Mango has been important herein the Ayurvedic and indigenous medical system for over 4000 years. It consists of different chemical constituents such as flavonoids, terpenoid and polyphenelic. It also consists of tenins and garlic acid derivatives. The bark of the plant is reported to have protocatethic acid, catechin, Mangifera, alanine, glycine, - amino butaric acid, tetracyclic, triterpenoids cycloart-24-en-2B and 27 triol, CD, indigocide. A & B, friedlin, mangolianon, cycloartan, 3-

B-30 diol and their derivatives, n-teteecosance, n-henai cosine, n- triacontane and mangiferolic acid, methyl ester, and other isolated product from the stem bark of *Mangifera Indica* (Etuh MA et al., 2019).

### 2.4.4. ***Syzygium Cumini***

*Syzygium Cumini* commonly known as Malabar plum, Java plum, black plum, Jaman or Jambolan is an evergreen tropical tree in the flowering plant family Myrtaceae and favored for its fruit, timber and ornamental value. It belongs to the family Myrtceae. It is commonly found in Indian Subcontinent and also extended to Bangladesh, Pakistan and Myanmar. Jamun is a rapidly growing tree which is as high as 100 feet and it gives fruits in Summer (Tejan, N. et al., 2018; Choudhary, S. et al., 2021). The fruits are produced in clusters and they are mostly round in shape. Fruits are also found in oblong in shape and about 2" long or less than that. The colour of the jamun found in India is generally purple and black in colour. The Jamun in India also known as Indian black berry, black plum, Duhat Jamun, Jaman, Jambul, Jambool, Java plum. Portuguese or Malabar plum (Coon, JT et al., 2004; Jiang, M., 2021) and the test is sweeties sour and it makes the tongue purple. Sometimes the taking of Jamun in excess amount is risky as its reduces the blood sugar level and eating of Jamun in empty stomach and after taking milk is strictly prohibited due to its adverse effect (Choudhary, S., et al., 2021). The excess amount also leads to cough, sputum accumulation in the lungs and fever (Choudhary, S., et al., 2021).

Along with the treatment of diabetes mellitus, *Syzygium cumini* is heavily used for the treatment of cancer as well. The plant syzygium species is a common basis of complementary

medicines because of its bioactive phytochemical several compounds derive from syzygium species such as polynolic acid, betulinic acid, dimethyle cardamonis and phenolics. The Jamun also known as nose apple and found in southeast Asia and ranges upto Kerala in India, Indochina and Yemen. These are densely populated in tropical climates which is nearly 10 to 12 m tall and more than 50 cm in dia. The leaves are dark green in nature. The flowers are greenish white with concave petals and supposed to be in clustered manner (Chua LK et al., 2019). The fruits of this Jamun are of pear shaped, yellowish skin when ripe and Jamun can be eaten fresh. Its leaves are used for the relief of patients suffering from diuretic rheumatism and sour eyes. Its seeds are used for diabetes diarrhea and dysentery. The bark of Jamun trees are also valuable and can be used for relieve of bronchitis and asthma.

### 2.4.5. ***Terminalia arjuna***

*Terminalia arjuna* is a tree of the genu Terminalia. It is commonly known as arjuna or arjun tree in English. Terminalia arjuna in the field of health started in time immemorial and the practice is still going on. This plant is large in size and deciduous and belongs to compretaceae family. The length of the plant is up-to 1000 feet. It has a buttressart trank and horizontally spreading branches and the branches are drop down wards. The smooth grey bark of the plant shows the presence of a single layer epidermic with hair like projection and few scattered lenticels. Generally the leates are conical in shape, oblonged or eliptic. It measures 10-15 cm in long and 4-7 cm broad. The upper side of the leaves happens to be pale green/ dark green and the lower side is pale yellow or pale brown. Petiole are arranged with 1 or 2 prominent guard at the top just

immediately below the leaf. The flowers are which in colour with short spikes and they ae biosexual. Each flower consists of 10 steams and an ovary which is disk-clothed with yellow or reddish hair. The calyx happens to be glabrous. Furits are 5- 12 cm long with fire hard angles or wins. Wings leaves are curved upwards or oblique.

The plant is used in drug formulation due to its effectiveness, cultural preferences and increasing cost of modern medicines. It is used for the treatment of cardiovascular diseases, diabetes, cough, excessive preparation of asthma, ulcer, tumour, inflammation, skin and many more (Marino B et al., 2001; and Matsui T et al., 1996). The bark stem powder of the Arjuna has been used in the case of "Hritshool" i.e. severe pain in the chest caused by not enough blood supply and other cardiac aliments by the ancient physician (Pallavi B et al., 2015; Raman BV et al., 2012). Arjunic acid, Arjujenin, Arjunan, Arjunalone and Ceutolin, Gallic acid, Allagic acid, oligomeric proanthocyanidins (OPCs), phytostenal are the major phytoconstituents.

### 2.4.6. ***Pterocarpus Marsupium***

*Pterocarpus marsupium* also known as Malabar kino, Indian kino, vijayasar or venkai is a medium to large, delicious tree that can grow up to 31 m (102 ft.) tall. It is a medium to large size plant, grow as long as 30 metres height. It bears leaves which have 3-7 oblongled leaflet, 3-5 inches in length with separated column margins. It gives fragrant yellow flower that occur in large penide and seed pots are orbicular, plant and winged. The outer surface of the plant is rough, grey and longitudinally fisher with scaly parts. It has been used as

traditional Ayurvedic medicine in India from time immemorial. The medicinal utility has been describe for leaf fruit and bark. These are brought for the treatment of cholera, dysentery, urinary complaints and tongue diseases. The gum excludes "Kino" B used as an astringent. The flowers are bitter and is being used for the appetite and cause flatulence. Most importantly *P. marsupium* has a long history in India for treatment against diabetes.

The constituent/ component of *P. marsupium* are pterosupin, pterostilbere, isoliquinitigenin, liquiritigenin, epicatechin, kinotonic acid, kinoin, kinored, B-eudeimol, marsupole, carpusin and marsupinal. Other chemicals include flavonoids and pterpinoids. There are some new flavonoids are also reported. C. glucosidase, 6- hydroxy, 2 cahydroxy benzyl-benzo furan, 3 methoxy, 4- hydroxy benzylidene, 6- hydroxy benzo, 2- (3H) furanon- 7- C- B- D- glucopyronocide, 1,2- bis (2.4 dihyroxy, 3-C-glucopyronasyl- ethane-dian (Keshaw Ram Aadil et al., 2014), 42 more known compounds such as B-D-glucopyranosil-2, 6 hydroxyl benzene (Powers AC et al., 2011) and sensquitorpene (Mamun-or-Rashid ANM et al., 2014) where isolated from an extract heart wood of P. marsupium tree (Pallavi B et al., 2015). There are also evidences which shows the presence of polyphendic compounds (B) and stilbene, pterostilbene, pteurosaprine and aromatic aldehyde, p-hydroxybenzaldehyde (Singh SP et al., 2015) and other phendic compounds (W. Brand-Williams et al 1995).

### 2.4.7. ***Carcuma longa***

*Curcuma longa* (commonly known as turmeric) belongs to the ginger family, zingiberaceae. The rhizomes of this plant are

used in coding. Petioles are arranged with 1 or 2 prominent glands at the top just immediately below the leaf. The flowers are white in colour with short spikes and they are bisexual. Each flower consists of 10 stamens and an ovary which is disk-clothed with yellow or radish basis. The calyx happens to be glabrous. Fruits are 5-12 cm with fire hand angles or wings. Wings lines are curved upwards or oblique.

### 2.4.8. ***Azadirachta indica***

*Azadirachta indica*, commonly known as neem, nimtree or Indian lileec is a tree in the mahogany family Meliaceae. It is very commonly used to treat diabetes in Indian system of medicines from time immemorial (Pallavi B et al., 2015). The leaf and bark extract of this plant is used to reduce/show to reduce blood glucose level. Dihydrochalcone, coumarine, Tannins and aliphatic compound (Akhila and Ranee, 1999, Viswash, 2002, Bramhachari, 2004). Out of the above-mentioned compounds, Nimbine specifically coming under the category of tritorperoids supposed to have the credit of biological activities of neem oil. It possesses anti-inflammatory, antipyretic, fungicidal, antihistamine, and antiseptic properties. Some more phytochemicals derived from neem include neembolite Azadirachtin, Azadiron, Azadiradion and Gedunin.

Neem is one of the vital tree and it is known to be the storehouse of several phytochemicals (Akhila & Rani, 1999, Viswash, 2002, CDQ, 1942, Subhpriya & Nayinee 2005). The neem leaves possess 0.13% of oil which provide a smell to the leaf (Puri, 1999) and it is believed that 2 major classes of phytochemicals, 1. Isoprenoids and 2. Non- isoprenoids have been isolated from different of neem such as bark, stem, flower, leaves etc. The most common isoprenoids include ditorpenoids,

tritoprpenoids, vilacimins, limonoids and G- cecomeliacins. The non-isotorpenoids mostly includes protein, polysarethils, sulphur compounds, polyphenolics.

### 2.4.9. ***Andrographis paniculata***

*Andrographis paniculata,* commonly known as creat or green chiretta is an annual herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is commonly known as Kalmegh in India. This plant has a wide history of treatment against cold influenza remedy. In Thailand and China people used this one for the treatment of fever associated with infectious diseases. The leaves of *Andrographis paniculata* are rich in Scandinavia. Specifically, this plant is also widely used for the treatment and prevention of upper respiratory tract infection due to its anti-inflammatory (Keshaw Ram Aadil et al., 2014; Chelladurai GRM et al., 2018), anti-pyratic (Powers AC et al., 2011; Mamun-or-Rashid ANM, et al., 2014), antiviral (Marino B. Arnao et al., 2001) and immunostimulator (Matsui T et al., 1996), properties.

So far more than 50 chemical constituent have been isolated and identified from paniculate such as Tenpinoids, Flavonoids, Inidoids, Ditorpinold, lactons, etc. Some special compounds are present such as Andrographolide (Ad) 14-deoxyandroyphalite DAD M-deoxy- 11,12- dehydrographalite (DAB) (Rofi et. al. 2020). The presence of this kind of chemicals makes *Andrographis paniculata* reach in natural antibiotic properties with diverse pharmacological effects which include antioxidant, anti-inflammatory, antidiabetic and anti-hepatorenal protective activity (Okhuarobo et. al. 2014). This plant is also being used for clearing heat resolving toxicity

(NMPA 2020). As its having a wide history of Ayurvedic medicinal treatment it may be extended to sore throat, cough and carbuncle. The typical system of heat and toxicity includes painful gums and the studies suggest that the inflammation of more organs is associated with various related diseases to a greater extent (Wong 2009). It also possesses a wide spectrum of antiviral activities [Co et. al. 2006 et.al. 2012, Ramlingum et. al 2018, Surname et.al. 2017).

### 2.4.10. ***Zingiber officinale***

Ginger (*Zingiber officinale*) is a herbaceous rhizomatous pereneal plant reaching upto more than 90 cm in height. Rhizomes are pale yellowish and do have an aromatic character. It has thick lobed and bears alternate narrow oblonged, lanceolate leaves. Gradually herb develops several lateral shoots in clumps, which get dry when the plant matures. Generally, the leaves of the ginger plants are long and 2-3 cm broad with sheathing bases. Flowers are rare rather small, calyx, superior, gemocepalous, three-tooth, open spilting on one side, corela of three sub-equal to laciolated connate greenish segments (Kawai 1994). It is commonly known as ginger root or ginger and is widely used as a spice and a folk medicine. The inflorescences bear flowers having pale yellow petals with purple edges and arise directly from the rhizome on separate shoots. This is widely used all over India and also the world in day-to-day life as a food spice. It has been an important ayurvedic ingredient used for various medicinal treatments such as rheumatisim, nervous diseases, asthma, stroke, constipation and diabetes (Awang 1992- Tapsell et. Al. 2006).

Ginger is commonly used in India and depending on their place of origin the properties and the chemical constituent littlely

varied. The oil of ginger depends mainly on effective yields and varies from 1 to 3%. Further more than 50 components of the oil have monoterpenoids and sesquitorpinoids, some of the oil components are converted to less odour-defined compounds by extraction and drying methods. (Langner et. al., 1998, Evans 2002) Ginger also contains a homogenous series of phenol. The most abundant is Gingeral. There are also smaller quantities of Gingerals with varied chain lengths are available. Some important features that contribute significantly to the enhancement of medicinal activity are due to presence of Acetoxic group, Alkalyl, ortho diphenoxyl, functionality of the Aromatic ring and unstructured herein moiety.

### 2.4.11. ***Pipper longum***

Long pepper (*Piper longum*) sometimes called Indian long pepper or thippali, is a flowering vine in the family piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning long pepper has a taste similar to, but hotter than, that of its close relative *Piper nigrum* from which black, green and white pepper are obtained. It is a small shrub with a large wordy root and creepy in nature. Jointed stems that are butchered at the nodes leaves are alternately arranged and spread over by another, without stipules and with blades varying greatly in size. The maximum leave size is 5-7 cm and leved are 2-3 cm in size. Flowers grows with solitary spikes. Fruits are (2.5 to 3.5 cm long with sleevely spikes are oblong, blunt and blackchin green. Commercial form is known as pippli (mature spikes) and root radix is known as pipalti mulla. There are 3 grads of piplamul and are classified such as grade 1- Thick roots and underground stem-grade-2, grade-3 Thin root, Stem and broken fragments. It is commonly used as household spice now-

a-days used as a well-known component of medicine as reflected in several studies. To name some fits for treating gonorrhea, menstrual pain, tuber colosis, sleeping problems, respiratory tract infecitons, chronic giant related pains and arthritic conditions (mehta et.al. 1998) (Courage of et. Al. 2000)

The fruit contains large number of alkaloids, the most abundant is preperine, then methyl preperine, pepernonaline, piporettine, asarinine, pellitorine, piperundealidine, piperlongumine, piperlonguminine, retrofractamide A, pergumidiene, brachystainide, a dimer of desmethoxypiplartina, N-isbutyl, decadieamids, brachyamide-A, brachystine, pipercide, piperderidine, longamide, dehydropipernonline, piperidine and tetrahydro piperine.

### 2.4.12. ***Piper nigrum***

Black piper (*Piper nigrum*) is a flowering vine in the family piper trees cultivated for its fruits, known as piper corn, which is usually dried and used as a spice and seasoning. It belongs to the family of piperceae which is a valuable medical plant most commonly used spice and continental as the king of spices. This is grown in many tropical countries like Brazil, Indonesia and India. Commonly it is also known as Kalimirch, pipply in Sanskrit, Melaga in Tamil and white pepper, green pepper, black pepper, mafagushar in English. Black pepper is used as medical agent as well as preservative. The fruit is a drupe (stone fruit) which is about 5 (mm) (020 in) in diameter (fresh and fully mature), dark red, and contains a stone which encloses a single piper seed.

*Piper nigrum* or its substantial active component are been used in different types of food and as medicine. It contains

alkaloid peperide which is known to possess many interesting pharmacological actions. Peperine (Arumugam G et al., 2013; Bräunlich J et al., 2013; Abhijit S et al., 2014; Anuradha Devi V et al., 2016; Chelladurai GRM et al., 2018) has been found to enhance the therapeutic efficacy of many drugs, vaccine and nutrients by increasing oral bioavailability by inhabiting various metabolizing enzymes (Mamun-or-Rashid ANM et al., 2014) to enhance cognitive action and fertility (Marino B. Arnao et al., 2001). It also stimulates the pancreatic and intestinal enzymes which add to digestion. The fruits is mainly used to produce white and green pepper and used as a flavouring agents.

The phytochemical investigations of *P. nirgum* revealed that it contains a variety of phytochemicals such as piperline (insulated from different members of pipeceae family), phenlics, flavonoids, alkamoids, amides and steroids, lignas, neoligans, chaliones etc. Some of the compounds are dihydro-pepercide, N-phermyle peperidyne, Guinineesine, Penta dienoyth-perridyne, Isobutyne- eicosadienamid, Thicholein, piperettine, pipercide, peperine, peprine B, cerementosine etc. The presence of life pharmacological activities was reported due to the presence of these different phytochemicals.

# CHAPTER-3

# METHODOLOGY

# METHODOLOGY

About 20.0 g of polyherbal formulation constituting of different medicinal plants (Table 2) was taken in a flask and 10 ml of 80% methanol was added. The mixture was soxhlet extracted at a temperature of $30^{0}$C for a duration of 48 hours. The solvent was evaporated in a rotary evaporator to make it concentrate followed by lypholzation to make a powder sample of the extract and kept at $4^{0}$C.

**Table 3.1-** Summary of pharmacological importance of selected plants.

| Sl No | Botanical Name | Local Name | Family | Pharmacological Importance |
|---|---|---|---|---|
| 1. | *Tinospora Cordifolia* | Guduchi | Menispermeaceae | anti-oxidant, anti-neoplastic, anti-stress, anti-dote, anti-spasmodic, anti-pyretic, anti-allergic, anti-leprotic, anti-hyperlypidaemia, anti-diabetic, anti-inflammatory, anticancer, Anti-Microbial Activity, Anti – HIV, Anti-bacterial activity, Anti-fungal. |
| 2. | *Gymnema Sylvestre* | Gudmari | Asclepiadaceae | Anti-diabetic, Anti-allergic, Anti-viral, Anti-dote against snake venom, Anti-obesity, Dental application, Anti-inflammatory, Hypolipidaemic, Anti-microbial, free radical scavenging, Antiarthritic, Anti-cancer, Anti- |

| | | | | |
|---|---|---|---|---|
| | | | | hyperlipidemic, Hepatoprotective, Anti-cataract. |
| 3. | *Mangifera indica* | Ambrabija | Anacardiaceae | Antioxidant, Anti-inflammatory, Immunomodulatory, Antidiabetic, Anti-obesity, Anticancer, Lipid-Lowering, Antiarthritic, Hepatoprotective activity, Anticaries, Anti-metastatic, Anti-bacterial, Anti-fungal, Anti-viral, Anthelmintic and Anti-allergenic, Antiparasitic, Anti-tumor-anti-HIV, Anti-diarrhoeal, Antispasmodic and antipyretic, Gastroprotective, Cardiotonic, Hypotensive,Anti-ulcer, Anti-amoebic, Laxative. |
| 4. | *Syzygiumcumimi* | Jambumanji | Myrtaceae | Antidiabetic, Antioxidant, Antihyperlipidaemic, Antiarthritic, Antiallergic,Antipyretic, Nephroprotective, Antifertility, Anti-inflammatory, Antibacterial, Antidiarrhoeal, Radioprotective, Hepatoprotective, Anti-ulcer, Chemopreventive, Radioprotective. |

| | | | | |
|---|---|---|---|---|
| 5. | *Terminalia arjuna* | Arjuna | Combretaceae | Anti-inflammatory, Analgesic, Anti-diabetic, Anti-depressant, Anti-inflammatory, Antihyperglycemic, Anti-bacterial, Cardioprotective, Antioxidant, Hepatoprotective, Anti tumor and cytotoxicity, Gastric, Anti-viral, Anthelmintic, Molluscidal. |
| 6. | *Carcuma longa* | Haridra | Zingiberaceae | Anti-diabetic, Anticancer, Antiinflammatory, Antibacterial, Antifungal, Antiviral, Cardiovascular, Hepatoprotective, Neuroprotective, Obesity, Allergy and asthma, Skin diseases, Alzheimer's disease, Chemoprotective, Antidermatophytic |
| 7. | *Azadirachta indica* | Neem | Meliaceae | Anti-oxidant, Anti-diabetic, anti-inflammatory, Anti-cancerous, Anti-Hyperglycemic, Antidyslipidemic, Anti-infertility, Anti-ulcer, Anti-parasitic, Anti-fungal, Anti-bacterial, Hepatoprotective, Skin Disorders, Anti-HIV/AIDS, Anti-dental caries, |
| 8. | *Amdrographis* | Bhuin neem | Acanthaceae | Anti-inflammatory, |

| | | | | |
|---|---|---|---|---|
| | *panicuate* | | | Antioxidant, Antidiabetic, Antileishmanial, Anti-diarrhoeal and intestinal, Anti-fertility, Antivenom, Anti-HIV, Antimalarial, Antifilaricidal, Antibacterial, Anti-bacterial, Anti-fungal, Cardiovascular, Hepatoprotective, Anticancer, Anti-pulmonary inflammation, Anti-neuroinflammation, Anti-obesity. |
| 9. | *Zingiber officinale* | Sunthi | Zingiberaceae | Anti-cancer, Anticoagulant, Antiemetic, Anti-Inflammatory, Antinociceptive, Antioxidant, Cardiovascular, Gastrointestinal, Antitussive, Immunomodulatory, Antiarthritic, Antigenotoxic, Radio Protective Activity, Antimicrobial Activities, |
| 10. | *Piper longum L* | Pippali | Piperaceae | anti-inflammatory, analgesic, anti-oxidant, anti-microbial, anti-cancer, anti-parkinsonian, anti-stress, nootropic, anti-epileptic, antihyperglycemic, hepatoprotective, anti- |

| | | | | |
|---|---|---|---|---|
| | | | | hyperlipidemic, anti-platelet, anti-angiogenic, immunomodulatory, anti-arthritic, anti-ulcer, anti-asthmatic, anthelmintic action, anti-amebic, anti-fungal, mosquito larvicidal and anti-snake venom. |
| 11. | *Piper nigrum* | Golmaricha | Piperaceae | Anti-microbial, Antioxidant, Anti-cancer, Neuroprotective, Hypoglycaemic, Anticonvulsant, Analgesic, Hypolipidemic, Anti-inflammatory, Gastro-intestinal Stimulant Anti-asthmatic, Anti-diabetic, To treat piles Antidiarrhoeal, Anti-platelet, Antithyroid activity, Antiasthmatic. |
| 12. | *Alianthusexcelsa* | Mahanimb | Simaroubaceae | Wounds and skin eruptions, Fevers, Bronchitis, Asthma, Diarrhea, Dysentery |
| 13. | *Withaniasomnifera* | Ashwagandha | Solanaceae | Anti-microbial, Anti-inflammatory, Anti-tumor, Anti-stress, Neuroprotective, Cardioprotective, Anti-diabetic |
| 14. | *Caeselpiniabonduc* | Karanj | Caesalpiniaceae | Anti-inflammatory, Antioxidant, Antitumor, Hepatoprotective, |

| | | | | |
|---|---|---|---|---|
| | | | | Antiviral, Antimalarial, Antibacterial |
| 15. | *Swertia chirayita* | Kirata tikta | Gentianaceae | Chronic fever, Malaria, Anemia, Bronchial asthma, Hepatitis, Gastritis, Constipation, Dyspepsia, Skin diseases, Worms, Epilepsy, Ulcers |
| 16. | *Holarrhenap ubescens* | Kurchi | Apocynaceae | Diarrhea, amoebic dysentery, liver disorders, irritable bowel syndrome, and bleeding piles |
| 17. | *Citrus lemon* | Lemon | Rutaceae | Antioxidant, Anti-inflammatory, Antibacterial, Neuroprotective, Anticancer, Antihyperlipidemic |
| 18. | *Plumeria alba* | Champa | Apocynaceae | Antifungal, Antioxidant, Antiacne, Hypolipidemic, Antiulcerogenic, Antibacterial, Cytotoxic, Anti-inflammatory, Hepatoprotective |
| 19. | *Murrayakoe nigi* | Curry-leaf | Rutaceae | Antioxidant, antidiabetic, anti-inflammatory, and antitumor properties. They also have anti-inflammatory, antioxidant, and antitumor properties |

### 3.1. Total flavonoid content

About 0.5 ml of crude extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 m sodium acetate and 2.8 ml of water. The volume of 10% aluminium chloride was substituted with distilled water in the blank. After incubation at room temperature for 30 min, the absorbance of the crude extract and standard solution reaction mixture was measured at 415 nm.

### 3.2. Total Phenol content

The total polyphenol content of the crude extract was measured by using Folin-ciocalteau reagent with little modifications. About 0.5 ml of diluted extract (1 mg/ml) was taken and mixed with 2.5 ml of 10 times diluted Folin-ciocalteau reagent. The mixture was allowed to stand at room temperature for 5 min. Then 2 ml of prepared 7.5 % $Na_2CO_3$ (w/v) solution was added to the above mixture. The reaction mixture was then incubated at 40 ◦C for 30 min and then cooled to room temperature. The absorbance was measured at 765 nm by taking distilled water as blank with the help of a spectrophotometer. Different concentrations (10, 20, 40, 60, and 80,100) µg/1ml of gallic acid solution were taken to make a standard calibration curve. The curve was used to determine the equivalents (GAE).

### 3.3. DPPH radical scavenging activity assay *(cross check)*

The DPPH-free radical scavenging assay was performed by the method described by Aadil et al. (2014) and Brand-Williams et al. (1995). The stock solution was prepared by dissolving 4.0 mg DPPH in 100 ml methanol and the working solution was raised by mixing 010 ml stock solution with 45 ml methanol so as to obtain an absorbance of 1.1 ± 0.05 at 517 nm. The reaction mixture was well shaked and incubated in the dark for near about 30 min at room temperature followed by measurement of absorbance at 517 nm using ascorbic acid as the standard. The scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = (A_0 - A_i) \,/\, A_0 \times 100$$

Where, $A_0$ was the absorbance of the control, and $A_i$ was the absorbance of the sample. The results were expressed in terms of $EC_{50}$ (concentration of phenolic compounds required to quench 50% of the initial DPPH radical).

### 3.4. ABTS•+ radical scavenging assay:

To calculate the $ABTS^{\bullet+}$ radical scavenging, the method proposed by Reference 7 and Aadil et al (2014) was followed. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7.4 mM ABTS stock solution with 2.6 mM potassium persulfate and allowing the mixture to stand in the dark @ room temperature for 12–16 h before use. A volume of 1 ml $ABTS^{\bullet+}$ solution was diluted with 50 ml methanol to $A_{734}$ = 0.70 and equilibrated at 30 $^0$C with the solvent used for the assay. The crude extract and the standard samples (150 µl) were allowed to react with 2850 µl of the $ABTS^{\bullet+}$ solution for 2 h in dark. The absorbance was taken at 734 nm using tannic acid as standard.

$$ABTS^{\bullet+}\text{scavenging activity (\%)} = (A_0 - A_i) / A_0 \times 100$$

Where, $A_0$ was the absorbance of the control, and $A_i$ was the absorbance of the sample.

### 3.5. Ferric reducing antioxidant power (FRAP) assay:

The FRAP assay was carried out by the method of Aadil et al (2014). The stock solutions included a 300 mM acetate buffer (3.1 g sodium acetate trihydrate in 16 ml glacial acetic acid, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM anhydrous $FeCl_3$ solution. The working solution was freshly prepared by mixing a 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3$ solution, followed by warming at 37 $^0$C before use. Samples (150 µl) were allowed to react with 2.85 ml of the FRAP solution for 30 min in the dark. The absorbance of the colored product (ferrous tripyridyltriazine complex) was taken at 593 nm. Result was expressed in terms of mol of GAE.

### 3.6. Alpha-amylase & Alpha-glucosidase inhibitory assay

The alpha-amylase inhibitory activity of the crude extract was carried out according to the standard method (Nickavar, Yousefian, 2009). The starch solution (0.5% w/v) was used as the substrate. The enzyme solution was prepared by dissolving 1mg of porcine pancreatic alpha amylase in 20 mM phosphate buffer (100 mL, pH 6.9). The sample solutions were prepared in DMSO (dimethyl sulfoxide) in different concentrations (10 to 100 mg/mL). The DNS solution (20 ml 96 mM 3,5-dinitrosalicylic acid, 12 g sodium potassium tartrate in 8 ml of 2 M NaOH and 12 mL deionized water) was used as the colouring reagent of the reaction. A mixture of 1 mL of each test and enzyme solution in a test tube was incubated at 2 5 °C for 30 min. Then, after taking out 1 mL from this mixture, 1 mL of the above mentioned starch solution was added and the mixture was incubated at 25 °C for 3 min. Finally, 1 mL of the DNS solution was added. The tube was then covered and heated in water bath at 85 °C for 15 min. After cooling the tube, the reaction mixture was diluted with distilled water (9 mL). It was mixed well and the absorbance was recorded at 540 nm. In the case of blank, the DNS solution was added prior to the addition of the starch solution, while the rest of the method was the same as for the test. For control, all procedure was again the same except that plant extract was replaced by 1 mL of DMSO. Acarbose, a well-known anti-diabetic medicine, was used as a positive control. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = [ (Ac - As ) /Ac] \times 100$$

Ac-absorbance for control; As-absorbance for standard.

The α-glucosidase inhibition was determined by the following modified methods (Matsui et al., 1996; Bräunlich et al., 2013). The α-glucosidase reaction mixture contained 2.9 mM p-nitrophenylα-glucopyranoside (pNPG), varying concentrations (10 mg/mL to 100 mg/mL) of crude extract and 1.0 U/mL α-glucosidase in sodium

phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls, acarbose replaced the sample extract. Mixtures without enzyme, sample extract and acarbose served as blanks. The reaction mixtures were incubated at 25 ºC for 5 min, after which the reaction was stopped by boiling for 2 min. The absorbance of the resulting p-nitro phenol (pNP) was determined at 405 nm using spectrophotometer and was considered directly proportional to the enzyme's activity. The $IC_{50}$ values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference drug for alpha glucosidase inhibition assay. All the tests were performed by triplicates.

### 3.7. In vivo anti-diabetic study:

Wistar rats were divided into four different groups, each are having six animals. Diabetes was induced in overnight-fasted rats by administering a single intraperitoneal (i.p.) injection of freshly prepared alloxan with a single dose of 120 mg/kg BW. Diabetes was confirmed in the alloxan treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of alloxan injection, the rats were given 5% w/v of glucose solution to prevent the mortality. The rats were fasted overnight, collection of blood samples and sera glucose determination were drawn from their tail tips. Sera glucose estimation was done by one touch electronic glucometer using glucose test strips, and the glucose level more than 250 mg/dl was used for the study.

**Group I:** Non diabetic normal control rats.

**Group II:** Negative control

**Groups III:** Diabetic rats administered with metformin (50 mg).

**Groups IV:** Diabetic rats administered with voglibose (1 mg).

**Groups V:** Diabetic rats was given the polyherbal extract of 250 mg/kg


Figure.3.1 The rats treated with oral doses of standard drugs (metformin and voglibose) and polyherbal formulation.

At the end of the experiment day-14, the rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and analysed. For the histological study, the organs were fixeded in 10% neutral buffered formalin.

**3.8. Acute and Sub-acute toxicity study test:**

Wistar rats were used for the study and fasted for 12 hours prior to dosing. Each animal was given a single dose of the polyherbal extract 5000 mg/kg body weight. After dosing they were observed first 30 mins for any behavioural changes, then were observed for another 24 hours and 72 hours subsequently. The parameters studied were body weight, somnolence, lacrimation, epistaxis, paralysis, pylorus, saliva, consumption of food, water, and death. From the above toxicity study, the polyherbal formulation was found non-toxic at a concentration >5000mg/kg body weight.

The animals were divided into four groupsnormal control, and three treatment groups, each containing three animals. Group-I was set as control and this group was given normal food and water. The other three groups received doses of 150mg/kg b.w/day, 250mg/kg b.w/day, and 500mg/kg b.w/day, respectively. The weight of the animals was measured daily and their behavioural and morphological

changes were observed on the 28th day of treatment. The animals were anesthetized in theAnaesthesia chamber containing isoflurane. Cardiac puncture was performed to collect blood sample and was analysed, histopathological study was done of different organ.

### 3.9. LCMS study results

**Chemicals, reagents, and samples**

The LCMS grade solvents acetonitrile and water were obtained from J. T. Baker, Avantor, Germany and Honeywell (Germany), respectively. LCMS grade reagents like formic acid were procured from Thermo Fischer Scientific, USA and Ammonium Formate was procured from VWR, Germany. Deionized water, purified by a Milli-Q system (Millipore, USA), was used throughout the study.

Ultra high performance liquid chromatography–mass spectrometry coupled with quadrapole time of flight analysis

***Preparation of sample solution***

50 mg of the methanolic extract was accurately weighed and transferred to a 25 mL volumetric flask and to it 25 mL of methanol is added and sonicated for 30 min. The solution was cooled and made up to volume with methanol. 5 mL of the solution was diluted to 20 mL with ethanol. Further, the solution was filtered using a 0.22 µm PTFE syringe filter. The filtered solution was used for the analysis.

***Instrumentation***

The analysis was performed on a Xevo G3 QToF Waters Corporation (MA, USA) with Acquity UPLC I Class Plus and MassLynx software (Waters Corporation, USA) and processed through Progenesis QI software (Waters Corporation, USA). The separation was carried out using Acquity UPLC HSS T3 column (100 x 2.1 mm, 1.8 µm) (Waters Corporation, USA). The column was maintained at 40°C and the sample temperature was kept at 15°C during the analysis. The main working parameters for MS were: ionization type- ESI, mode-MSE, acquisition time 23 min, mass range (*m/z*) 50−1200 *m/z*, low collision

energy 6 eV, high collision energy 10−40 eV (ramp), cone voltage 40 V, capillary voltage 3.0 kV for, source temperature 130°C, desolvation temperature 500°C, cone gas flow 50 L/h, desolvation gas flow 750 L/h. Mass was corrected during acquisition, using an external reference (Lock–Spray) consisting of 200 pg/mL solution of leucineenkephalin (Waters , USA) infused at a flow rate of 10 µL/min via a lock–spray interface called Zspray, generating a reference ion for the positive ion mode [(M+H) *m/z* 556.2771] to ensure mass correction during the MS analysis. The Lock–Spray scan time was set at 0.5 s with an interval of 10 s. The elution was carried out in positive mode [ES+] at a flow rate of 0.4 ml/min using gradient mobile phase, 0.1% formic acid in water (solvent A), and 0.1% formic acid in acetonitrile (solvent B). The volume ratio of solvent B was changed as follows, 5% B for 0–1 min, 5–25% B for 1–5 min, 25–35% B for 5–8 min, 35–45% B for 8–11 min, 45–55% B for 11–14 min, 55–90% B for 14–20 min, 90–5% B for 20–20.1 min, and 5% B for 20.1–23 min. 5 µL of the test solution was injected for the phytochemical screening and the chromatographs were recorded for 23 min.

# CHAPTER-4

# RESULTS

# &

# DISCUSSION

Polyherbal formulations were got an extremely important place in the Ayurvedic medicine due to their therapeutics efficacy. The natural herbal products are the secondary metabolites which are produced from /by plants through their metabolic pathway (Chelladurai and Chinnachamy, 2018). There are plenty of active natural chemical constituents present in the herbal formulation, which work against Diabetes mellitus (DM) type –II. DM is an endochronological disorder in line with prolonged hyperglycemia, higher level of glaciated hemoglobin with more complexity on comorbid condition. The DM is simply a metabolic disorder associated with high blood sugar and chronic in nature (Pallavi et al 2015). This is more often exist due to physical inactivity on healthy diets, raise of blood level in chlosterol (Singh and Kumar, 2015). The present life style leads to many suffering and severity of diseases forced the doctoral and scientific community to look after the reasons. The existence of DM comes into play when pancreas does not produce enough insulin or the body unable to use it in proper way (Arumugam et al.2013). It can be classified by four different ways such as Type – I DM, Type – II DM, Gaestasanal DM and non classical cause of DM, on the basis of pathogenic pathway (Powers AC, 2011). Type – II DM falls on the category of disorder where the cells present in the body do not responds to insulin (Mamun-or-Rashind et al 2014). So for many synthetic medicine are discover and available in the market but the use of such medicine do have many side effects (Ranjit et al. 2012). These side effects leads to other critical illness, so aroused the scientific community to work further alternative herbal treatment with low cost and of no side effects. Natural herbal medicine extracted from plants were investigated and used to control over the glucose production and absorption. Polyherbal formulation collected from the plants play a vital role in the medicinal field due to its healing capacity from time immemorial. The phytochemical screening methods and pharmacological importance brings about the wonderful potential

of plant to be used for treatment. The plant metalitessich as flavnoids, terpenoind, tannins, saponins, piperines, azarinines etc., were responsible for the said potential activity (Nitya, 2016). The plant extract reach in flanoids for responsible for multipotential activity against the treatment of various diseases (Vivekananda et al. 2013). The present study emphasized on alpha amylase and glucosidase inhibition assay, phytochemicals properties and antioxidant activity of polyherbal formulations of 15 medicinal plants were investigated.

**4.1 Flavonoids content**

Flavonoids are a group of polyphenolic compounds with known properties that include free radical scavenging inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. These are vital in combating the free radicals which damage human cells. Numerous epidemiological studies confirm a significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk. The total Flavonids content of the polyherbal formulation was found to be 6.545 ± 0.048 mg/g.

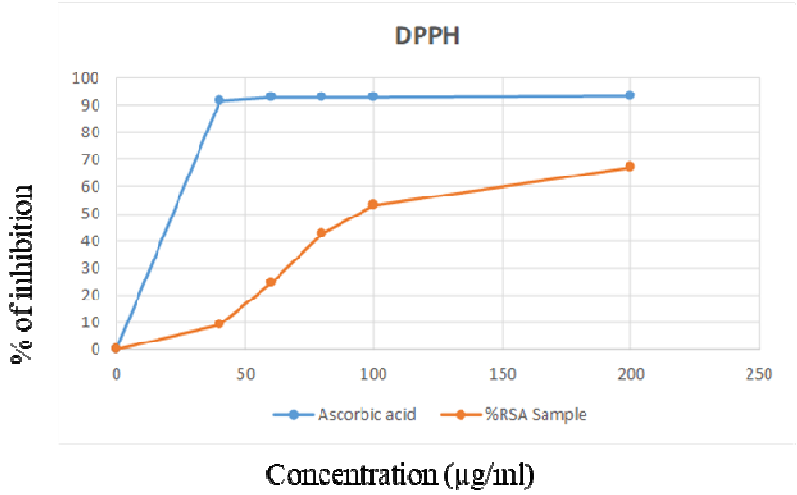
**4.2 Phenol content**

Phenol was ubiquitous secondary metabolites in plants and comprise a large group of biologically active ingredients. Around 8000 of phenols have been identified so far in plants. These phenolic compounds possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, and anticarcinogenic and can modify the gene expression. The total phenol content of the polyherbal formulation was found to be 11.071 ± 0.184 mg/g.

**4.3 DPPH antioxidant activity**

DPPH radical scavenging activity is an easy, rapid, and sensitive way to estimate the antioxidant activity of a specified compound or plant extract. DPPH scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids, and tannins.

Antioxidant activity in terms of percentage of inhibition was shown in Figure 4.1. The highest antioxidant activity of 92.22% was found in the polyherbal formulation. The high antioxidant activity was due to the presence of high amounts of phenols and flavonoids.



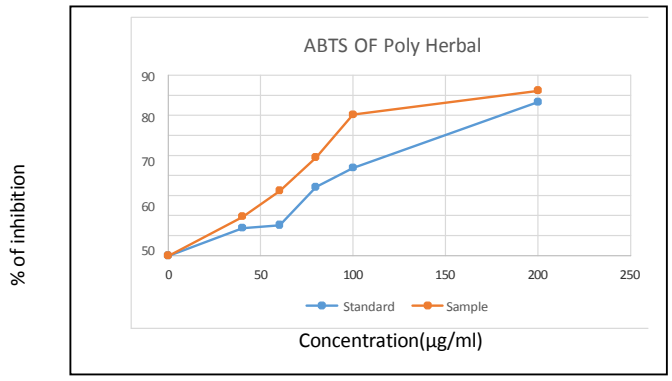

**Graph 4.1-** DPPH radical scavenging activity

### 4.4. $ABTS^{\bullet+}$ radical scavenging assay:

To calculate the $ABTS^{\bullet+}$ radical scavenging, the method proposed by Reference 7 and Aadil et al (2014) was followed. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7.4 mM ABTS stock solution with 2.6 mM potassium persulfate and allowing the mixture to stand in the dark @ room temperature for 12–16 h before use. A volume of 1 ml $ABTS^{\bullet+}$ solution was diluted with 50 ml methanol to $A_{734}$ = 0.70 and equilibrated at 30 $^0$C with the solvent used for the assay. The crude extract and the standard samples (150 µl) were allowed to react with 2850 µl of the $ABTS^{\bullet+}$ solution for 2 h in dark. The absorbance was taken at 734 nm using tannic acid as standard (Figure 4.2).

$$ABTS^{\bullet+}\text{scavenging activity (\%)} = (A_0 - A_i) / A_0 \times 100$$

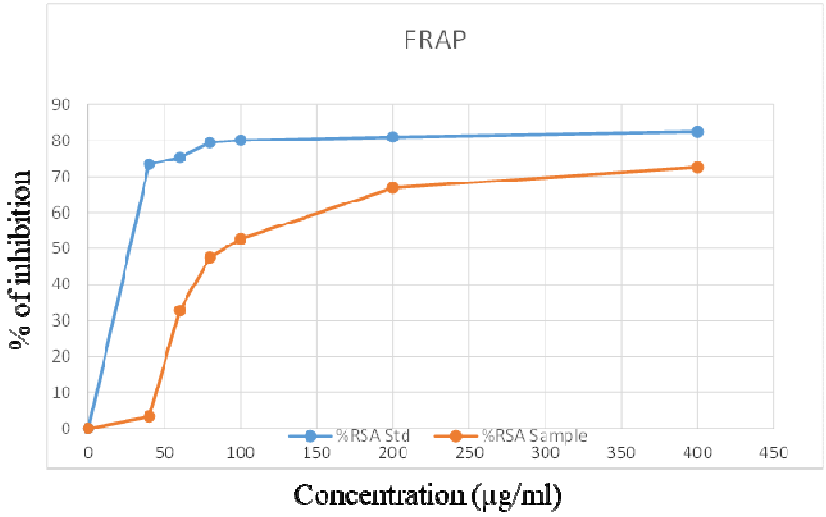
Where, $A_0$ was the absorbance of the control, and $A_i$ was the absorbance of the sample.




**Graph 4.2: ABTS•+ radical scavenging assay**

### 4.5 FRAP antioxidant activity

A relatively higher absorbance value indicated more reduction of ferric ions to ferrous ions. Samples having a higher reducing power had a higher absorbance value at 700 nm. High reducing antioxidant activity of 4.84±0.0487% was noted for the crude extract of polyherbal formulation. (Figure- 4.3) The high FRAP reduction value indicates its good scavenging property.




**Graph- 4.3** FRAP scavenging activity

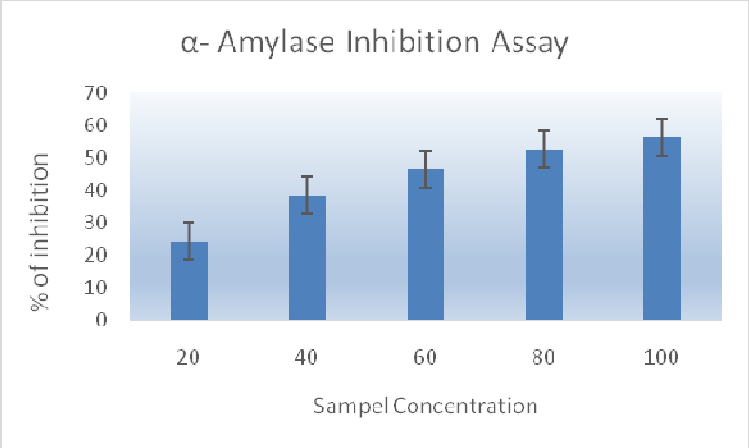
### 4.6 Alpha amylase and alpha glucosidase:

*In vitro* antidiabetic activity was carried out in the polyherbal formulation by using alpha – amylase and alpha glucosidase inhibition assay. The polyherbal formulation showed potential inhibitory effects on these enzymes. Different concentration (20, 30, 40, 60, 80, 100 mg/mol) of polyherbal extract were subjected to α-amylase and α-glucosidase assay.

The maximum inhibition of 56.21% for α-amylase was obtained at a concentration of 100 µg/ml solution of polyherbal formulation. In contrast, the standard drug, ascorbate has an inhibition of 88.92% at a concentration of 100 µg/ml. The $IC_{50}$ value of α-amylase activity for ascorbate and polyherbal extract was found to be 39.086 µg/ml & 6.195 µg/ml, respectively. Similarly the effective inhibition of α-glucosidase with the treatment of standard and polyherbal extract were carried out. The maximum percentage of inhibition of α-glucosidase assay was found to be 76.42 % at a concentration of 100 µg/ml of polyherbal extract. The standard drug ascorbate was shown a maximum inhibition of 81.91% of at a concentration of 100 µg/mol. The $IC_{50}$ value for standard drug was 65.692 µg/ml and for polyherbal extract was 87.714 µg/ml. From the result it was revealed that the polyherbal extract effectively inhibit the action of a α-amylase and α-glucosidase enzyme.

**Table 4.1:** The inhibition of α-amylase enzyme activity with polyherbal formulation and α-glucosidase enzyme activity with polyherbal formulation.

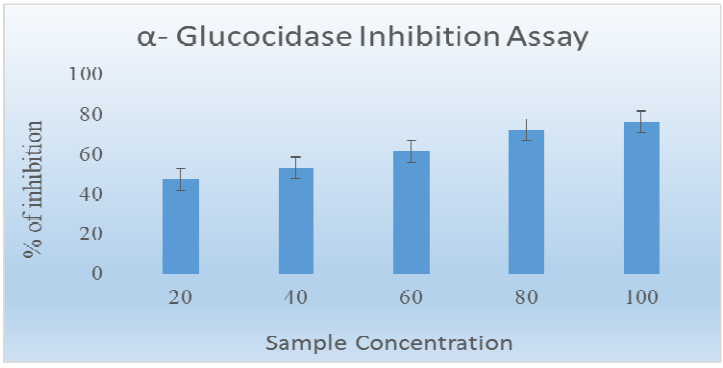
| Concentration (µg/ml) | % of inhibition |
|---|---|
| 20 | 24.32 |
| 40 | 38.41 |
| 60 | 46.36 |
| 80 | 52.48 |
| 100 | 56.21 |




**Graph 4.4-** The inhibition of α-amylase enzyme activity with polyherbal formulation

**Table 4.2:** The inhibition of α-glucosidase enzyme activity with polyherbal formulation

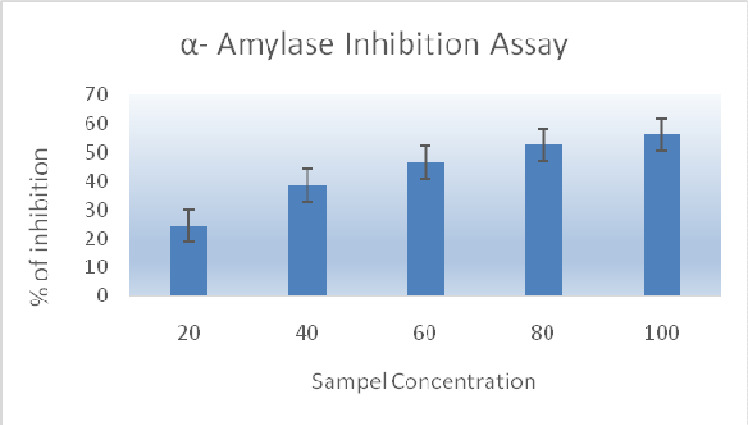
| Concentration (µg/ml) | % of inhibition |
|---|---|
| 20 | 32.48 |
| 40 | 58.49 |
| 60 | 64.48 |
| 80 | 59.51 |
| 100 | 74.23 |




**Graph 4.5:** The inhibition of α-glucosidase enzyme activity with polyherbal formulation

**Table 4.3:** α-amylase for 3mg/ml

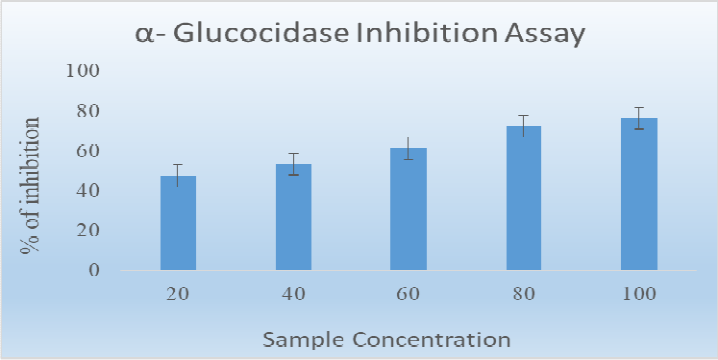
| Concentration | % of inhibition |
|---|---|
| 20 | 24.32 |
| 40 | 38.41 |
| 60 | 46.36 |
| 80 | 52.48 |
| 100 | 56.21 |




**Graph-4.6-** Alpha amylase inhibition assay

**Table 4.4** α-glucosidase for 3mg/ml

| Concentration | % of inhibition |
|---|---|
| 20 | 32.48 |
| 40 | 58.49 |
| 60 | 64.48 |
| 80 | 59.51 |
| 100 | 74.23 |




**Graph-4.7-** α-glucosidase nhibition assay

The rate of inhibition of α-amylase & α-glucosidase enzyme in response to gradient concentrations of the polyherbal formulation is represented in Table 1& 2. It indicates that the polyherbal extract has enhanced rate of inhibition of α-amylase and α-glucosidase activity. The enzyme α-amylase and α-glucosidase enzyme are responsible for the carbohydrate breakdown into glucose. Further, α-amylase is also responsible for hydrolyzing the starch, which breaks into glucose before absorption (Abhijit et al 2014). The inhibition of α-amylase can reduce postprandial hyperglycemia (Raman et al 2012). It is believed that the cleavage of disaccharides into glucose is due to the α-glucosidase enzyme in the small intestine. The inhibition of α-glucosidase is very challenging to control over diabetes (Anuraddha Devi and Malikarjuna, 2016). The polyherbal extract has a plethora of phytochemicals that can inhibit α-glucosidase activity, leading to minimum absorption of monosaccharides. The aqueous methanolic extract of polyherbal formulation showed potential percentage of α-amylas and α-glucosidase inhibition.

Table-4.5 Presents Shapiro-Wilk test of normality for distribution of FBS for different treatment groups. It revealed that data of different time points for different treatment groups do not confirm to normality in many cases.

**Table 4.5. Tests of Normality: FBS (Shapiro-Wilk) ('P' value)**

| Test of Normality: FBS | | | | | | |
|---|---|---|---|---|---|---|
| Group | Week 0 | Week 3 | Week 12 | Week 16 | Week 20 | Week 24 |
| Placebo | 0.104 | 0.004* | 0.015* | 0.417 | 0.1 | 0.000* |
| Voglibose 1 mg/kg | 0.966 | 0.483 | 0.726 | 0.801 | 0.11 | 0.605 |
| Metformin 50 mg/kg | 0.715 | 0.273 | 0.005* | 0.056 | 0.011* | 0.002* |
| PHF **(Polyherbal Formulations)** | 0.073 | 0.753 | 0.053 | 0.009* | 0.204 | 0.248 |

**Table 4.6 Descriptive statistics of FBS, PPBS before induction of the rabbits**

| Time Period | Mean | Std. Error | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Day 0, 0 hr. | 125.72 | 1.294 | 7.767 | 114 | 140 |
| Day 0, 1 hr. | 145.47 | 1.317 | 7.905 | 129 | 162 |
| Day 0, 2 hr. | 157.25 | 1.173 | 7.036 | 147 | 170 |

Descriptive statistics of FBS, PPBS before induction of the rabbits

**Table 4.7**

**Descriptive Statistics of Fasting Blood Sugar for different time period of Study**

| **Time Period** | **Mean FBS+SE (mg/dl)** | | | |
|---|---|---|---|---|
| | **Placebo** | **Vog. 1mg/Kg** | **Met. 50 mg/kg** | **PHF (polyherbal formulations)** |
| Week-0 | 182.50 ± 4.01 | 182.00 ± 1.93 | 191.5 ± 5,72 | 181.67 ± 4.44 |
| Week-1 | 172.83 ± 2.65 | 141.17 ± 2.47 | 144.83 ± 6.10 | 142.667 ± 4.99 |
| Week-2 | 170,83 ± 6.49 | 139.50 ± 3.75 | 141.50 ± 7.16 | 136.167 ± 6.74 |

| | | | | |
|---|---|---|---|---|
| Week-3 | 170,67 ± 2.93 | 135.00 ± 2,49 | 140.17 ± 6.43 | 136.333 ± 5.21 |
| Week-4 | 174.00 ± 3.99 | 136,67 ± 2.33 | 138.17 ± 3.83 | 135.167 ± 3.88 |
| Week-6 | 173,67 ± 3.62 | 133.00 ± 1,93 | 134,83 ± 5.02 | 131.333 ± 2.70 |
| Week-8 | 172.17 ± 2.73 | 134.33 ± 1.52 | 137,16 ± 4.83 | 130.833 I 4.27 |
| Week-10 | 173.50 ± 3.51 | 135,17 ± 2.27 | 131.83 ± 3.44 | 130.333 ± 3.27 |
| Week-12 | 173.17 ± 2.50 | 130.33 ± 3,07 | 133.83 ± 6.43 | 127.833 ± 1.08 |
| Week-14 | 172.83 ± 2.63 | 131.83 ± 2.39 | 131.33 ± 2,99 | 124.833 ± 1.19 |
| Week-16 | 170.33 ± 3.25 | 128.33 ± 1.89 | 129.17 ±4 2,57 | 125.667 ± 1.31 |
| Week-20 | 169,67 ± 2.29 | 128.67 ± 2.03 | 127,33 ± 2,77 | 125.333 ± 2.17 |
| Week-24 | 168.33 ± 2.75 | 126.00 ± 1.98 | 126,33 ± 2.91 | 123.000 ± 1,69 |

**Table 4.8**
**Kruskal-Wallis Test of significance of treatment effect on FBS**

| Group | Mean Rank:Fasting Blood Sugar | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | Week 3 | Week 12 | Week 16 | Week 20 | Week 24 |
| Placebo | 12 | 33.5 | 31.83 | 33.5 | 33.5 | 33.5 |
| Voglibose 1 mg/kg | 24.75 | 15.17 | 16.75 | 16.67 | 15.25 | 13.25 |
| Metformin 50 mg/kg | 20.92 | 18.17 | 19.17 | 21 | 17.58 | 18 |
| PHF (Polyherbal formulations) | 21.25 | 15.58 | 14.42 | 10.42 | 12.5 | 9.25 |
| "p" Value | 0.222 | 0.01 | 0.006 | 0.003 | 0.008 | 0.002 |

Table 4.8 presented Kruskal-Walis test of significance of treatment effect at Week 0, Week 3, week 12, week 16, week 20 and week 24. In the Week 0 mean ranks for different groups did

not vary significantly (p = 0.222). This indicated at Week 0 the treatment effects are more or less the same for all the groups. In Week 3 and onward still week 24, significant difference was found among the mean rank of treatment groups with p value 0.01. Kruskal-Wallis test simply tells there is either a significant difference or no significant difference among the treatment groups but it does not pin point which pairs of treatment groups do differ significantly or not. Therefore this was followed by Mann-Whitney test.

**Table 4.9**

**Tests of Normality for distribution of PPBS (Shapiro-Wilk) ('p'value)**

| Test of Normality at 1 hour | | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **Week 0** | **Week 3** | **Week 12** | **Week 16** | **Week 20** | **Week 24** |
| Placebo | 0.259 | 0.268 | 0.015* | 0.88 | 0.512 | 0.073 |
| Voglibose 1 mg/kg | 0.31 | 0.521 | 0.23 | 0.528 | 0.5 | 0.045* |
| Metformin 50 mg/kg | 0.88 | 0.878 | 0.313 | 0.883 | 0.96 | 0.052 |
| PHF (polyherbal formulations) | 0.039* | 0.153 | 0.018* | 0.138 | 0.449 | 0.571 |

**Table 4.10**

**Tests of Normality PPBS (Shapiro-Wilk) ('p'value)**

| Test of Normality at 2 hour | | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **Week 0** | **Week 3** | **Week 12** | **Week 16** | **Week 20** | **Week 24** |
| Placebo | 0.383 | 0.013* | 0.351 | 0.017* | 0.020* | 0.033* |
| Voglibose 1 mg/kg | 0.008* | 0.691 | 0.795 | 0.773 | 0.268 | 0.172 |
| Metformin 50 mg/kg | 0.744 | 0.274 | 0.864 | 0.24 | 0.051 | 0.178 |

| | | | | | | |
|---|---|---|---|---|---|---|
| PHF (polyherbal formulations) | 0.241 | 0.338 | 0.196 | 0.085 | 0.031* | 0.014* |

Table 4.12 and 4.13 presents the Shapiro-Wilk test of normality for distribution of PPBS at 1hour and 2hour respectively for different treatment groups at different time interval. At many time points the distributions do not confirm into normality and as such the sample size is also small. Therefore Kruskal-Walis test followed by Mann-Whitney test have been performed. To ascertain the significant difference among treatment groups and between the pair of treatment groups respectively.

**Table 4.11**

**Summary Statistics of PPBS at 1hour (Mean±S.E.)**

| Group | Placebo | Vog. 1mg. | Met. 50 mg. | PHF (polyherbal formulations) |
|---|---|---|---|---|
| **Week 0** | 218.00 ± 2.70 | 195.17 ± 2.80 | 210.00 ± 2.73 | 191.17 ± 3.97 |
| **Week 1** | 202.67 ± 2.22 | 149.33 ± 4.62 | 165.17 ± 3.65 | 142.33 ± 4.27 |
| **Week 2** | 206.17 ± 3.20 | 146.50 ± 4.35 | 158.33 ± 3.71 | 137.83 ± 6.01 |
| **Week 3** | 202.33 ± 5.02 | 146.83 ± 6.07 | 159.33 ± 4.21 | 137.50 ± 5.25 |
| **Week 4** | 203.17 ± 3.12 | 147.67 ± 4.32 | 157.17 ± 2.57 | 135.50 ± 3.64 |
| **Week 6** | 203.83 ± 2.70 | 144.83 ± 3.39 | 150.33 ± 3.58 | 133.83 ± 3.87 |
| **Week 8** | 201.00 ± 2.07 | 143.00 ± 3.10 | 156.00 ± 5.70 | 131.17 ± 4.05 |
| **Week 10** | 200.67 ± 3.25 | 141.17 ± 5.10 | 147.00 ± 3.62 | 130.67 ± 3.51 |

| | | | | |
|---|---|---|---|---|
| **Week 12** | 199.83 ± 2.68 | 140.83 ± 3.88 | 149.83 ± 5.94 | 126.83 ± 1.11 |
| **Week 14** | 199.00 ± 2.86 | 139.00 ± 6.06 | 145.67 ± 3.73 | 124.50 ± 0.72 |
| **Week 16** | 198.00 ± 3.25 | 135.00 ± 4.83 | 148.17 ± 3.47 | 126.17 ± 1.45 |
| Week 20 | 199.17 ± 4.05 | 132.33 ± 3.93 | 145.83 ± 2.90 | 123.83 ± 1.42 |
| Week 24 | 198.17 ± 4.25 | 134.00 ± 6.13 | 140.50 ± 2.88 | 123.00 ± 1.57 |

Table 4.14 presented summary statistics of PPBS atI hour. The mean PPBS level for all the subject before the induction was l50.47±1.31 in the Placebo group the mean PPBS level has maintained the steady level much above the pre inductionvalue. The Voglibose 0.25mg/kg the mean PPBS level declined from 200.17 ± 6.27 to 145.83 ± 2.60 Voglibose 0.5mg/kg brought about the reduction from 202.83 ± 2.90 to 138.00 ± 3.39. Vogliboselmg/kg resulted in the reduction of PPBS at Ihour from 195.17 ± 2.80 to 134.00 ± 6.13. Metformin 50mg/kg reduced the PPBS at I hour from 210.00 ± 2.73 to 140.50 ± 2.88and the combination reduced it from 191.17± 3.97 to 123.00 ± 1.57.

Scatter diagram of PPBS at 1 hour with respect to time for different treatment group provide the opportunity to look at the

impact on individual observation. It is clearly seen that Voglibose 1mg/kg has impacted to bring down the PPBS at 1hour. Most of the study subjects at an earlier period with clear advantage over Metformin 50mg/kg. The combination therapy has alsosimilar kind of result.

**Table 4.12**

**Kruskal-Wallis Test for significance of treatment effect on PPBS**

| Group | Mean Rank PPBS at 1 hour | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week0 | Week 1 | Week 3 | Week 12 | Week 16 | Week 20 | Week 24 |
| Placebo | 31.170 | 33.500 | 33.500 | 33.500 | 33.500 | 33.500 | 33.500 |
| Vog. 1 mg/kg | 11.750 | 11.330 | 13.670 | 15.500 | 12.830 | 11.920 | 12.670 |
| Metformin 50 mg/kg | 24.670 | 23.830 | 22.420 | 20.000 | 23.000 | 22.420 | 18.670 |
| PHF (polyherbal formulations) | 8.500 | 6.670 | 8.580 | 3.920 | 5.580 | 4.830 | 4.580 |
| p' Value | 0.002 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 |

Table 4.15 furnished the result of Kruskal-Walis test for significance of treatment effect on PPBS at 1 hour among the groups. Kruskal-Walis test revealedsignificant treatment effects among the groups from Week 0 onwards (p <0.0 I).

**Table 4.13**

**Summary Statistics of PPBS at 2-hour (Mean±S.E.)**

| Group | Placebo | Vog. 1 mg/kg | Met. 50 mg/kg | PHF (polyherbal formulations) |
|---|---|---|---|---|
| Week 0 | 212.17 ± | 203.33 ± 2.23 | 215.33 ± 2.82 | 198.67 ± 3.24 |

| | | | | |
|---|---|---|---|---|
| | 3.17 | | | |
| **Week 1** | 168.33 ± 2.08 | 151.50 ± 2.81 | 169.17 ± 2.69 | 150.00 ± 5.24 |
| **Week 2** | 155.00 ± 3.12 | 147.33 ± 3.6 | 167.83 ± 2.59 | 142.67 ± 6.09 |
| **Week 3** | 155.00 ± 3.34 | 143.50 ± 4.66 | 163.67 ± 2.47 | 142.17 ± 4.72 |
| **Week 4** | 157.83 ± 5.68 | 141.33 ± 1.12 | 159.83 ± 3.01 | 135.33 ± 2.57 |
| **Week 6** | 149.50 ± 3.5 | 146.33 ± 3.72 | 159.33 ± 4.45 | 140.33 ± 5.51 |
| **Week 8** | 149.50 ± 4.31 | 139.83 ± 1.4 | 157.33 ± 4.54 | 136.17 ± 5.19 |
| **Week 10** | 151.17 ± 4.05 | 139.83 ± 3.16 | 154.50 ± 4.52 | 137.50 ± 4.54 |
| **Week 12** | 153.67 ± 4.31 | 139.67 ± 3.13 | 158.17 ± 5.45 | 139.33 ± 5.86 |
| **Week 14** | 152.OO ± 3.24 | 135.17 ± 1.99 | 155.50 ± 5.25 | 129.00 ± 2 |
| **Week 16** | 154.50 ± 3.1 | 136.33 ± 2.75 | 149.83 ± 4.19 | 129.17 ± 2.14 |
| **Week 20** | 149.67 ± 4.57 | 137.00 ± 3.23 | 151.33 ± 4.15 | 127.33 ± 1.65 |
| **Week 24** | 151.00 ± 2.48 | 132.17 ± 2.89 | 150.67 ± 4.06 | 122.67 ± 1.17 |

Table 4.16 furnished summary statistics of PPBS at 2 hour for different treatment groups for different time periods. Voglibose 0.25mg/kg could reduce the PPBS at 2 hour from121.17 ± 3.17 to 151.00 ± 2.48. Voglibose 0.5mg/kg declined the PPBS at 2 hour from 208.83 ± 1.68 to 142.00 ± 3.52. VogliboseI mg impact the PPBS at 2hour to reduce from 203.33 ±2.23to132.17 ±2.89. Metformin 50mg/kg brought down the PPBS at 2hour from 215.33±2.82 to 150.67±4.06 and the combination therapy resulted in a decline from 198.67±3.24 to 122.67±1.17.

**Table 4.14**

**Kruskal-Wallis Test for test of significance of treatment effect on PPBS**

| Mean Rank PPBS at 2 hour | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | Week 0 | Week 1 | Week 3 | Week 12 | Week 16 | Week 20 | Week 24 |
| Placebo | 33.3 | 33.5 | 33.5 | 33.5 | 33.5 | 33.5 | 33.5 |
| Voglibose 1 mg/kg | 10.8 | 7.67 | 9.58 | 9.67 | 10.6 | 11.6 | 10.4 |
| Metformin 50 mg/kg | 24 | 23.58 | 24.75 | 21.92 | 20.2 | 21.4 | 22.8 |
| PHF (Polyherbal formulations) | 6.67 | 8.33 | 9.25 | 10.33 | 5.08 | 4.25 | 3.83 |
| p' Value | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 |

The Kruskal-Walistest (Table 4.17) revealed significant difference treatment effects among the groups right from week O to week 24.

**Table 4.15**

**Tests of Normality for Glycosylated haemoglobin**

| Shapiro-Wilk test 'p' value | | | |
|---|---|---|---|
| Study Group | Glycosylated Hb 0 day | Glycosylated Hb 80 day | Glycosylated Hb 160 day |
| Placebo | 0.88 | 0.68 | 0,448 |
| Voglibose 1 mg/kg | 0,264 | 0.50 | 0.41 |
| Metformin 50 mg/kg | 0.29 | 0.07 | 0.08 |
| Voglibose 1 mg/kg & Metformin 50 mg/kg | 0.98 | 0.67 | 0.94 |

Table 4.18 furnished the test of normality for glycosylated haemoglobin through Shapiro-Wilktest at 3time periods O day, 80

day and160 day for different treatment groups. The distribution of glycosylated haemoglobin confirms that the distribution is more or less normal. Fig. 20 represented comparative distribution of glycosylated haemoglobin by different treatment groups of different time periods. It revealed that no extreme values for all the treatment groups. Only out lairs are present in Placebo group. Therefore mean treatment was compared through ANOVA followed by post-hoc Bonferroni test.

**Table-4.16**

**Comparison of Mean for different treatment groups (ANOVA)**

| Group | Glycosylated Hb 0 day | Glycosylated Hb 80 day | Glycosylated Hb 160 day |
|---|---|---|---|
| | Mean ± SE | | |
| Placebo | 4.450±0.340 | 6.950±0.247 | 6.583±0.280 |
| Voglibose 1 mg/kg | 4.383±0.279 | 6.650±0.452 | 5.167±0.453 |
| Metformin 50 mg/kg | 4.550±0.488 | 6.783±0.513 | 4.783±0.514 |
| PHF (polyherbal formulations) | 4.517±0.317 | 7.250±0.293 | 4.650±0.267 |
| **ANOVA** (F value) | 0.235 | 0.419 | 3.504 |
| (P value) | 0.944 | 0.832 | 0.013 |

Table 4.19 presented the mean level of glycosylated haemoglobin at different time periods for different treatment groups along with ANOVA 'F' and 'p' values. The mean glycosylated

haemoglobin at O day and also at 80 day do not differ significantly among different treatment groups. At 160 day mean glycosylated haemoglobin differ significantly among the treatment group (p= 0.013).

The Vogliboselmg/kg, combination therapy and Metformin have been able to bring down glycosylated haemoglobin level to the pre-induction level around the time period of 160 days. The significant feature of this analysis is that Voglibose 1mg/kg, Metfonnin 50mg/kg and the combination are effective in controlling the glycosylated haemoglobin level.

# Discussion

The present study was carried out in the Department of BioTechnology and Bioinformatics & Sambalpur University. It was a randomized placebo-controlled, open comparative study spanning a period of 24 weeks to compare the blood glucose levels. The study population was 6 groups (n = 6) of alloxan-induced diabetic rats. Four parameters of blood glucose level - fasting (FBS), 1 hour post-prandial (PPBG1) and 2 hour post-prandial (PPBG2) blood glucose level and glycosylated Hb%(GHb%) was taken at the interval of 80 days for comparison. The effect of a-glucosidase inhibitor, voglibose 1 .0 mg/Kg) was compared with metformin (50mg/Kg), as well as PHF and all these 3 groups were compared with placebo. The pattern of

alternation of the blood glucose level at different weeks was compared in each group with that of pre-induction level i.e. before the rats were made diabetic.

No significant difference in FBS, PPBG I and PPBG2 was found among the, groups before induction of diabetes. But all these parameters were increased significantly after induction of diabetes in all groups. Again no significant difference was found between groups after the induction of diabetes by alloxan.

**Effect on FBS**

The group treated with Voglibose 1 mg/kg, attained the pre-induction level from 2 weeks onwards and maintained consistently till 24 weeks. Metformin 50mg/kg, Attained the pre-induction level and maintained it consistently from 10 weeks onwards.

**Table 4.17: Treatment effect of different groups on FBS in comparison to pre-induction value**

| Group | Day 0 | Day 1 | Day 7 | Day 14 | Day 21 | wk 4 | wk 6 | wk 8 | wk 10 | wk 12 | wk 14 | wk 16 | wk 20 | wk 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean + S.D. : Wilcoxon Signed Ranks Test | | | | | | | | | | | | | |
| Placebo | 127.67 ± 9.61 | 182.50 ± 9.81 | 172.83 ± 6.49 | 170.83 ± 6.34 | 170.67 ± 7.17 | 174.00 ± 9.78 | 173.67 ± 8.87 | 172.17 ± 6.68 | 173.50 ± 8.60 | 173.17 ± 6.11 | 172.83 ± 6.43 | 170.33 ± 7.97 | 169.67 ± 5.61 | 168.33 ± 6.74 |
| Voglibose 1mg. | 130.33 ± 8.87 | 192.00 ± 4.73 | 141.17 ± 6.05 | 139.50 ± 9.18 | 135.00 ± 6.10 | 136.67 ± 5.72 | 133.00 ± 4.73 | 134.33 ± 3.72 | 135.17 ± 5.57 | 130.33 ± 7.53 | 131.83 ± 5.85 | 128.33 ± 4.63 | 128.67 ± 4.97 | 126.00 ± 4.86 |
| Metformin 50 mg. | 121.17 ± 7.44 | 191.50 ± 14.01 | 144.83 ± 14.93 | 141.50 ± 17.55 | 140.17 ± 15.74 | 138.17 ± 9.37 | 134.83 ± 12.29 | 137.17 ± 11.84 | 131.83 ±8.42 | 133.83 ± 15.74 | 131.33 ± 7.31 | 129.17 ± 6.31 | 127.33 ± 6.77 | 126.33 ± 7.12 |
| PHF (Polyherbal Formulations) | 121.00 ± 3.03 | 191.67 ± 10.88 | 142.67 ± 11.99 | 136.17 ± 16.51 | 136.33 ± 12.75 | 135.17 ±9.50 | 131.33 ± 6.62 | 130.83 ± 10.46 | 130.33 ± 8.02 | 127.83 ± 2.64 | 124.83 ±2.93 | 125.67 ±3.20 | 125.33 ± 5.32 | 123.00 ± 4.15 |

## Table 4.18 : Mann -Whitney Test for Comparison of FBS for different treatment groups

| Group | p' value for comparison of FBS for different pairs of treatment groups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week0 | Week1 | Week2 | Week3 | Week4 | Week8 | Week 10 | Week 12 | Week 14 | Week 16 | Week20 | Week24 |
| Placebo &Voglibose 1 mg. | 0.078 | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Placebo & Metformin 5O mg. | 0.172 | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| PHF (Polyherbal formulations) | 0.108 | 0.004* | 0.006* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Voglibose 1mg. & Metformin 50 mg. | 0.872 | 0.336 | 0.809 | 0.687 | 0.335 | 0.809 | 0.294 | 0.744 | 0.872 | 0.517 | 0.624 | 0.261 |
| Vogilbose 1mg and PHF (Polyherbal formulations) | 0.418 | 0.872 | 0.420 | 0.936 | 0.746 | 0.469 | 0.135 | 0.744 | 0.010* | 0.373 | 0.419 | 0.466 |
| Metformin 50 mg. &PHF (Polyherbal formulations) | 0.871 | 0.747 | 0.575 | 0.572 | 0.686 | 0.294 | 0.935 | 0.507 | 0.125 | 0.144 | 0.463 | 0.102 |

In the PHF there is no consistency in attain1nent of pre-induction FBS level . In the Placebo group never attained the pre-induction FBS level in this 24 week of study period.(Table 4.20)

All the alloxan-treated diabetic rats, the FBS showed a downward trend in each •.week till 6 months i.e. at the end of the observation period in all groups of rats including the placebo treated group. Of course the decrease has never significant in placebo-treated group as it never regained its pre-induction level. In the first week none of the pair of treatment groups exhibited significant difference (p<.0 I ).But from 3rd week Onward there is significant difference (p<.0 I) between placebo and all other groups. None of the other pairs did not exhibit any significant difference(p<.05) in treatment effect.(Table 4.21)

After the rats were made diabetic the FBS level showed decrease with passage of time in all groups, though the decrease has not significant in all groups when compared to their pre-induction level. Voglibose Yl .0 mg/kg from second week normalized FBS to the pre- induction level and maintained it consistently up to 6 , month. But it took 4 weeks time to normalize the FBS in case of Metformin but the level fluctuated around the pre-induction level for another 4 more weeks to achieve pre-induction level consistently from 8 weeks onwards up to 6 months. Interestingly, the PHF exhibited fluctuation of FBS level around their pre- induction level all throughout the 6 months of observation. In the placebo-treated group FBS

decreased but the decrease was not significant ($p<.05$) at any point of time in comparison to the pre-induction level.(Table 4.20)

**Table 4.19: Treatment effect of different groups on PPBS at 1hour in comparison to pre-induction value**

| Group | Day 0 | Day 1 | Day 7 | Day 14 | Day 21 | wk 4 | wk 6 | wk 8 | wk 10 | wk 12 | wk 14 | wk 16 | wk 20 | wk 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean + S.D.: Wilcoxon Signed Ranks Test | | | | | | | | | | | | | |
| Placebo | 144.00 ± 10.20 | 218.00 ± 6.60 | 202.67 ± 5.43 | 206.17 ± 7.83 | 202.33 ± 12.31 | 203.17 ± 7.65 | 203.83 ± 6.62 | 201.00 ± 5.06 | 200.67 ± 7.97 | 199.83 ± 6.56 | 199.00 ± 7.01 | 198.00 ± 7.95 | 199.17 ± 9.93 | 198.17 ± 10.40 |
| Voglibose 1mg. | 151.83 ± 8.01 | 195.17 ± 6.85 | 149.33 11.33 | 146.50 ± 10.65 | 146.83 ± 14.87 | 147.67 ± 10.58 | 144.83 ± 8.31 | 143.00 ± 7.59 | 141.17 ± 12.48 | 140.83 ± 9.50 | 139.00 ± 14.85 | 135.00 ± 11.83 | 132.33 ± 9.63 | 134.00 ± 15.02 |
| Metformin 50 mg. | 142.50 ± 8.96 | 210.00 ± 6.69 | 165.17 ± 8.93 | 158.33 ± 9.09 | 159.33 ± 10.31 | 157.17 ± 6.31 | 150.33 ± 8.76 | 156.00 ± 13.97 | 147.00 ± 8.88 | 149.83 ± 14.54 | 145.67 ± 9.14 | 148.17 ± 8.50 | 145.83 ± 7.11 | 140.50 ± 7.06 |
| PHF (polyherbal formulations) | 142.17 ± 4.58 | 191.17 ± 9.73 | 142.33 ± 10.46 | 137.83 ± 14.72 | 137.50 ± 12,85 | 135.50 ± 8.92 | 133.83 ± 9.48 | 131.17 ± 9.91 | 130.67 ± 8.60 | 126.83 ± 2.71 | 124.50 ± 1.76 | 126.17 ± 3.55 | 123.83 ± 3.49 | 123.00 ± 3.85 |

**Table 4.20: Mann -Whitney Test for Comparison of PPBS for different treatment groups**

| Group | p' value for comparison of PPBS for different pairs of treatment groups at 1 hr | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week0 | Weekl | Week2 | Week3 | Week4 | Week8 | Week 10 | Week 12 | Week 14 | Week 16 | Week 20 | Week 24 |
| Placebo &Voglibose 1 mg. | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Placebo & Metformin 50 mg. | 0.054 | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Placebo &PHF (polyherbal formulations) | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Voglibose 1mg. & Metformin 5O mg. | 0.005* | 0.016* | 0.065 | 0.127 | 0.065 | 0.297 | 0.054 | 0.423 | 0.337 | 0.078 | 0.037* | 0.109 |
| Voglibose 1mg. &PHF (polyherbal formulations) | 0.199 | 0.260 | 0.200 | 0.258 | 0.109 | 0.090 | 0.053 | 0.076 | 0.010* | 0.195 | 0.077 | 0.055 |
| Metformin 50 mg. &PHF (polyherbal formulations) | 0.016* | 0.006* | 0.025* | 0.016* | 0.004* | 0.020* | 0.008* | 0.024* | 0.004* | 0.004* | 0.004* | 0.004* |

Effect on PPBG 1:

Voglibose 1 mg/kg, attained the pre-induction level from first week and maintained consistently till 8 weeks. Then the PPBG at 1hr showed significant downward decrease and the trend continued from 8 weeks onwards till end of the study of 24 weeks. The group treated with Metformin 50 mg/kg attained the pre-induction level and maintained it consistently from 6 weeks onwards. In contrast PHF (polyherbal formulations) attained the pre-induction level from first week and maintained consistently 10 weeks after which significant decrease from the pre induction level was observed and the trend continued from 10 weeks onwards till end of the study of 24 weeks. But the Placebo group never attained the pre-induction FBS level any point of observation.(Table 4.21)

All the alloxan-treated diabetic rats, the PPBG I showed a downward trend ineach week till 6 months i e. at the end of the observation period in all groups of rats including the placebo treated group. Of course the decrease was never significant in placebo-treated group as it never regained its pre-induction level. Like the FBS, the PPBG 1 showed one-week earlier in improvement in groups treated Voglibose(1.0 mg/kg) i.e. from **first week** onwards as they achieved their pre-induction value. The improvement was continued consistently upto 6 months in group treated with Voglibose1.0 mg/kg. However, the group showed statistical significant (p=0.05) **hypoglycemia from 8 weeks** onwards till the end of the observation period i.e. 6 months. Metformin

took 6 weeks to normalize the PPBGto pre-induction level and maintained it as such without exhibiting hypoglycemia. The PPBG I level was brought down to pre-induction level in PHF (polyherbal formulations) group from first week and was maintained upto 10$^{th}$ week when hypoglycemia was found which continued upto end of 6 months. (Table 4.22 and 4.23).

**Table 4.21: Treatment effect of different groups on PPBS at 2hour in comparison to pre-induction value**

| Group | Day 0 | Day 1 | Day 7 | Day 14 | Day 21 | wk 4 | wk 6 | wk 8 | wk 10 | wk 12 | wk 14 | wk 16 | wk 20 | wk 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean + S.D.: Wilcoxon Signed Ranks Test | | | | | | | | | | | | | |
| Placebo | 157.83 ± 9.28 | 230.00 ± 4.60 | 215.83 ± 7.91 | 213.67 ± 9.97 | 212.00 ± 10.53 | 217.83 ± 7.31 | 214.83 ± 9.77 | 214.33 ± 9.18 | 213.00 ± 11.51 | 214.50 ± 9.79 | 215.33 ± 10.84 | 214.33 ± 11.57 | 213.33 ± 10.86 | 209.50 ± 11.78 |
| Voglibose 1mg. | 161.33 ± 8.38 | 203.33 ± 5.47 | 151.50 6.89 | 147.33 ± 8.82 | 143.50 ± 11.42 | 141.33 ± 2.73 | 146.33 ± 9.11 | 139.83 ± 3.43 | 139.83 ± 7.73 | 139.67 ± 7.66 | 135.17 ± 4.88 | 136.33 ± 6.74 | 137.00 ± 7.90 | 132.17 ± 7.08 |
| Metformin 50 mg. | 155.67 ± 6.59 | 215.33 ± 6.92 | 169.17 ± 6.59 | 167.83 ± 6.34 | 163.67 ± 6.06 | 159.83 ± 7,36 | 159,33 ± 10.89 | 157.33 ± 11.11 | 154.50 ± 11.08 | 158.17 ± 13.35 | 155.50 ± 12.85 | 149.83 ± 10.27 | 151.33 ± 10.17 | 150.67 ± 9.95 |
| PHF (Polyherbal formulations) | 152.17 ± 4.54 | 198.67 ± 7.94 | 150.00 ± 12.84 | 142.67 ± 14.92 | 142.17 ± 11.55 | 135.33 ± 6.28 | 140.33 ± 13.49 | 136.17 ± 12.70 | 137.50 ± 11.11 | 139.33 ± 14.36 | 129.00 ± 4.90 | 129.17 ± 5.23 | 127.33 ± 4.03 | 122.67 ± 2.88 |

**Table 4.22: Mann -Whitney Test for Comparison of PPBS for different treatment groups**

| Group | p' value for comparison of PPBS for different pairs of treatment groups at 2 hr | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **WeekO** | **Weekl** | **Week2** | **Week3** | **Week4** | **Week8** | **Week** 10 | **Week 12** | **Week 14** | **Week 16** | **Week20** | **Week24** |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Placebo &Voglibose 1 mg. | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Placebo & Metformin 50 mg. | 0.008* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Placebo & PHF (Polyherbal formulations) | 0.004* | 0.004* | 0.004* | 0.004* | 0.003* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* | 0.004* |
| Voglibose 1mg. & Metformin 50 mg. | 0.012* | 0.010* | 0.010* | 0.010* | 0.004* | 0.045* | 0.013* | 0.020* | 0.013* | 0.016* | 0.024* | 0.004* |
| Voglibose 1mg. &PHF (Polyherbal formulations) | 0.197 | 0.810 | 0.422 | 0.810 | 0.072 | 0.470 | 0.688 | 1.000 | 1.000 | 0.090 | 0.019* | 0.009* |
| Metformin 50 mg. &PHF (Polyherbal formulations) | 0.010* | 0.010* | 0.008* | 0.006* | 0.004* | 0.037* | 0.013* | 0.020* | 0.045* | 0.004* | 0.004* | 0.004* |

**Effect on PPBG2:**

The group treated with Voglibose 1 mg/kg attained the pre-induction level from first week and maintained till consistently 3 weeks after which significant decrease from the pre-induction level was observed and the trend continued from 3 weeks onwards till end of the study of 24 weeks. The group treated with Metformin 50 mg/kg, attained the pre-induction level and maintained it consistently from 4 weeks onwards. The PHF (polyherbal formulations) treated group attained the pre-induction level from first week and maintained consistently 3 weeks after which significant decrease from the pre-induction level was observed and the trend continued from 3 weeks onwards till end of the study of 24 weeks. But the decrease pattern exhibited fluctuation. But the placebo group never attained the pre-induction PPBG level. (Table 4.24 and 4.25)

The pattern of change in PPBG2 was in the similar fashion with PPBG I. Only difference is the normalization of PPBG2 to pre-induction level is 1 week earlier in the group treated with Voglibose, 1mg/Kg body weights. In contrast, Metformin achieved pre-induction level of PPBG2 from 4th week and never showed hypoglycaemia. The PHF group exhibited hypoglycaemia 3 weeks earlier (4th week) but the hypoglycaemia was not consistently maintained.(Table 4.24)

Alpha-glucosidase inhibitors competitively block small intestine brush border enzymes that are necessary to hydrolyze oligosaccharides and polysaccharides to monosaccharides. Normally, carbohydrates are primarily and rapidly absorbed in the first half of the small intestine. With a-glucosidase inhibitions, carbohydrate absorption and digestion occurs throughout the small intestine This results in slower absoption of ingested carbohydrates and consequently, the postprandial plasma glucose rise is blunted in animals and humansf' 161. Voglibose is an a-glucosidase inhibitor used clinically for lowering the post-prandial blood glucose levels in patients with diabetes mellitus. In the present study, voglibose lowered the post-prandial blood glucose level at 1 and 2 hour in alloxan-treated diabetic rats.

Alpha-glucosidase inhibitors, like voglibose delay carbohydrate absorption, which results in an increase in sugar absorption in the lower gut. Sugar absorption plays a critical role both in increase and prolonging the secretion of glucagon-like peptide-I (GLP-1), a gut hormone derived from enteroendocrine L cells, in normal individuals and patients with type-2 diabetes. GLP-1 secretion and that GLP-1 producing cells are abundant in the lower gut, delayed carbohydrate absorption is considered to be an acceptable contribution factor for stimulating GLP-1 secretion by alpha-glucosidase inhibition.

Chronic treatment of voglibose also had an impact on enteroendocrine cells, which may contribute to the observed increase in plasma active G LP- 1 level.

Yusuke Moritoh et al also found that when administered chronically (3-4 weeks), Voglibose resulted in a 40-51% decrease in plasma DPP-4 activity and decreased its plasma concentration by 31 - 43%. This decrease in plasmaDPP-4 activity was likely to be sufficient to increase plasma active GLP-1 level Chronic treatment with Voglibose resulted in an increase in active GLP-1 content in the lower intestine (1.5 to 1.6-fold increase) and colon (1.4 to 1.6-foldincrease). The increased active GLP-1 content in the colon was positively correlated with an increase in gut gene expression levels of Neurod I (1,3 to 1.4- fold increase) and Glucagon (2.6- to 3.1-fold increase). The increased gene expression of NeurodI, which is known to be an essential regulator forenteroendocrine cell differentiation, suggests that Voglibose may induce differentiation of enteroendocrine cells.

Thus, Voglibose-generated undigested carbohydrates may play a role in the increase in gene expression of Neurodl and Glucagon, and GLP-l content in the lower gut.

GLP-1 has anti-apoptotic action on cells which is essential for cell survival in response to cellular injury. Cell also exhibits significantly

increased insulin content and improved glucose dependent insulin secretion. In addition to direct effects on inhibition of -cell apoptosis, GLP-1 exerts simultaneous effect on control of glucose homeostatis and islet cell growth. GLP-1 inhibits glucagon secretion and enhances glucose-dependent insulin secretion, actions that promote lowering of glycemia and restoration of normal metabolic milieu. Furthermore, activation of GLP-1 receptor signaling expands islet mass via stimulation for islet neogenesis and induction of cell proliferation in both young and old, normal and diabetic animal in multiple different experimental paradigms. GLP-1 may alsoindirectly attenuates islet glucotoxicityand lipotoxicity, thereby resulting in healthier cell and reduced cell death. As decrease in post-prandial glucose level by Voglibose is withoutinduction of insulin hypersecretion, it is not associated with exhaustion of β- cells.

In this study the improvement of PPGH was observed from the first week oftherapy and is maintained throughout the treatment period of 6 months. Thisfinding is same as was observed by Remi Rabasa-Lhoret et al. But the significant decrease in PPBG was observed at 4 and 8 weeks by ApichatiVicharyanrat et al. This delay and inconsistency of PPBG control was not found in the present study.

The improvement in the Post Prandial hyperglycaemia (PPGH) due to Voglibose has its beneficial effect on FBG. In the present study improvement of PPGH occurred from the first week of continuous

Voglibose therapy but the raised FBS started normalizing after 2 weeks of Voglibose therapy. This finding is in contrast to the finding by ApichatiVicharyanrat et al. who found no significant reduction of FBG with voglibose and acarbose at 4 and 8 weeks after treatment.

The lowering of FBS as observed in the present study observed from second week which was one week after the improvement PPGH. This was rather an expected outcome following the improvement associated with the stimulation of GLP-1 secretion with its accompanied improvement of f3-cell population and activity and turnover and decrease in glucagon secretion.

Voglibose may have a more favorable effect on increasing active GLP-1 levels compared to acarbose by increasing the GLP-1 secretion. Acarbose inhibits alpha-amylase whereas voglibose has a much weaker effect on it when given in a pharmacologically effective dose range. The lack of inhibitory activity of voglibose on a-amylase activity, which seems to generate more disaccharides in the lower gut than acarbose, may contribute to the increase in GLP-1 secretion in diabetic mice. Acarbose has lower efficacy at increasing Glucugon gene expression in the colon in comparison to voglibose. Taken together, these results suggest that the ability of voglibose to increase GLP-1 secretion and gut GLP-1 content accompanied with decreasing plasma DPP-4 activity may explain the reason why voglibose exhibits a more favourable effect on increasing

GLP-1 levels compared to acarbose . Voglibose is a more potent and tolerant α-glucosidase inhibitor as compared to acarbose and miglitol.

| **Table 4.23: Pairwise Multiple Comparison of Glycosylated Haemoglobin after 23Weeks of Treatment Between Groups (Bonferroni)** | | | | |
|---|---|---|---|---|
| **(I) Group** | **(J) Group** | **Mean Diff. (I-J)** | **S. E** | **p' value** |
| | Voglibose 1mg/kg | 1.717 | 0.555 | 0.241 |
| | Metformin 50 mg/kg | 1.8 | 0.555 | 0.044 |
| | PHF (polyherbal formulations) | 1.933 | 0.555 | 0.023 |
| Voglibose 1mg per kg | PHF (polyherbal formulations) | 0.517 | 0.555 | 1 |
| Voglibose 1mg per kg | Metformin 50 mg/kg | -0.167 | 0.517 | 1 |
| Metformin 50 mg/kg | PHF (polyherbal formulations) | 0.133 | 0.595 | 1 |
| *. The mean difference is significant at the 0.05 level. | | | | |

| **Table 4.24- Comparison of Hb with Pre-induction level at day 80 & 60 for different treatment groups (Paired't' test)** | | | |
|---|---|---|---|
| **Group** | **Day 0** | **Day 80** | **Day 160** |
| | **Mean± SD (Paired 't'test)** | | |
| Placebo | 4.45+0.837 | 6.95+0.60 | 6.58+0.69 |
| Voglibose 1mg per kg | 4.38+0.68 | 6.65+1.10 | 5.16+1.10 |
| Metformin 50 mg | 4.55+1.20 | 6.78+1.25 | 4.78+1.25 |
| PHF (polyherbal formulations) | 4.51+0.78 | 7.25+0.71 | 4.65+0.65 |

PPGH is the major determinant of HbAlc levels. Reduction in PPHG significantly reduces HbA levels in Type 2 OM patients. This is also reflected in this study. The group of diabetic rats treated with higher dose of Yogi ibose 1mg/kg the HbA 1c returned to pre-induction value after 23rd weeks of treatment. The HbAlC decreased by 1.5% from 6.65±1.10 to 5.16±1.10.The groups of diabetic rats treated with established oral anti diabetic drug metformin 50 mg/kg normalizes the glycosylated haemoglobin after 23rd weeks of treatment. There is decline in HbAlC from 6.78±1.25 to 4.78±1.25 i.e. 2% decline in glycosylated Hb which is significant (p<.05). The effect of PHF (polyherbal formulations) of metformin and voglibose on glycosylated Hb observed in this study of 24 week and. the HbA1C decrease by 2.6% which is also significant (p<.05) .Long-term reduction of PPBG, as reflected by reduction ofHbAlc below 7% not only caused reduction in the risk of progression to IGT/IFG patients to frank OM, but also caused a 34% risk reduction in development of new cases of hypertension and 49% risk reduction in CVD.(Table 4.26 and 4.27)

So the results obtained in this study and from the support of the literatures cited above it may be inferred that the beneficial effect of voglibose is primarily on the post-prandial blood glucose level. It blunted the postprandial plasma glucose rise by slower absorption of ingested

carbohydrates and increased GLP-1 secretion and decreased OPP-4 level and improvement of β-cell population and activity.

The beneficial effect in the post-prandial hyperglycaemia is reflected in decrease in HbAlc level and in reduction in fasting blood sugar level due to GLP-1- mediated regulation of insulin secretion, glucagon secretion, β-cell turnover in alloxan-induced diabetes mellitus in Rats.

The improvement in PPHG was observed at least 3 weeks earlier with 0.5 mg/Kg than with metfonnin 50 mg/Kg. But once the improvement was achieved the beneficial effect continued equally with both of the drugs. While the PPHG improvement by voglibose is due to delay in absorption of carbohydrate and stimulation of GLP-1 release, the improvement seen by metformin is mainly due to improvement in the peripheral utilization of glucose which may be responsible for the delay in the beneficial effect. However, as both the drugs are not associated with rise of the insulin level, the beneficent effect continued after achievement and later caused lowering of raised FBS due to improved basal secretion from the rejuvenated -cell mass. As expected, the improvement in FBS with metfonnin was observed from $10^{th}$ week of continuous administration.

In this study conducted on alloxan-induced diabetic rat model, Voglibose1.0 mg/Kg produced improvement in PPBG from first week onwards and FBS from second week onwards. The beneficial effect was maintained upto 6 month till the end of the study. Hb1 Ac was significantly decreased after 23 weeks (l 60 days). So it may be concluded that voglibose can be tried as a drug for preventing the diabetes at the stage of IGT. It may be a better alternative to metformin as the beneficial effects comes earlier and is not associated with any serious systemic adverse effect.

**Blood Glucose level of Diabetic Animals:**

**Table- 4.25-** Blood glucose level of diabetic animals

| Sl No | No of Day of Treatment | Normal untreated group (Unit mg/dL) | Metformin Treated group (Unit mg/dL) | Polyherbal Treated group (Unit mg/dL) |
|---|---|---|---|---|
| 1. | Day 0 | 102 | 549 | 497 |
| 2. | Day 2 | 98 | 389 | 298 |
| 3. | Day 4 | 106 | 351 | 176 |
| 4. | Day 6 | 103 | 349 | 132 |
| 5. | Day 8 | 96 | 272 | 118 |
| 6. | Day 10 | 100 | 187 | 101 |
| 7. | Day 12 | 107 | 143 | 94 |
| 8. | Day 14 | 111 | 132 | 88 |

### 4.6.4 Histopathological study:

The study of tissues at a microscopic level contributes significantly to our understanding of developmental processes. Histological examination revealed normal tissue architecture with well-organized cellular

structures. The staining pattern observed in the histological sections indicated a high degree of cellular differentiation. Abnormal histological findings, including increased cellularity and disorganized tissue structure, were indicative of pathological changes.

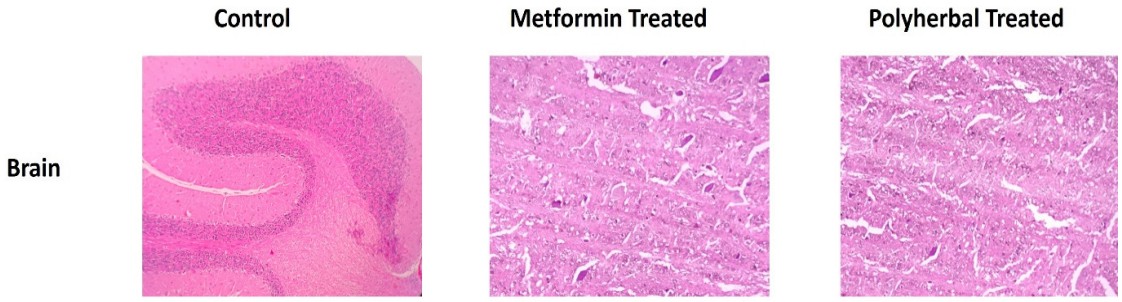



**Figure- 4.8-** Histopathology study of Rats brain during the Acute study of Control, Metformin treated & polyherbal formulation treated

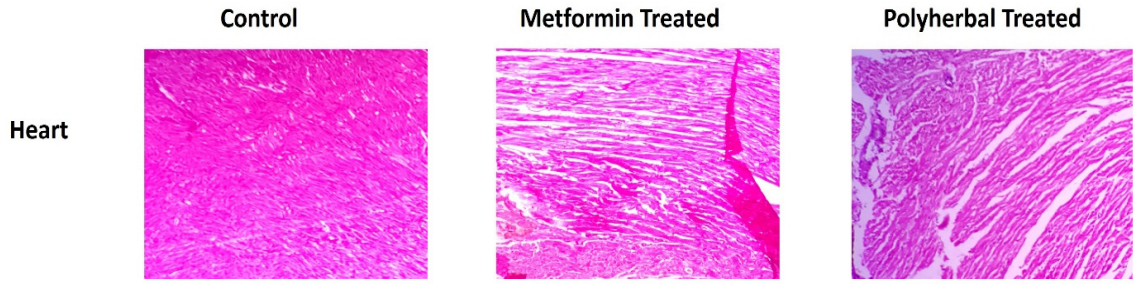



**Figure- 4.9-** Histopathology study of Rat's Heart during the Acute study of Control, Metformin treated & polyherbal formulation treated

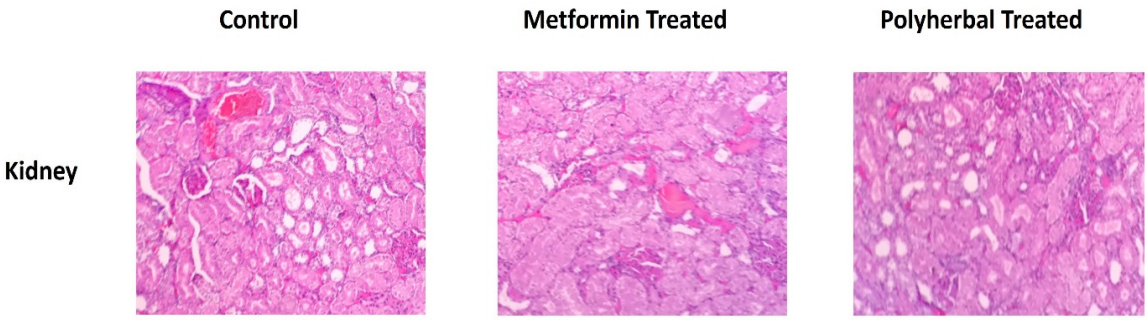



**Figure- 4.10-** Histopathology study of Rat's Kidney during the Acute study of Control, Metformin treated & polyherbal formulation treated

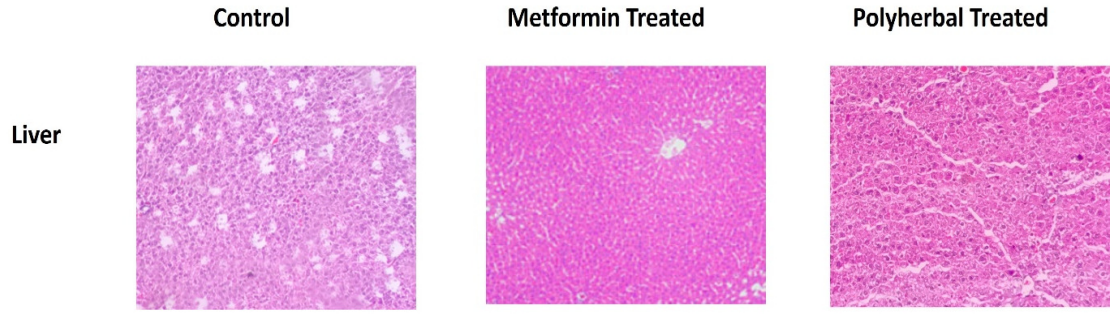



**Figure- 4.11-** Histopathology study of Rat's Liver during the Acute study of Control, Metformin treated & polyherbal formulation treated

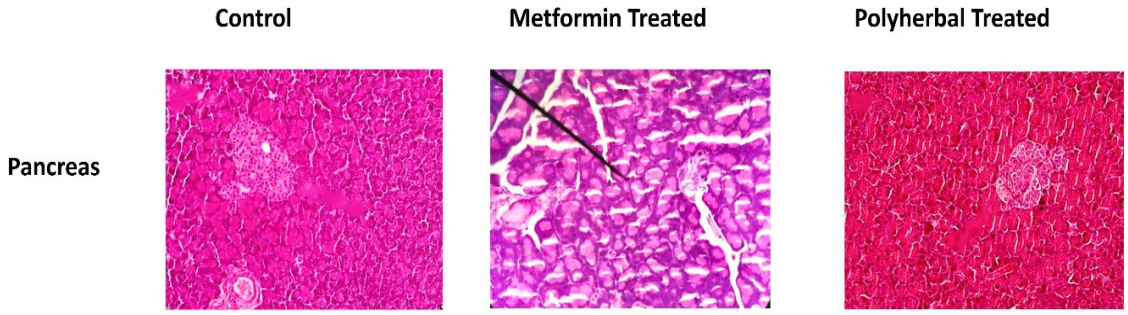



**Figure- 4.12-** Histopathology study of Rat's Pancreas during the Acute study of Control, Metformin treated & polyherbal formulation treated

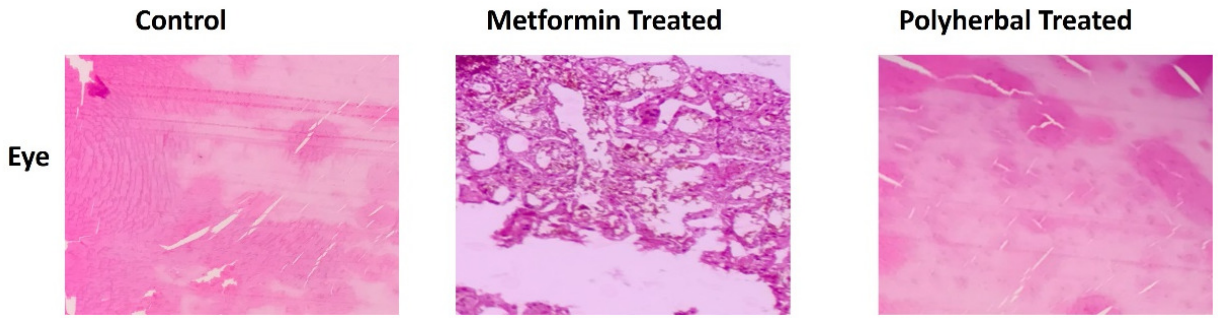



**Figure- 4.13-** Histopathology study of Rat's Eye during the Acute study of Control, Metformin treated & polyherbal formulation treated

**Haematological parameters of rats of Diabetes study:**

The haematological parameters between the treated (metformin and polyherbal formulation) and untreated control groups did not show any side effects (Table 4.27).

**Table 4.26-** Haematological parameters of rats for diabetic study

| Sl NO | Test Name | Normal | Metformin | Polyherbal | Ref Range |
|---|---|---|---|---|---|
| 1. | ALB (Albumin blood test) | 3.2 | 3.6 | 3.87 | 2.3 – 4.0 g/L |
| 2. | UREA | 32.76 | 88.9 | 67.92 | 15.0 – 58.0 g/L |
| 3. | CREA (Creatinine) | 1.2 | 1.6 | 1.3 | 0.5 – 1.8 g/L |
| 4. | Alt (Alanine transaminase) | 29.66 | 1.95 | 4.79 | 10.0 – 12.0 g/L |
| 5. | SGOT | 13 | 22.9 | 23.98 | 0 – 50.0 g/L |
| 6. | TP | 6.4 | 10 | 4.21 | 5.2 – 8.2 g/L |
| 7. | DBIL | 0.32 | 3.90 | 0.43 | 0 - 0.50 g/L |
| 8. | TBIL | 0.56 | 3.78 | 0.85 | 0 – 0.90 g/L |
| 9. | ALP | 113 | 98.78 | 17.09 | 23.0 – 212.0 g/L |
| 10. | HbA1C | 4.3 | 12.8 | 9.7 | 4.0 – 5.6 g/L |

***Identification of marker components***

The marker components in the polyherbal formulation methanol extract were identified and confirmed by their respective mass ion, fragmentation pattern, offline and online mass spectral database, and related literature. Data acquisitions were executed under positive (+ve) and negative (−ve) mode of ionization utilizing full spectrum scan.

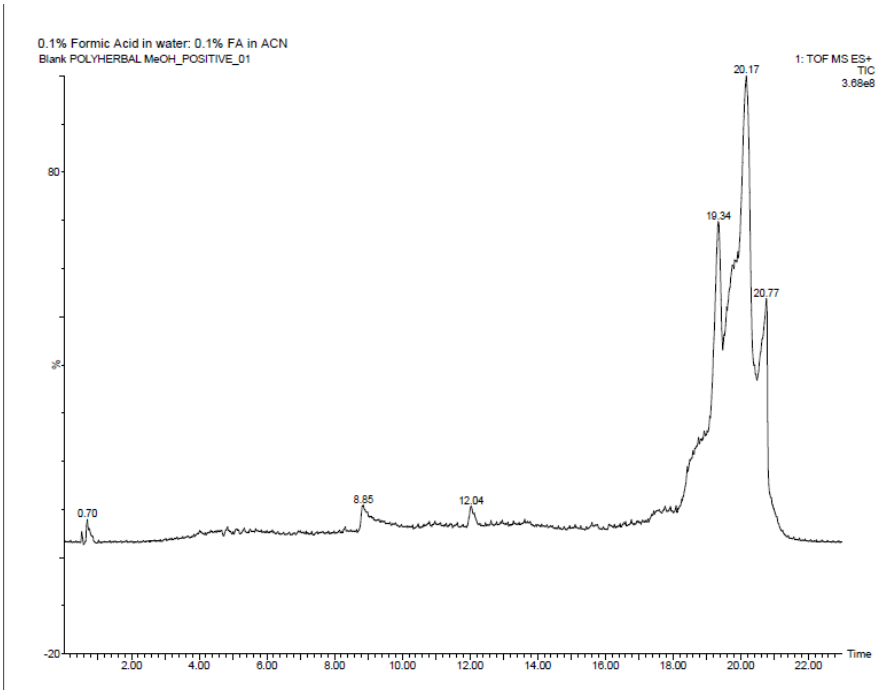



**Figure 4.14** Polyherbal formulation Blank TIC

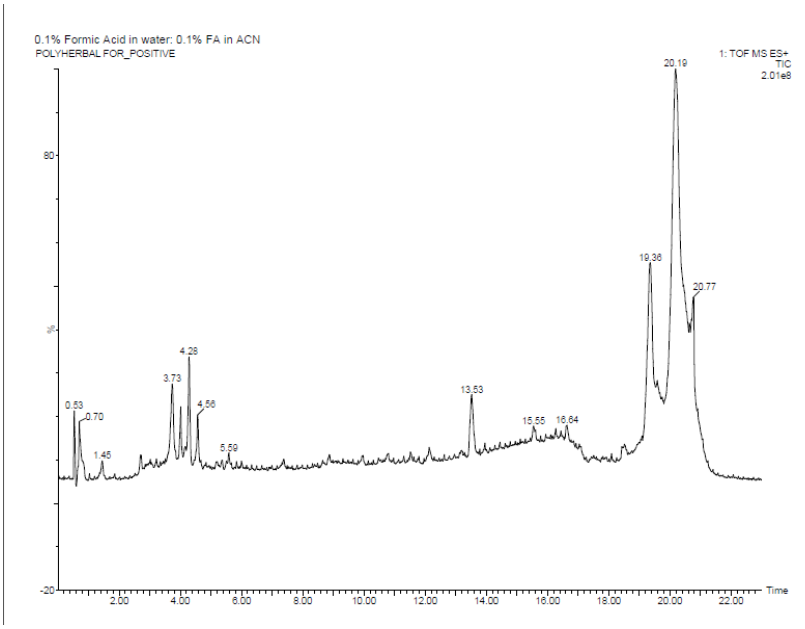



**Figure 4.15** Polyherbal formulation TIC

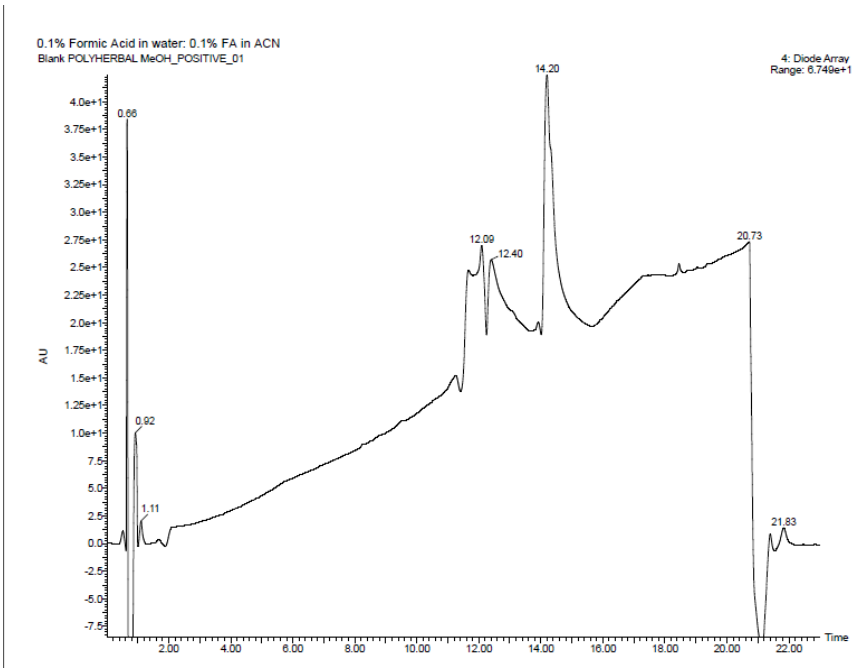



**Figure 4.16** Polyherbal formulation Blank UV Chromatogram

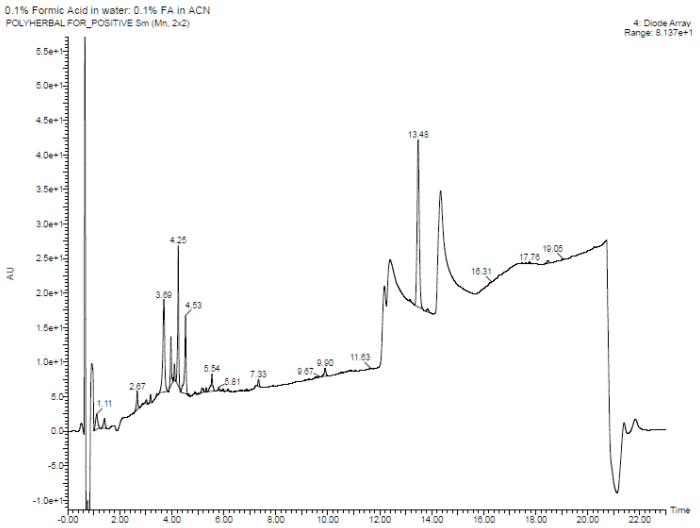



**Figure 4.17** Polyherbal formulation Sample UV Chromatogram

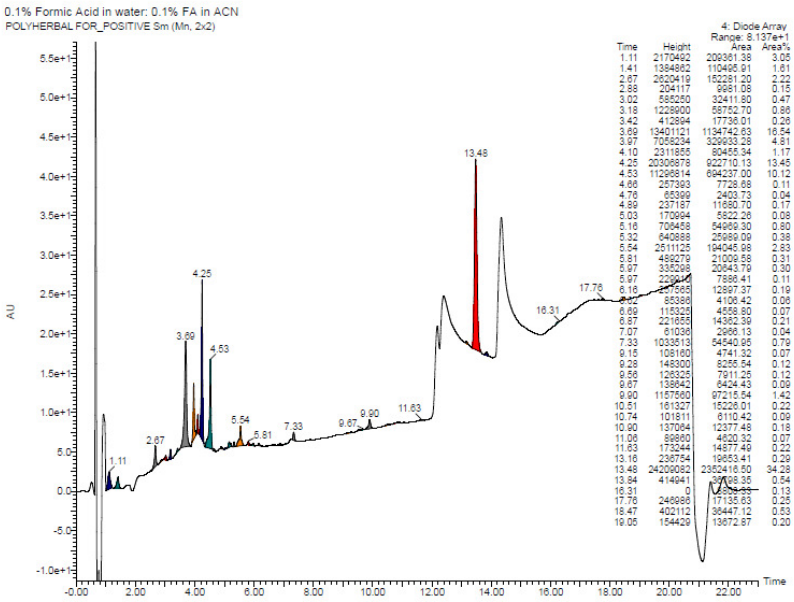
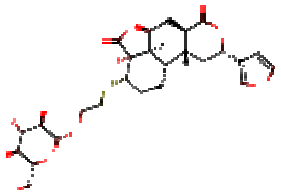
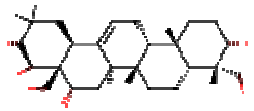
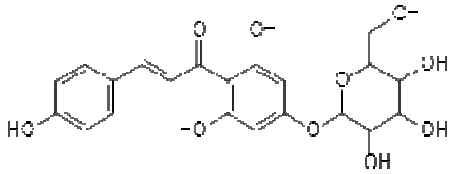
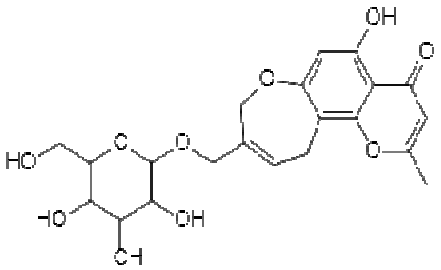
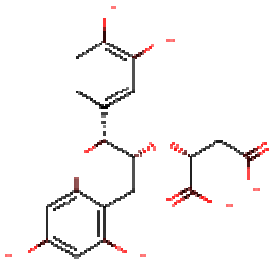
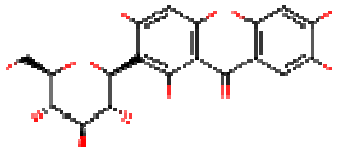
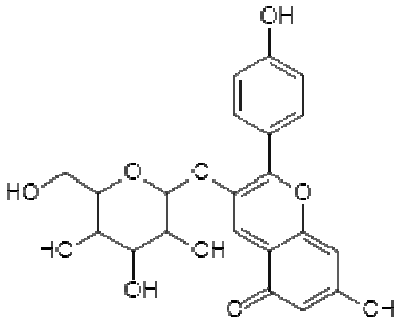
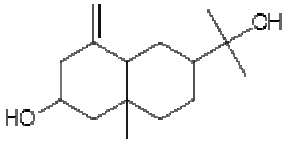
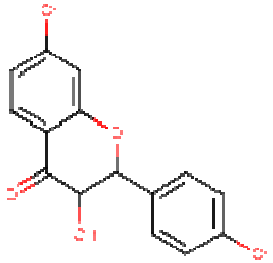
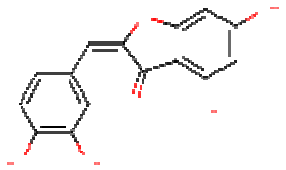
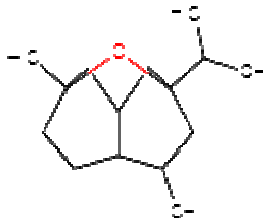
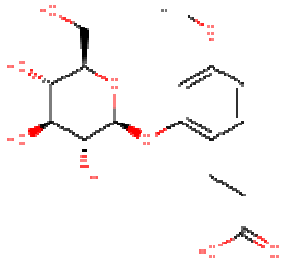


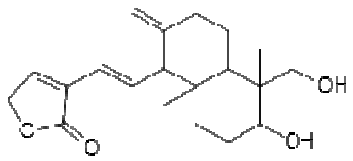

**Figure 4.18** Polyherbal total peak list

| **Table-4.27** Molecular properties description of all isolates | | | | | | |
|---|---|---|---|---|---|---|
| **Description** | **RT (min)** | **Formula** | **Mode** | **Neutral mass (Da)** | ***m/z*** | **Structure** |
| Cordifolide A | 1.45 | $C_{28}H_{38}O_{12}S$ | +ve | 598.66 | 599.6673 |  |
| Gymnemagenin | 3.72 | $C_{30}H_{50}O_6$ | +ve | 506.724 | 507.7313 |  |
| Naringenin 4'-glucoside | 3.73 | $C_{21}H_{22}O_{10}$ | +ve | 434.1223 | 435.1296 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5-hydroxy-2-methyl-9-({[(2r,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}methyl)-8h,11h-oxepino[2,3-h]chromen-4-one | 4.01 | $C_{21}H_{24}O_{10}$ | +ve | 436.1379 | 437.1452 |  |
| (2r)-2-{[(2r,3r)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2h-1-benzopyran-3-yl]oxy}butanedioic acid | 4.06 | $C_{19}H_{18}O_{10}$ | +ve | 406.0914 | 407.0987 |  |
| Mangiferin | 4.33 | $C_{19}H_{18}O_{11}$ | +ve | 422.0849 | 423.0922 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7-hydroxy-2-(4-hydroxyphenyl)-3-{[(2s,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}chromen-5-one | 4.56 | $C_{21}H_{20}O_{10}$ | +ve | 432.1075 | 433.1148 |  |
| Pterocarpol A | 9.48 | $C_{27}H_{46}O$ | +ve | 238.1932 | 239.2005 |  |
| 3,7,4'-Trihydroxy flavonone | 11.67 | $C_{15}H_{12}O_5$ | +ve | 272.0685 | 273.0758 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cernuine | 13.53 | $C_{15}H_{10}O_6$ | +ve | 286.0477 | 287.0550 |  |
| Curcumin-L | 13.79 | $C_{15}H_{26}O$ | +ve | 222.1984 | 223.2057 |  |
| 2-O-β-d-glucosyloxy-4-methoxybenzenepropanoic acid | 15.55 | $C_{11}H_{19}N_3O_3$ | +ve | 358.1264 | 359.1337 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 14-Deoxy-11,12-didehydroandrographolide | 17.14 | $C_{22}H_{24}O_7$ | +ve | 332.1981 | 333.2053 |  |

# *CHAPTER-1*

# CONCLUSIONS

## Conclusion

- A polyherbal formulation consisting of raw powder from 15 different medicinal plants collected from Gandhamardan was developed.
- The methanolic extract of this polyherbal formulation was used to determine the total phenols, total flavonoids and antioxidant activity. The polyherbal formulation was very rich in flavonoids and phenols content and found to have very good antioxidant activity.
- The antidiabetic potential of this polyherbal formulation was assessed in vitro based on inhibition to α-amylase and α-glucosidase enzyme activity. The formulation has very good inhibitory activity against both the enzymes, α-amylase and α-glucosidase.
- The in vivo animal study of the formulation using diabetic model revealed very promising results in terms of reducing the blood sugar significantly without any side effects.
- Therefore, this polyherbal formulation may have a potential herbal formulation for the treatment and management of diabetes.

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# ANNEXURES