MICROARCHITECTURE OF PANCREATIC ISLET AND ITS MODULATION IN DIABETIC ANIMAL DUE TO HERBAL DRUG THERAPY

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by

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CERTIFICATE

This is to certify that the research work entitled, "MICROARCHITECTURE OF PANCREATIC ISLET AND ITS MODULATION IN DIABETIC ANIMAL DUE TO HERBAL DRUG THERAPY" submitted by Abhijit Sahu bearing registration no:55/2022/Biotechnology at Sambalpur University, Odisha, India is a bonafide record of his 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 for the award of degree of ***Doctor of Philosophy in Biotechnology.***

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DECLARATION

I hereby declare that, the work reported in the Ph.D. thesis entitled "**MICROARCHITECTURE OF PANCREATIC ISLET AND ITS MODULATION IN DIABETIC ANIMAL DUE TO HERBAL DRUG THERAPY"** submitted at **Sambalpur University** is the original report of my research, under the joint guidance of **Dr. Pradeep Kumar Naik**, Professor, Dept. of Biotechnology & Bioinformatics, Sambalpur University and **Dr. Pravash Ranjan Mishra,** Professor, Dept. of Anatomy, All India Institute of Medical Sciences (AIIMS), Bhubaneswar. I have not submitted this work previously to any other organization for any degree or professional qualification. I have confirmed the norms and guidelines given in the ethical code of conduct of the university. Whenever I have used materials (data, theoretical analysis and text) from other sources, I have given due credit to them by citing them in the text of the thesis and given their details in the references.

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disorder that has reached pandemic levels globally, affecting millions at an alarming rate and contributing to significant morbidity and mortality. The disease is characterized by persistent hyperglycemia resulting from insulin resistance and/or a progressive loss of pancreatic β-cell function. Islets of Langerhans, a highly vascularized micro-organ of the pancreas, are scattered throughout the exocrine acinus and account for approximately 1–2% of the total pancreatic mass. These consist of 15–20% α-cells (glucagon-producing), 60–65% β-cells (insulin-producing), and the rest are polypeptide P.P. cells and δ cells. Maintaining an optimal islet size is crucial for enhanced functionality. The mantle-core architecture of the cell subtypes within the islets enhances insulin secretion via a paracrine effect. Normal insulin secretion from pancreatic β-cells is facilitated by calcium influx and membrane depolarization.

Motor proteins have garnered significant interest in this field because of their active role in membrane and organelle trafficking. Among the myriad motor proteins, myosin Va stands out for its functions in mRNA trafficking, exocytosis of secretory vesicles, and organelle transport in neuroendocrine cells. This motor protein is highly expressed in endocrine organs and is directly involved in the localization and secretion of endocrine granules. By reviewing these characteristic features of myosin Va, the present study examined the differential expression patterns of the myosin Va motor protein in the endocrine region of diabetic and herbal drug-treated animals.

Despite the availability of various pharmacological treatments, including biguanides, sulfonylureas, thiazolidinediones, and GLP-1 receptor agonists, the long-term use of these synthetic drugs often results in adverse side effects, such as cardiovascular risk (associated with sulfonylureas and thiazolidinediones), gastrointestinal complications, and liver and kidney dysfunctions (associated with biguanides and GLP-1 receptor agonists). These side effects can significantly impact daily activities. Consequently, interest in alternative therapies, particularly those based on natural products and traditional medicine systems, which offer the potential for minimal side effects and have been used for centuries in various cultures to manage chronic health conditions, including diabetes, is increasing.

Anecdotal evidence suggests that ethnopharmacological agents may have a favourable impact when used as adjunct therapies for managing various ailments, including diabetes. Recent WHO reports indicate that many developing countries (70–80%) rely on traditional

methods of treatment involving the use of medicinal plants to manage various chronic health conditions, including diabetes, due to their lower risk of side effects than modern therapies.

Current research focuses on identifying potent ethnopharmacological agents with promising biological activity. Natural products, especially those derived from medicinal plants, have shown potential antidiabetic properties through various mechanisms, including enhancing insulin secretion, improving insulin sensitivity, inhibiting carbohydrate-digesting enzymes, and protecting pancreatic β-cells from oxidative stress.

This study aims to validate the antidiabetic efficacy of polyherbal formulations and individual plant extracts, including *Tinospora cordifolia* and *Mangifera indica*, through a multitiered approach involving *in silico, in vitro,* and *in vivo* antidiabetic models. It also explores the microarchitectural changes in pancreatic islets in diabetic animal models and their modulation with herbal drug therapy. Additionally, we assessed the potential restoration of pancreatic β-cell function and the expression of the myosin Va motor protein following treatment with these natural products. Furthermore, the present study also examined the active role of myosin Va in the transportation of pancreatic β-cells toward the final stage of exocytosis.

The primary objective of the present research was to evaluate the antidiabetic efficacy of the developed polyherbal formulation by utilizing *in silico, in vitro,* and *in vivo* antidiabetic models. To investigate the effects of standardized ayurvedic formulations (prepared from selected plants) on the morphology of the pancreatic islets in diabetic rodents. To study the secretion capacity of endocrine cells on the basis of the intensity of the Myosin Va motor protein after treatment with standardized formulations.

Materials and Methods:

The sixteen selected plant parts were freshly collected from the flora of Gandhamardhan Hill, Bargarh, Odisha, and validated by the Taxonomist Regional Plant Research Centre (RPRC) Bhubaneswar. After drying in a tray dryer, the samples were powdered with a versatile pulverizer and passed through a sieve of 10 mesh for uniformity. The polyherbal formulation was prepared by adding all sixteen plants in equal proportions and subjected to aqueous extraction (APE) with a Soxhlet apparatus. After standardization, three plants (*T. cordifolia, S. cumini* & *M. indica*) were further used to prepare another aqueous polyherbal formulation by mixing in equal proportions and then subjected to aqueous extraction (APF) via a Soxhlet apparatus. For individual preparations (*T. cordifolia* & *M. indica*) of plant

extracts, the powdered plants were subjected to hydroalcoholic extraction (ethanol:water) via a microwave extraction system. After extraction, the yield extract was lyophilized and used in the present study.

Quantitative and biochemical analysis

The lyophilized extracts (APE, APF, *T.C.*, *M.I.*) were quantified for identification of the bioactive compounds via advanced quantitative techniques such as Fourier transform infrared spectroscopy (FTIR), ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS), and Gas chromatography–mass spectrometry (GC–MS). The total phenolic content (TPC) was quantified using gallic acid equivalent (GAE), and the total flavonoid content (TFC) was quantified using quercetin equivalent (Q.E.). All the extracts were subjected to biochemical analyses, including free radical scavenging activity (DPPH, ABTS, and FRAP) and *in vitro* enzyme inhibition assays (such as α-amylase, α-glucosidase, and β-glucosidase). The results were expressed as IC_{50} values to determine the potency of the extracts.

In silico study

The identified bioactive compounds from the analytical studies of the extracts were prepared. Insulin-like growth factor-I (IGF-I) and glucose transporter 4 (GLUT 4) proteins were selected for docking studies against the selected bioactive compounds. The Schrodinger molecular environment was utilized for the docking and prediction of the ADME properties of these proteins and ligands. This analysis helped to elucidate potential mechanisms of action for the phytochemicals.

In vitro cell assays

The *in vitro* cell assays were performed using 3T3L1 (adipocytes) and MIN6 β (isolated from pancreatic islets) cell lines. The cell cytotoxicity assay was performed using the 3T3-L1 cell line. A cellular glucose uptake assay utilizing the 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG) fluoroprobe was performed in the MIN6 β cell line. This assay was performed to evaluate the effects of the extracts on insulin-mediated glucose uptake. The 3T3L1 adipocyte line was used for the lipid accumulation study. The oil red O staining technique was used to detect the accumulated lipid droplets.

In vivo antidiabetic study

Wistar rats were selected for the present antidiabetic study. Acute and subacute oral toxicity studies were performed prior to the experiment to determine the safe dose. Alloxan

was injected intraperitoneally *(i.p.)* for the development of diabetic models. The rats were randomly divided into control, untreated diabetic control, positive control, and herbal drug-treated groups. Diabetic animals were treated with either metformin or herbal extracts for four weeks. Blood glucose and HbA1c levels were measured to assess long-term glucose control. Serum markers, including glucose, albumin, urea, creatinine, cholesterol, triglycerides, and liver enzymes (SGPT, SGOT, ALP), were evaluated to assess the safety and efficacy of the treatment. Upon completion of the experiment, the animals were sacrificed by mild anaesthesia (isoflurane), blood was collected via cardiac puncture for biochemical parameter studies, and the pancreatic tissues were fixed in 10% neutral buffered formalin (NBF). The fixed tissues were processed, and paraffin blocks were prepared. Each block was subjected to H&E and IHC staining with primary antibodies, such as anti-synaptophysin, anti-insulin, anti-glucagon, and anti-myosin. The stained slides were observed under a microscope in the bright field region and quantified using Image J software.

Results

UHPLC-Q-TOF-MS analysis of the aqueous polyherbal formulation (APE) revealed 60 bioactive compounds, of which 39 compounds exhibited antidiabetic activity. The APE has 6.465 mg/Q.E. g D.W. and 10.089 mg/GAE g D.W. of total flavonoids and phenolic content, respectively. APE scavenges oxygen free radicals, with IC_{50} values of 24.15 µg using DPPH, 25.485 µg using ABTS, and 39.95 µg using FRAP methods. A molecular docking study demonstrated the strong interaction of verbacoside B with the target proteins insulin-like growth factor-I (IGF-I) and GLUT4. This interaction suggests that the compound enhances insulin secretion and inhibits carbohydrate-digesting enzymes, subsequently increasing glucose uptake into cells and improving insulin sensitivity and overall glucose metabolism, thereby providing a multifaceted approach to managing diabetes. *In vitro* carbohydrate digestive enzyme inhibition assays revealed that APE significantly inhibited α-amylase and α-glucosidase at a minimal concentration. The calculated IC_{50} values were 54.26 ± 0.14 and 26.47 ± 0.12 µg/ml, respectively. Glucose uptake assays in MIN6 cells demonstrated that APE enhanced insulin secretion and glucose uptake in a dose-dependent manner, further supporting its antidiabetic efficacy. *In vivo,* acute and subacute oral toxicity studies revealed nontoxic nature of APE. At a dose of 500 mg/kg body weight, APE significantly decreased blood sugar and HbA1c levels and had no side effects on liver or kidney function, as measured by blood serum parameters. A histopathological study (H&E) revealed structural alterations in the

pancreatic islets of the untreated diabetic control; however, a significant restoration near that of the normal control was observed in the APE-treated group. An immunohistochemical study revealed a 47% restoration of pancreatic β-cells following APE treatment in diabetic animal models, demonstrating the ability of APE to restore pancreatic function.

Gas chromatography–mass spectrometry (GC–MS) and Fourier transform infrared (FT–IR) spectroscopic analysis revealed the presence of several bioactive compounds in the ayurvedic polyherbal formulation (APF). The quantitative analysis revealed a total phenolic content of 8.957 mg GAE/g DW and a total flavonoid content of 5.786 mg QE/g DW. The antioxidant efficacy of APF was assessed via DPPH, ABTS, and FRAP. The calculated IC_{50} values were 27.07, 41.31, and 25.92 µg/ml, respectively. *In vitro,* a carbohydrate digestive enzyme inhibition assay revealed that APF inhibits the α-amylase and α-glucosidase enzymes, with calculated IC_{50} values of 42.92 ± 0.24 and 30.60 ± 0.17 µg/ml, respectively. An oral toxicity study revealed 250 mg/kg body weight as the effective dose of APF, which does not affect other vital organs. The treated diabetic animals had significantly decreased blood glucose and HbA1c levels, suggesting that APF is capable of controlling postprandial blood glucose levels. A significant decrease in the size of islets was observed in the untreated diabetic control group (60–80 µm) compared with the normal control group (130–200 µm). In contrast, the APF-treated group presented significantly larger islet sizes (110–185 µm). Immunostaining with an anti-insulin antibody revealed 49.07% regeneration of pancreatic β-cells after APF treatment. The expression of the myosin Va molecular motor protein was significantly lower in the untreated diabetic control group (11.265 ± 2.15) than in the normal control group (70.285 ± 2.38). However, APF treatment restored its expression to 57.082 ± 1.28. The restoration of the myosin Va protein indicates its role in facilitating the movement of insulin granules towards the final stage of exocytosis, thereby normalizing hyperglycemia. Notably, the expression of myosin Va in the exocrine pancreas remained consistent across both the diabetic and treated groups (30.81 ± 1.44).

Fourier transform infrared (FT-IR) spectroscopy and gas chromatography–mass spectrometry (GC–MS) analysis of *Tinospora cordifolia* revealed the presence of diverse functional groups and a number of bioactive compounds with numerous biological activities, including antidiabetic activity. Quantitative analysis revealed that the extract contained 27.85 mg GAE/g DW of total phenolic content and 4.138 mg QE/g DW of total flavonoid content. The antioxidant activity of the extract was calculated in terms of DPPH free radical scavenging

activity, with a calculated IC_{50} value of 5.40 µg/ml. *T. cordifolia* extract inhibited carbohydrate digestive enzymes (α-amylase and β-glucosidase) at a minimal concentration, which was comparable with standard ascorbic acid. The calculated IC_{50} values were 49.32 µg/ml and 44.36 µg/ml for α-amylase and β-glucosidase, respectively. A lipid accumulation assay using a 3T3-L1 cell line revealed that *T. cordifolia* extract inhibited lipid accumulation in a dose-dependent manner, and the calculated IC_{50} value was 48.68 µg/ml. Prior to the *in vivo* antidiabetic study of *T. cordifolia* extract, oral toxicity tests were conducted to determine the safe dose and evaluate the toxic nature of the extract. The safe dose was calculated to be 250 mg/kg body weight, and the extract did not affect the other vital organs after administration. A physiochemical parameter study revealed that, compared with the untreated diabetic control group (123 ± 1.06 g), the extract-treated group (209.3 ± 1.24 g) presented a significant increase in body weight, confirming the positive effects of the extract on physiochemical parameters. A substantial reduction in blood glucose levels was observed in the extract-treated group (118 ± 5.35 mg/dl) compared with the diabetic control group (492 ± 0.98 mg/dl), illustrating its antidiabetic efficacy. The elevated kidney and liver functions observed during diabetes were restored to normal with the extract treatment, suggesting its broad spectrum of beneficial effects on various metabolic and organ-specific parameters under diabetic conditions. Histopathological and immunohistochemical studies revealed significant restoration of both synaptic integrity and insulin production in the pancreatic islets. In the extract-treated group, 34% of the β-cells regenerated.

Quantitative analysis of the *Mangifera indica* extract revealed total phenolic and flavonoid contents of 17.02 mg GAE/g and 2.35 mg QE/g D.W., respectively. The DPPH free radical scavenging activity assay revealed the highest scavenging activity of 97.09%, with a calculated IC_{50} value of 50.54 µg/ml. The *in vitro* carbohydrate digestive enzyme (β-glucosidase) inhibition assay revealed better inhibition activity with the extract treatment. The IC_{50} value was calculated to be 76.77 µg/ml, which was comparable with the standard. The oral toxicity study suggested that the safest dose for treatment was 200 mg/kg body weight, which is nontoxic to other vital organs. A positive effect of extract treatment was observed in managing elevated parameters such as body weight, blood glucose levels, and HbA1c levels. The biochemical serum marker levels were also well controlled with the extract treatment, suggesting its beneficial characteristic features of various metabolic and organ-specific parameters under diabetic conditions. Histopathological analysis revealed significant

restoration of the structural architecture of the pancreatic islets in the extract-treated animals compared with those in the diabetic control animals. This result signifies the regenerative potential of the lost pancreatic β-cells.

Discussion & Conclusion

This study highlights the potential of polyherbal formulations (APE and APF) along with hydroalcoholic extracts of *T. cordifolia* and *M. indica* as promising adjunct therapies for diabetes management. Our *in silico, in vitro,* and *in vivo* analyses revealed that these natural products exert substantial antidiabetic effects through diverse mechanisms of action. Secondary metabolites, including phenols and alkaloids, play a key role in inhibiting carbohydrate-digestive enzymes and improving lipid metabolism, further enhancing the therapeutic potential of these extracts.

A remarkable finding was the significant regeneration of β-cells and restoration of myosin Va expression in the treated groups compared with those in the controls. This regeneration within the pancreatic islets and recovery of β-cell function, along with the expression of myosin Va, a marker of insulin secretory potential, suggest an enhanced exocytotic capability within β-cells. These results underscore the ability of these herbal formulations to restore pancreatic architecture and functionality. Collectively, our findings support the use of these natural products as valuable therapeutic candidates for diabetes. Their multifaceted benefits complement conventional treatments, offering a powerful, natural option in diabetes management.

1. Introduction

As a leading cause of morbidity and mortality, Diabetic Miletus (DM) imposes a substantial burden on healthcare systems, necessitating a comprehensive understanding of its pathophysiology and management. Chronic hyperglycemia as a key attributor arises from impaired insulin secretions and resistance are the major characteristic features of D.M. Lifestyle changes have significantly contributed to the rising incidence of DM globally, with many developing countries now confronting a burgeoning diabetic 'epidemic' that poses substantial public health challenges. Chronic diabetes leads to a spectrum of complications including micro (retinopathy, nephropathy, and neuropathy) and macro (cardiovascular) vascular which collectively contribute to the damage/dysfunctions of various vital organs. Recent diabetes data from the International Diabetes Federation (IDF 2021), has a global incidence rate of about 537 million cases and 2.2 million deaths contributed by this disease every year. India has become the capital of diabetes, contributing around 77 million cases yearly. Clinically DM is categorized as Type1 Diabetic Miletus (T1DM), Type2 Diabetic Miletus (T2DM), Maturity onset Diabetic Miletus, and Gestational DM Diabetic Miletus. Out of these T1DM and T2DM are the most prevalent forms. Regardless of the type of diabetes the proper control of the blood glucose levels and its associated complications management are the major tasks for a good healthy lifestyle. In addition, this polygenic disease has some more common symptoms like excessive thirst, frequent urination, sweating, blurred vision, sudden weight loss, fatigue, and slow-healing sores. The digestive enzyme including pancreatic lipase (PL), pancreatic α-amylase (PA), and α-glucosidase plays a crucial role in regulating the blood glucose levels in diabetes. Inhibiting these key enzymes, thereby reducing D-glucose liberation and absorption, represents a promising strategy for developing potent new drugs to manage hyperglycemia and control plasma glucose levels (Aierken *et al.,* 2017; Cai *et al.,* 2016; Darenskaya *et al.,* 2021; R. Kumar *et al.,* 2020). Type-I, type-II, and maturity-onset diabetes mellitus is the most prevalent form of DM, approved by the American diabetes association (ADA). The details classifications and their symptoms are described in the table below (Table 1.1).

Table 1.1. Types of Diabetic Miletus and their mechanism.

Sl No	Type of Diabetes	Brief description of mechanism action
1.	Type-I DM	T-helper cells, cytotoxic lymphocytes, and autoantibodies contribute to the autoimmune destruction of pancreatic β-cells in the endocrine pancreas, resulting in diminished insulin secretion and the onset of Type 1 Diabetes Mellitus (T1DM).
2.	Type-II DM	The most prevalent one among all other types of DM. It is clinically characterized by varying degrees of β-cell dysfunction and insulin resistance and is frequently associated with overweight and obesity.
3.	Maturity onset DM (MOD)	The clinical feature of MOD embraces the age of onset before 45 years. The autoimmunity loss of pancreatic β-cells, features of metabolic syndrome, sustained endogenous insulin production, and a strong family history.
Diagnostic criteria for diabetes: Fasting blood sugar level of ≥126 mg/dL; 2-hour post-load plasma glucose ≥200 mg/dL & HbA1C ≥6.5%.		

1.1. Islets of Langerhans

Islet biology plays a crucial role in maintaining a healthy lifestyle. The pancreas is an inimitable gland situated in the digestive system, comprised of different components including exocrine, endocrine, ducts, and connective tissues. These pancreatic lobules consist of a blend of ductules and well-vascularized epithelial cell clusters under the microscope, exhibiting the dual role of the pancreas including digestion and glucose homeostasis. For specific tissue functions within the organ, parenchyma (islets of Langerhans) that, consist of only the endocrine cell mass are important and responsible for the maintenance of glucose homeostasis. The endocrine parenchyma is invaded from the capillary network by the vascular cells, which are effectually connecting the islets for systematic circulations. Additionally, the microenvironment of the parenchyma plays a

crucial role in regulating the function of the immersed cells. The glucose concentration in the parenchyma directly relies upon the contradictory secretions of insulin (β-cells) and glucagon (α-cells). Insulin incorporates the glucose molecules into the liver, muscles, and adipose tissue anabolically, and accumulates it as fats and glycogen. In contrast, glycogen helps in releasing glucose molecules into the bloodstream through its catabolic effects via activating liver glycogenolysis and gluconeogenesis (Almaça *et al.,* 2020; Bano, 2013; Quesada *et al.,* 2008).

The exocrine cells, which make up 98% of the pancreatic parenchyma, secrete a combination of digestive enzymes and bicarbonate into the duodenum. The endocrine cells, which make up 1-2% of the total pancreatic mass, are dispersed throughout the pancreatic parenchyma, situated between the acini and ductal structures. These cells have been extensively researched concerning Diabetes Mellitus (DM) due to the hormones they produce and secrete into the portal vein, which plays a crucial role in maintaining glucose homeostasis. Following the discovery of the direct role of insulin in regulating blood glucose levels, research has focused on the biology of insulin-producing β-cells within the islets, as their dysfunction and subsequent loss of insulin production are closely linked to diabetes. The Islets of Langerhans comprise five distinct types of endocrine cells. In 1907, Lane first identified glucagon-releasing α-cells and insulin-secreting β-cells based on their histochemical staining properties. Somatostatin-producing δ-cells were later identified by Bloom in 1931. The discovery of pancreatic polypeptide-producing PP-cells and Ghrelin cells was facilitated through immunocytochemistry techniques. The secreted hormones from islets are more crucial for regulating the blood glucose level (decreases of insulin & increases of glucagon proportion). Hence, a tight regulation of these hormones effectively responds to metabolic demands. This regulation involves multiple mechanisms, including intrinsic cellular glucose sensing, paracrine interactions between islet cells, autonomic nervous inputs, and hormones from the gastrointestinal system (Da Silva Xavier, 2018; In't Veld & Marichal, 2010; Koh *et al.,* 2012).

Pancreatic β-cells a 51-aminoacid peptide, are the main cellular component of the islets of Langerhans and occupy 70-80% of the total endocrine region. It regulates the whole-body metabolism by secreting insulin in response to elevated glucose levels. Insulin is stored in secretory granules complexed with zinc. It is released when glucose levels are high and is further stimulated by neurotransmitters. Additionally, the presence of incretin hormones enhances insulin secretion. Understanding the cellular mechanisms of insulin secretion is of great interest, as defective insulin secretion can lead to disorders of blood

glucose homeostasis, such as diabetes mellitus. The release of islet's hormones is primarily associated with the glucose-sensitive islet cells. A higher glucose concentration triggers the exocytosis of the pancreatic β-cells that requires the influx of Ca^{2+} ions from the extracellular space. During the exocytosis process a series of reactions take place i.e., i) glucose is imported and metabolized within the cell, by increasing the ATP/ADP ratio; ii) elevated ATP levels block the K_{ATP} channels, subsequently causing the membrane depolarization and iii) this depolarization activates the voltage-gated Ca^{2+} channels, leading to an influx of Ca^{2+} ions, which in turn mediates the Ca^{2+} dependent exocytosis of insulin granules. α-cells oppositely respond to glucose compared to β-cells, this may be due to the membrane potential-dependent inactivation of voltage-gated Ca^{2+} channels (Göpel *et al.,* 2000; Koh *et al.,* 2012; Ramracheya *et al.,* 2010).

1.2. β-cells dysfunction and development of Diabetes

Approximately 80% of the endocrine parenchyma consists of β-cells. Within the pancreatic islets, these cells engage in a delicate hormonal dance through homologous and heterologous interactions, facilitated by paracrine effects. Disruptions in the function of these parenchymal cells result in impaired insulin secretion, ultimately, leading to β-cell exhaustion, which precedes the demise of these crucial cells, a central factor in the development of diabetes. The major risk factors for the dysfunction of this β-cell include oxidative stress, endoplasmic reticulum (ER) stress, islets capillary, obesity, cytokines, and inflammation (Almaça *et al.,* 2020; Cerf, 2013).

At the basal level, reactive oxygen species (ROS) play a significant role in the secretions of insulin via moderate $Ca2^{+}$ influx, and activating ryanodine receptors (RyRs) channels. The mitochondrial electron transport chain (ETC) is the leading contributor to the production of ROS. However, excessive ROS accumulation subsequently leads to oxidative stress and impacts the secretory capacity of the pancreatic β-cell. Increased demand for insulin during hyperglycemic conditions significantly affects the proinsulin misfolding and disturbs the unfolded protein response (UPR). This, in turn, plays a pivotal role in β-cell dysfunction, emphasizing the importance of managing ER stress. The parenchyma is a highly vascularized micro-organ of the pancreas, receiving about 15-20% blood supply. The abnormalities in the islet's capillary morphology including capillary hypertrophy, irregular morphology, thickening, and fibrosis play an immortal role in dysfunction. Obesity influences insulin sensitivity and subsequently modulates the function of β-cell and decreases the secretion of insulin for the maintenance of hyperglycemic conditions. The concentration of nonesterified fatty acids (NEFAs), hormones, cytokines, proinflammatory substances, and glycerol influence insulin

resistance and ultimately, β-cell function may deteriorate, leading to impaired insulin secretion. The pro-inflammatory cytokines are related to obesity via an inflammatory response triggered by these cytokines, which subsequently leads to the dysfunction of pancreatic β-cell. In addition to this, mitochondrial stress and the immune cells can also directly lead to the dysfunction of β-cell (Al-Goblan *et al.,* 2014; Eguchi *et al.,* 2021; Hogan & Hull, 2017).

Pancreatic β-cells play a crucial role in regulating insulin release, the regulation fluctuates due to their quantity, nature, and route of stimulus administration. Continuous failure of these β-cell functions is the leading cause of the development of diabetes characterized by elevated insulin secretion, fasting blood glucose, and postprandial blood glucose. High blood glucose levels resulting from glucotoxicity aggravate the severity of DM and harm the peripheral tissue sensitivity and insulin uptake capacity. In addition to this higher plasma NEFA levels are another contributing factor for the dysfunction of these β-cells via modulating the glucose-stimulated insulin secretion pathways which subsequently reduces insulin biosynthesis (Boden, 1996; Gallagher *et al.,* 2000; Kahn, 2001; Kahn *et al.,* 2006).

1.3. Myosin: Molecular motor protein

Unlike machines play a direct role in the virtual movement of all aspects of life, myosin molecular motor protein converts the cellular free energy into mechanical energy. This molecular motor protein is crucial for various cellular processes including reproduction, childbirth, growth, development, and immune responses. In addition to this, it also contributes to various disease susceptibility. For the executions of specific and dedicated cellular function variety of motor proteins are there i.e., nucleic acid (RNA and DNA polymerases, Helicase, topoisomerases, RSC, SW1/SNF complex, SMC, viral DNA packaging protein), polymerization (actin, microtubule, dynamin), rotary (F0F1-ATP synthase), and cytoskeletal (myosin, kinesin, dynein) motor protein. Among these, myosin stands out due to its active role in organelle trafficking and high expression in the endocrine region of various vital organs including skeletal muscles, and neuroendocrine cells. It is a major component of the cytoskeletal system constituted of proteins encoded by 441 genes (Betapudi, 2014; Chen *et al.,* 2014; Pecci *et al.,* 2014).

Out of the 20 super classes of myosin motor protein Myosin V attracted the researchers due to their structural homology, organization, and different functional properties. It was initially known as a calmodulin-binding protein and later revealed that they have motor activity. Myosin V helps in the transport of vesicles and other secretory granules along with F-actin; in addition to this, it also helps in maintaining the cell shape,

mortality, and several biological processes such as the establishment of cell polarity, signal transduction, and rDNA transcription in nucleoli. It engenders the energy for cellular movement by interacting with the actin filament. Myosin V encodes for three paralogous genes (i.e., Va, Vb, and Vc). Myo Va is highly expressed in the endocrine region and is most abundantly found in mammals. This distinctive feature of the motor protein has garnered significant research interest in the field of diabetes research.

Myosin Va most abundantly situated near the pancreatic β-cells region of the endocrine parenchyma, is a molecular motor that binds to vesicles containing a range of cellular macromolecules, including hormones, receptors, and neurotransmitters. Myosin Va works as a vesicle transport motor protein in the various cells of the brain and neuroendocrine cells (Madhuvrata *et al.,* 2012). It also promotes the exocytosis of the insulin granules by releasing ATP and regulates purinergic signalling (Figure 1.1). Along with this it also regulates movement of the insulin-containing granules at the terminal stage of exocytosis in the beta cells. Very few studies have shown knowledge of the role of myosin Va in the pathogenesis of diabetes. In the *in vitro* study in PC12 cell lines Myosin Va was found to reduce insulin secretion through controlled exocytosis. The movement of Myosin Va-driven vesicles along the cortical actin network is quite essential to controlling membrane muffling (Madhuvrata *et al.,* 2012; Sweeney & Holzbaur, 2018; Varadi *et al.,* 2005).

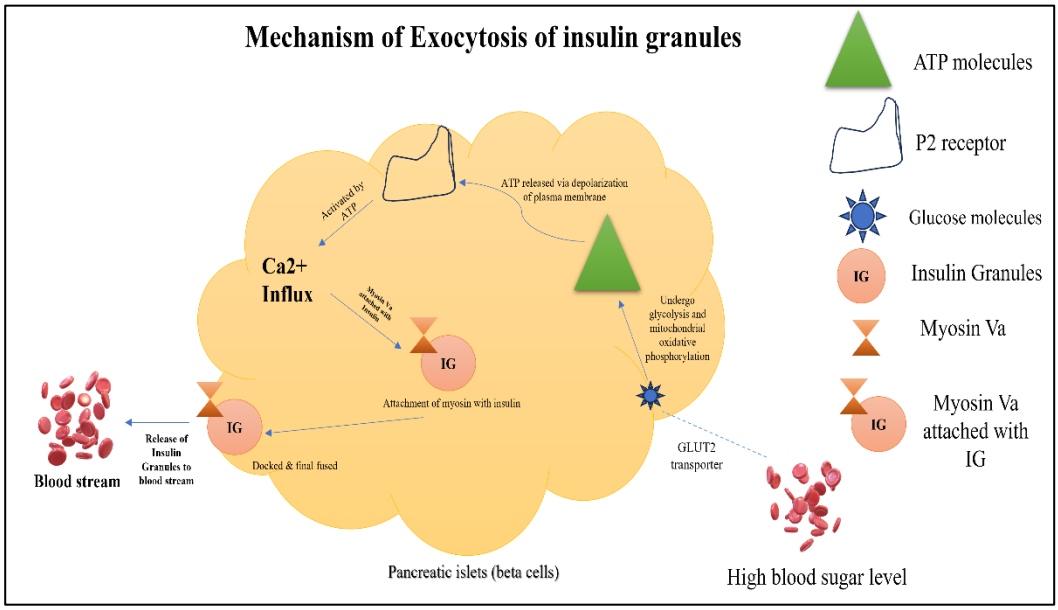


Figure 1.1: Showing the mechanism of insulin exocytosis via releasing ATP and regulating purinergic signalling.

1.4. Current treatment strategies and complications

Long stand diabetes leads to several complications including neurovascular complications, retinopathy, and neuropathy. For the management of DM, several synthetic drugs have been used such as biguanides, sulfonylurea, thiazolidinedione, GLP-1 receptor agonists, etc. However, upon long-term use, these synthetic drugs exhibit many adverse side effects. Sulfonylureas and thiazolidinediones are associated with cardiovascular risk, however, biguanides, GLP-1 receptor agonists, and thiazolidinediones cause gastrointestinal complications, and liver, and kidney dysfunctions. In addition to this, these oral hyperglycemic drugs provide only symptomatic relief by lowering elevated blood glucose levels without addressing the underlying cause, which is insulin resistance (IR). Consequently, the disease progresses over time, leading to various complications associated with chronic hyperglycemia. Additionally, these drugs often suffer from poor aqueous solubility, limited bioavailability, and a range of side effects (Campbell, 2009; Chaudhury *et al.,* 2017; Khursheed *et al.,* 2019).

Due to these adverse side effects of the use of these synthetic drugs, there is an urgent need to discover a new novel, and more effective antidiabetic formulation with less/no side effects.

1.5. Natural Products: An Alternative Source of Medication

Over the past several decades, herbal medications have attracted research interest in drug development due to their natural origin and minimal side effects. India being the botanical garden of the world, contributes around more than 2500 species of medicinal plants. WHO has categorized a total of 21,000 medicinal plants globally as being used for the treatment of various alignments. More than 100 species of medicinal plants are traditionally being used for the management of diabetes in India, but the mechanism of action of these herbal drugs is still controversial. These plants are well documented in the traditional system like Ayurveda, Siddha, and Unani. These herbal drugs are more effective and have long-standing health complaints along with cost-effective than the currently used conventional drugs (K. Kumar *et al.,* 2014; Modak *et al.,* 2007; Samad *et al.,* 2009).

The phytoconstituents extracted from the medicinal plants have a diverse mechanism of action for their glucose-lowering potential. The proposed mechanism of actions of these plants are as follows, β-cell regenerating, insulin mimicking, creatine kinase increasing, glucose utility enhancing, glucose absorption reducing herbs, glucagon inhibiting, glyoxalase 1 activity enhancing, glycogen metabolic enzyme inhibiting plants (Figure 1.2) (Collip, 1923; Lee, 2002; Modak *et al.,* 2007; Parsadanian *et al.,* 1989).

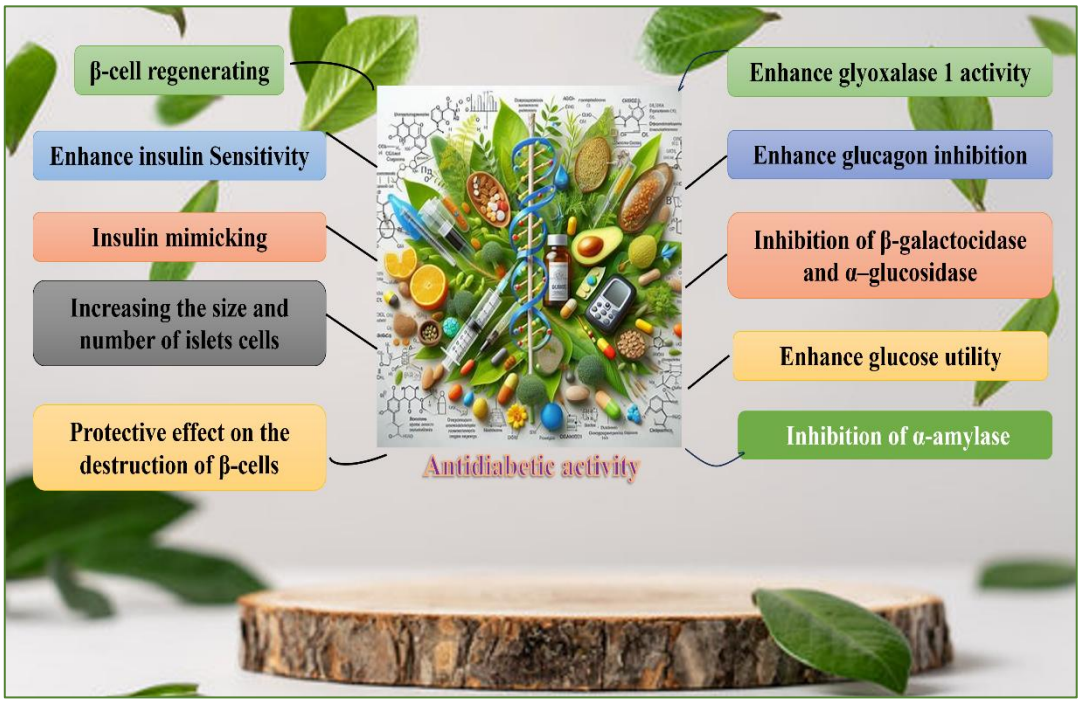


Figure 1.2: Illustrating the proposed mechanism of action of the herbal drugs possessed in antidiabetic activity.

1.6. Ethnomedicinal Antidiabetic Plants of Odisha: Traditional Wisdom

The remarkable advancement of medicinal plants lies in their availability and fewer side effects. Odisha boasts a diverse array of medicinal flora, though it is rich in the tribal lands but has less attention from the ethnobotanical community. Consequently, many folklore plants are not being considered for inclusion in the ayurvedic pharmacopeia due to a lack of their identifications and scientific evidence. Historically, these plants have been a fundamental source of pharmaceuticals, culinary ingredients, and other essentials of tribes, with most of the current medications being derived directly or indirectly from them (Dash *et al.,* 2019; Jena *et al.,* 2024; Jethi *et al.,* 2023). The medicinal plants and their traditional usage by the Vaidya in managing diabetes over many decades in Odisha are described below (Table 1.2).

Table 1.2: Ethnomedicinal Plants Used for the Treatment of Diabetes in Odisha

Sl No	Plant Name	Local Name	Traditional usages
1.	*Aegle marmelos* (L.)	Bela	Fresh leaves crushed with black pepper and consumed on an empty stomach effectively help in the control of diabetes.

2.	*Ilanthus excelsa* Roxb.	Mahanim	Bark powder (2 tsp) twice daily on an empty stomach for 1-2 months regularly lowers the blood sugar level.
3.	*Andrographis paniculata* (Burm.f.)	Chireita	2-3 fresh tender leaves are chewed in the morning on an empty stomach to lower the diabetes level.
4.	*Artocarpus heterophyllus* Lam.	Panas	A decoction was made of the leaves of this plant and taken on an empty stomach twice a day for two months to help control diabetes.
5.	*Azadirachta indica* A. Juss.	Neem	A tablespoon of dry leaf powder taken with water on an empty stomach once a day during the morning helps in diabetic activity.
6.	*Cajanus cajan* (L.) Millsp.	Harad	One teaspoon of sundried leaf powder if taken on an empty stomach controls the diabetes
7.	*Casearia tomentosa* Roxb.	Khokra	Decoctions made up from the bark help in reducing blood sugar levels.
8.	*Catharanthus roseus* (L.) G. Don	Sadabihari	Fresh leaves chewed on an empty stomach help in reducing blood glucose levels.
9.	*Curcuma longa* L.	Haldi	Intake of half a spoon of haldi powder taken on an empty stomach for a long period controls diabetes.
10.	*Tinospora sinensis* (Lour.)	Duluchi	The dried stem of this plant mixed with the powder of c longa, taken on an empty stomach helps in reducing high sugar level.
11.	*Terminalia arjuna* (Roxb. ex DC.)	Arjuna	The bark of this plant made up of decoction and taken on an empty stomach helps in reducing the sugar levels.

12.	*Mangifera indica*	Aam	A decoction was made of the fresh leaves of the plant and taken twice daily on an empty stomach to control diabetes.
13.	*Holarrhena pubescens* Wall. ex G. Don	Indrajau	3 gm of dried flowers were given to the patient to control the diabetes.
14.	*Gymnema sylvestre* (Retz.) R.Br. ex Sm	Gudmar	4-5 leaves are chewed daily which changes the taste of the mouth and helps in lowering sugar levels in the body.
15.	*Limonia acidissima* Groff	Kaintha	The leaf extract taken twice during morning and evening on an empty stomach helps control the sugar level
16.	*Moringa oleifera* Lam.	Munga	10 gm leaf powder given to diabetic patients daily for 6 months in the morning helps to get relief from sugar level.
17.	*Saraca asoca* (Roxb.)	Asoka	Leaves of this plant mixed with the leaves of Mangifera. Decoctions were made and taken on an empty stomach for 2 months to help in the sugar level.
18.	*Cardiospermum halicacabum* Linn.	Phutiphutika	The root powder helps in reducing the overweight.
19.	*Biophytum sensitivum* (L.)	Lokchanna	Leaves decoctions were made and taken on empty stomach to cure diabetes.
20.	*Wrightia tinctoria* (Roxb.) R.Br.	Khirna	Decoctions made from the seeds and bark powder (2-6 g) were taken once daily for two weeks, especially in acute Instances.

21.	*Annona squamosa* L.	Badhal	Fruits and leaves were taken to lower the blood sugar level.
22.	*Ficus religiosa* L.	Peepal	Decoctions was made from the root bark of the plant and taken on an empty stomach for 8 weeks to lower the blood sugar levels.
23.	*Momordica charantia* L.	Kalara	pulp and fruit have been used for the management of diabetes.
24.	*Syzygium cumini* (L.)	Jamun	The dried seed powder is mostly used for lowering blood sugar levels.
25.	*Citrus grandis* (L.)	Tava	The leaf decoction was made and taken on an empty stomach, helps in maintaining the elevated blood sugar levels.

1.7. Concept of Polyherbal Formulations

The traditional medicinal system reported a good number of plants that possess antidiabetic activity via different mechanisms of action, but they suffer a lot due to their lack of scientific validation and standardization. The concept of polyherbalism, highlighted in the Sharangdhar Samhita, an Ayurvedic text from 1300 AD, suggests that polyherbal formulations can enhance therapeutic efficacy. Compared to single herbs, polyherbal formulations offer enhanced and extended therapeutic potential, as well-documented in ancient literature ((Bisht *et al*., 2021; Deore *et al*., 2018; Petchi *et al*., 2014)).

For the management of diabetes, currently, a number of polyherbal formulations are marketed and have shown more effective results than modern drugs. Like, Dianex is a polyherbal formulation prepared by mixing aqueous extract of eight medicinal plants and has a remarkable result in diabetic rats. Similarly, Hyponidd, another polyherbal formulation comprising extracts from ten medicinal plants, has been investigated for its potential antihyperglycemic and antioxidant effects. Triphala is the most well-known formulation marked for the management of DM. This formulation is highly effective in managing oxidative stress conditions and enhancing cell-mediated immune responses (Bisht *et al.,* 2021; Deore *et al.,* 2018; Petchi *et al.,* 2014).

By revising the characteristic features of polyherbal formulation, in the current research a polyherbal formulation is developed by mixing sixteen medicinal plants found in the flora of Gandhamardan hill range, Bargarh district, Odisha. Based on the documented traditional uses and literature review, these plant and their respective parts (leaves, flower, bark, seed, stem, root and rhizome) are selected for inclusion into the development of the formulation to achieve a better therapeutic effect via an alternative mechanism to combat diabetes without any adverse effects. The selected plants and used parts are *Tinospora cordifolia* (Willd.) (stem), *Mangifera indica* L. (seed), *Syzygium cumini* (L.) (bark), *Terminalia arjuna (Roxb. ex DC.)* (bark), *Curcuma longa* L. (rhizome), *Alianthus excelsa* Roxb. (leaf), *Caesalpinia bonduc* L. (Roxb.) (seed), *Swertia chirayita* (Roxb.) (stem), *Holarrhena pubescens* Wall. ex G. Don (root), *Azadirachta indica* A.Juss. (leaf), *Murraya koenigii* (L.) Spreng. (leaf), *Withania coagulans* (flower), *Salacia oblonga Wall. ex Wight & Arn.* (root), *Cedrus deodara (Roxb. ex D. Don)* (bark), *Picrorhiza kurroa* Royle ex Benth. (root), and *Pterocarpus marsupium* Roxb. (bark).

2.1. History of Diabetes Mellitus

Diabetes Mellitus has been well-known since ancient times i.e., 5th century BC. It is a chronic metabolic disorder, clinically characterized by enduring hyperglycemia due to impaired insulin production/action, or both by the pancreatic β-cells. Diabetes is manifested by decreased insulin levels, leading to various complications such as weight loss, polyuria, polydipsia, blurred vision, and polyphagia.(Sarkar & Rajamani, 2022; Verma, 2019). For the first time, the renowned Indian surgeon Sushruta used the term "madhumeha" in Samhita to describe diabetes, he also stated that it mainly affects the rich social group people as they consume disproportionate food like cereals, sweets, rice, etc. In the 2nd century, the well-known physicians of Greco-Roman antiquity Aretaeus of Cappadocia coined the term "Diabetes" and in 17th century the English anatomist and physician Thomas Willis added the term "mellitus" to describe the exceptionally sweet taste of urine (Karamanou *et al.,* 2016; Verma, 2019). After the discovery of the direct relation between the pancreas and diabetes by AD Thomas Cawley (in 1788) and AD Joseph von Mering & Oscar Minkowski (in 1889), diabetes research was directed towards this field. In 1869, Paul Langerhans specifically described the role of pancreatic islet in diabetes and normal individuals, after this novel discovery conferring his name, it was renamed as "islets of Langerhans". The hormone "insulin" secreted from the islets of Langerhans maintained the blood glucose levels was coined by de Mayer and Schaefer in 1909-1910. Best and Collip (in 1921) in their experimental model they established the deficiency of insulin causes Diabetes (Alam *et al.,* 2017; Lakhtakia, 2013). Banting inspired by the previous hypothesis that a hormone produce from pancreas can reduce the blood glucose levels. There after Banting and Macleod started the experiment in diabetes dogs. They extracted that substance from a normal dog pancreas followed by injected it to the diabetic dog. This resulted in a substantial declined in the blood sugar levels. After this experiment James Collip help them to purify the extracted substance and make it safe for further use. This purified extract substance was renamed as insulin. In January 1922 for the first time, they treated a 14-year-old diabetic boy who was dying from diabetes with insulin, which subsequently helps in lowering the blood glucose level in the body. After achievement, insulin gained a major focus area in diabetic research. In diabetes the number of pancreatic islets significantly reduced and a disturbed cellular composition has been observed i.e., a. the proportion of β-cells was significantly decreased in diabetic cases as compared to the normal. b. there is a remarkable variation in the proportion of β-cells in both types of diabetes. The reduction of beta cell number may be due to the auto destruction by, immune system felicitates by T-cells to attacks and destroyed the pancreatic β-cells

(T1DM), long stand insulin resistance and increased demands to insulin results in the apoptosis and dysfunction of β-cells (T2DM), in addition to this, islets plasticity and inflammation in diabetes is also a key factor for destruction of these cells subtype. Approximately 80% of the β-cells were lost in progressive diabetes (S.J. Cooperstein & Watkins, 1981).

2.2. Development of diabetes and mechanism action of insulin

Glucose homeostasis process is mediated by several factors like neuronal signals, glucose transporter, hormones, and a good physiological condition of the body. Any fluctuations in this process can directly lead to impaired glucose homeostasis leads to diabetes, that is characterized by the impaired insulin secretions or actions, leading to a series of metabolic dysfunction including a significant increase in the endogenous glucose production, elevated blood glucose levels, dyslipidaemia, increased insulin resistance, and chronic inflammation (Figure 2.1). Overt diabetes is often developed by elevated metabolic abnormalities that are common in individuals prior to the onset of diabetes. This relative progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and ultimately to the development of diabetes (Chen *et al.,* 2014; Dabur *et al.,* 2018; Schofield & Sutherland, 2012; Weyer *et al.,* 1999).

The lower concentration of insulin level causes a significant rise in the blood glucose level which leads to polyuria, polyphagia, weight loss, polydipsia, blurred vision, etc. (Sarkar & Rajamani, 2022). Glucagon, another hormone released from the pancreatic α-cells, playing a crucial role in diabetes i.e., downregulation of this hormone leads to hyperglycemia, it helps in reducing food intake and increase energy expenditure (Campbell & Drucker, 2015; Lee *et al.,* 2016). Figure 2.3 below illustrates the mechanism of insulin and the role of the pancreas in maintaining glucose homeostasis. Briefly, when the blood glucose level falls it releases the hormone glucagon, however, an uncontrolled blood glucose level stimulates the secretions of insulin from the pancreas followed by the uptake of glucose from the bloodstream which results in a decreased blood glucose level.

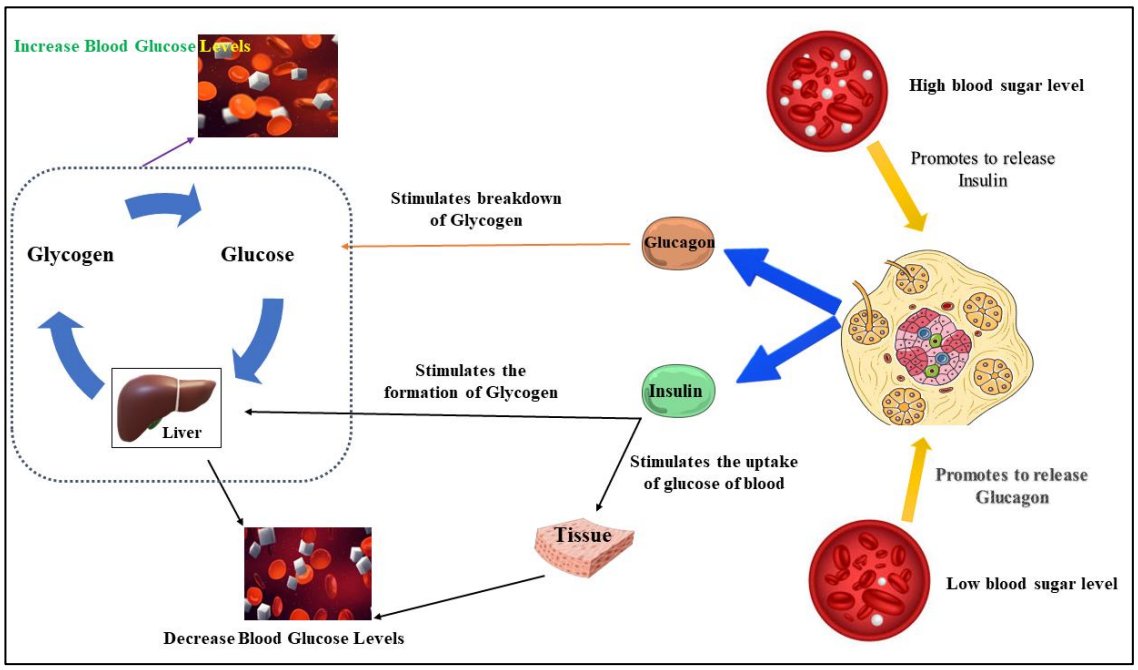


Figure 2.1: Role of insulin and glucagon during glucose homeostasis.

The intracellular signalling of the insulin is initiated after binding it with the cell surface of the transmembrane receptor. Insulin receptor belongs to the class of tyrosine kinase, and it contains 2 alpha and 2 beta subunits. The insulin granules bind to the α-subunit followed by the autophosphorylation initiated by β-subunit finally leads to the activation of the intrinsic tyrosine kinase (ITK) activity. This ITK activity phosphorylated many downstream signal proteins (like insulin receptor substrate, protein kinase B and Akt complex, phosphatidyl inositol-3-kinase (PI3K), and protein kinase C). Among all the above proteins PI3K plays an important role in the insulin signalling mechanism for the transportation of glucose-by-Glucose Transporter 4 (GLUT4) from the intracellular secretory vesicle (ISV) to the plasma membrane. Insulin promotes the absorption of glucose via PI3K independent pathway, that involves the tyrosine-mediated phosphorylation of cb1 protooncogenes. The cb1 interacts with the adopter protein cr1k11 to activate the G protein TC 10 that initiates the signal to GLUT4. Besides the above, this phosphorylated insulin activates the Ras/MAP kinase cascade, that directly involved in the mitogenic effects of insulin (Elmendorf, 2002; Patti & Kahn, 1998; Saltiel, 2021).

2.3. Global and Indian Scenario of Diabetes

Diabetes is one of the fast-growing global health burdens of the 21st century, by making both the rural and urban environment more prone to this disease. This continuing emergence of the disease directly affects the nation's health and economic progress, excellence, and expectancy of human life (Sen *et al.,* 2015). According to the latest international federation of diabetes (IFD) data 2021, a total of 537 million individuals has

diabetes which will be projected to 643 million in 2030 and 783 million by 2045. This life-threatening disease contributes 6.7 million deaths. In 2021, over 1.2 million children and adolescents have diabetes (Sun *et al.,* 2022). The highest incidence rate of diabetes was observed in China, India, Indonesia, Mexico and the United States. Besides the low-middle, middle, and high-middle income countries were high burden of the disease. The prevalence percentage of low-middle, middle and high-middle income countries are 6.7%, 7.1% and 8.2% respectively. Several metabolic and behavioural factors directly affect the epidemiology of diabetes globally, i.e., metabolic risks (high body mass index) and behavioural factors (poor diet, smoking, and less physical activity) (Figure 2.2) (Verma, 2019).

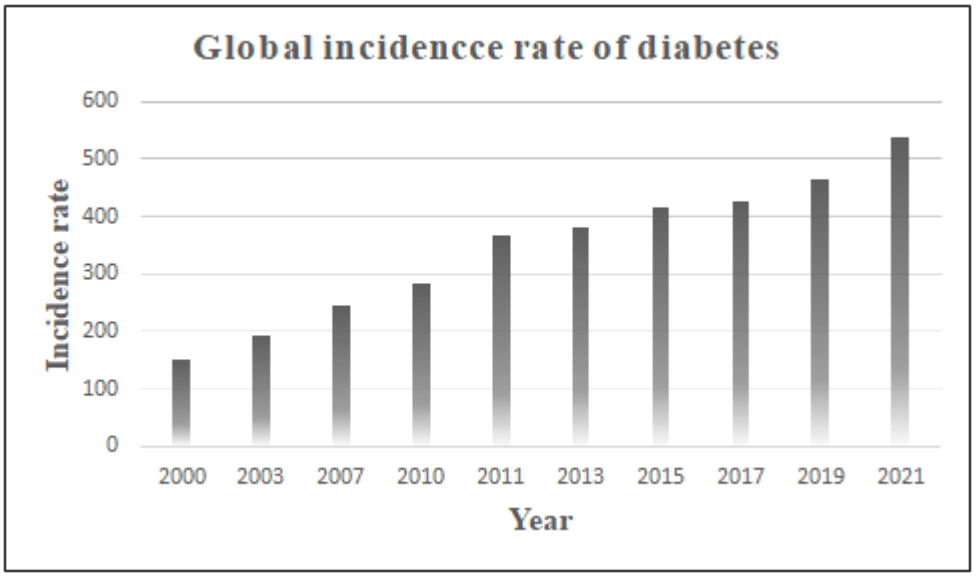


Figure 2.2: Graphical presentation of the global incidence rate of diabetes (Source: Sen *et al.,* 2015; Sun *et al.,* 2022).

2.3.1. Epidemiology of diabetes in India and risk factors

India is the diabetic capital of the world, contributing an estimated 77 million people with diabetes and will be projected to 124.9 million by 2045, followed by 0.6 million deaths annually. Also, it has been reported by IFD data 2021 that, India accounts for 1 in 7 of all adults living with diabetes worldwide. In India, the metabolic factor like mitochondrial dysfunction, endoplasmic reticulum stress, dyslipidaemia, obesity, insulin resistance, hypertension etc are the major risk of diabetes as compared to the behavioural factor contributing about 20% of the total diabetes prevalence and the rate has increased gradually day by day in every state of the country. Besides, several other risk factors that affect the prevalence of diabetes are tobacco use, occupational exposure (second hand smoke), use of alcohol, and dietary pattern. The recent report on diabetes suggests that the

metabolic factors affecting this disease are seen to be more in females as compared to males, whereas the other risk factors are comparatively high in men.

2.3.2. Sexual dimorphism in diabetes

In accordance to sex dimorphism male are more prone to diabetes compared with the female, except the region like Middle East and North Africa. In the middle east and north Africa region the prevalence rate is almost same both in male and female (Tramunt *et al.,* 2020). The *in vivo* animal model also showed a higher prevalence of the development of diabetes in the male animals which is due to obesity, insulin resistance, and hyperglycemia in response to nutritional challenges compared to females. In addition to this psychosocial factor also impact in development and progression of diabetes and coping in a gender-dimorphic way. The existing report also signifies that men are diagnosed with diabetes at a lower Body Mass Index (BMI) compared to women across various age groups (Logue *et al.,* 2011). This finding suggests that men may have a higher susceptibility to diabetes even with less adiposity. Besides this, glucose homeostasis and energy regulation notably differ in both sexes which have a directly relation with this metabolic disorder. A case study revealed that, female pancreas biopsy had a 6% more pancreatic β-cells than male donor, which suggesting a functional disparity (Lamri *et al.,* 2022). The sex hormone like endogenous estrogen helps in enhancing the insulin secretions, protects the pancreatic β-cells from damage in female islets. This also helps in preserve β-cells function and prevent apoptosis caused by metabolic stressors such as oxidative stress and lipotoxicity, underscoring the sex-specific regulation of islet biology (Tramunt *et al.,* 2020).

In addition, the above-mentioned metabolic and behavioural factors many other aspects directly affect the overall health issues related to diabetes, i.e.,

a. *Lack of awareness*: In India, among the population, there has been a lack of awareness about the diseases as reported by the ICMR-INDIAB study. According to the report, about 43.2% of the total population were affected by the disease (36.8 % in rural regions and 58.4% in urban regions). The dietary habit change, physical inactivity, genetic predisposition are the major cause of the development of diabetes in the rural region (Little *et al.,* 2017).

b. *Lack of proper diagnosis:* Development of diabetes in India mainly affected by several factors including lack of proper diagnosis. This has led to a substantial portion of the population remaining undiagnosed 50% in rural areas and 30% in urban areas resulting in many individuals only seeking medical help when complications arise. Additionally, there is a widespread lack of awareness and knowledge about diabetes including insufficient

counselling, and inadequate adherence to evidence-based medicine. These factors collectively contribute to the high prevalence and poor management of diabetes in India, highlighting the urgent need for improved proper diagnosis for the management of diabetes.

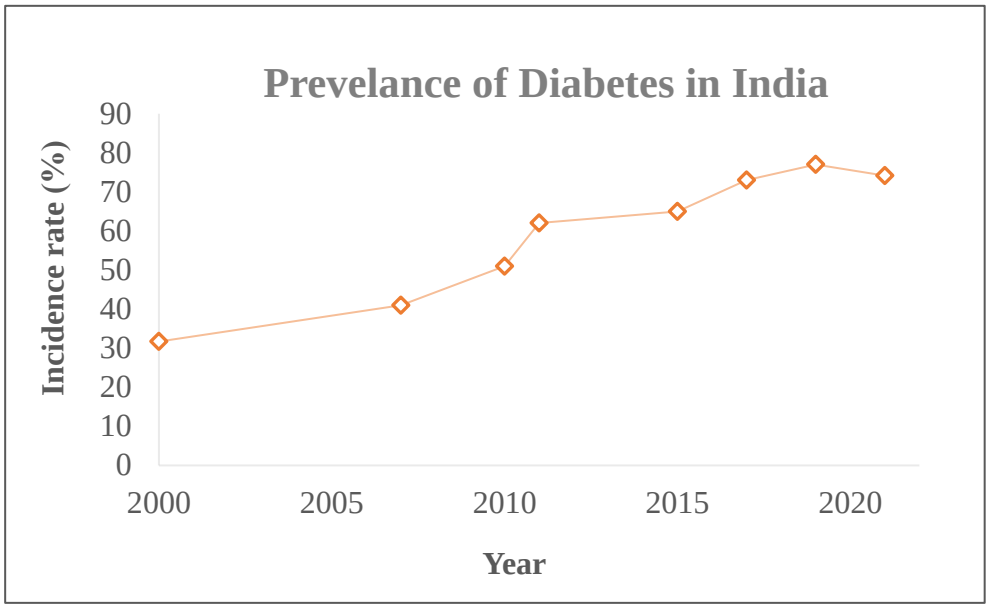


Figure 2.3: Graphical presentation of the Indian incidence rate of diabetes (Source: Kaveeshwar & Cornwall, 2014; Pradeepa & Mohan, 2021; Sen *et al.,* 2015; Sun *et al.,* 2022).

2.4. Types and Etiology of DM

Diabetes is classified into the following categories such as type-I, type-II, gestational diabetes, which is approved by the American diabetes association (ADA). The major affected types of diabetes are type - I and II, latter on this type are renamed as juvenile-onset, ketosis-prone/non-ketosis-prone, and insulin-dependent/non-insulin-dependent according to their respective phenotypical features (Committee *et al.,* 2024; Genuth *et al.,* 2021; Maraschin, 2013).

a. *Type-I diabetes (T1DM)*: T1DM is an autoimmune disorder, where the body's immune system bouts the insulin-producing pancreatic β-cells, resulting in a decline in insulin production or very little insulin produced. The glucagon producing α-cells generally not affected, but their normal function is indirectly altered due to loss of pancreatic β-cells, which subsequently dysregulates the glucose homeostasis. This type represents approx. 5% of the total incidence of diabetes globally. The molecular mechanism behind this destructive process is still unclear, a conventional explanation attributes it to a confluence of hereditary predisposition and environmental stimuli, such as viral

infection, which set off these kinds of autoimmune reactions. This type of DM can develop at any age period but are most common in childhood and young adult. The individual having type-I DM are typically of peripubertal age 50 per 100,000 individuals. The highest incidence is often observed in the peripubertal age group, particularly between 10 and 14 years with a short duration symptom of polyuria (frequent urination), polydipsia (excessive thirst or drinking), weight loss, lack of energy or fatigue, blurred vision, diabetic ketoacidosis, etc. The central characteristic feature of this type is insulin deficiency. Autoantibodies to glutamic acid decarboxylase (GAD), insulin, insulinoma-associated protein 2 (IA-2), and zinc transporter 8 (ZnT8) are a well-established marker for characterizing type I and type II diabetes. The presence of these multiple antibodies is the main characteristic feature of T1DM while the absence of these antibodies is typical for T2DM. T1DM is further divided into two subtypes i.e., Type A1 and Type A2. Type A1 is well described as an autoimmune disease that targets particular organs and is mostly genetically susceptible by the major histocompatibility locus located on chromosome 6. It has been associated with other autoimmune diseases, such as Hashimoto's thyroiditis and Addison's disease. The illness is inclined in those who have certain human lymphocyte antigen (HLA) haplotypes. In addition to being more prone to ketosis and lacking HLA association, type A2 is diabetes is distinguished by the absence of autoimmune responses that typically cause the death of β-cells. The apoptosis of β-cells may be due to some other factors, such as genetic predispositions, environmental triggers, or metabolic stress. (Genuth *et al.,* 2021; Maraschin, 2013; Sun *et al.,* 2022)

b. *Type-II diabetes (T2DM):* T2DM is one of the most common forms of diabetes, account for an overall incidence of 90% globally and most of the affected individuals belong to the adult category (20-79 years). An uncontrolled increase in this incidence is mainly due to hereditary, smoking, hypertension, high cholesterol levels, obesity, sedentary lifestyle, socio-economic status and stress.

Insulin resistance due to hyperglycemic conditions is the main cause of the development of T2DM. The clinical symptoms of T2DM are similar to those of T1DM, but some patients may be asymptomatic. Additionally, another major cause of diabetes development is the progressive failure of β-cells due to chronic hyperglycemia, lipotoxicity, and oxidative stress. This failure is characterized by a lack of immune-mediated destruction and can infrequently advance to the point where the patient needs insulin to survive. However, long-standing T2DM convert to T1DM this progressive stage of requires insulin therapy. Although rare, ketoacidosis is typically linked to a serious

coexisting condition. Though the condition is more prevalent after age 40, research has indicated that it is also becoming more prevalent in young people and adolescents as obesity rates rise. Concerning the pathophysiology of T2DM, a fault arises in the feedback loop that links insulin secretion to the behaviours (insulin action) that cause elevated blood glucose levels. The dysfunction of the pancreatic β-cells leads to inadequate insulin secretion and fails to compensate for the increased demand for insulin due to insulin resistance. In contrast, juvenile diabetes (Type 1 Diabetes Mellitus) is an autoimmune disorder that primarily affects children and young adults, leading to the destruction of insulin-producing β-cells. These long-standing cases of Type II diabetes can progress to a stage where the patient needs insulin to survive, similar to the insulin dependence seen in Type I diabetes. The existence of both β-cell dysfunction and insulin resistance increases hyperglycemia, which accelerates the development of T2DM.

If diabetes remains untreated it directly leads to several traditional complications like kidney dysfunction, neuropathy, peripheral artery disease, foot ulcer, stroke, coronary heart disease, and heart failure. Moreover, several other emerging complications are also seen such as liver dysfunction (non-alcoholic fatty liver, fibrosis, non-alcoholic steatohepatitis), functional disability, cognitive disability, affective disorder (depression, anxiety, Alzheimer disease, vascular dementia).

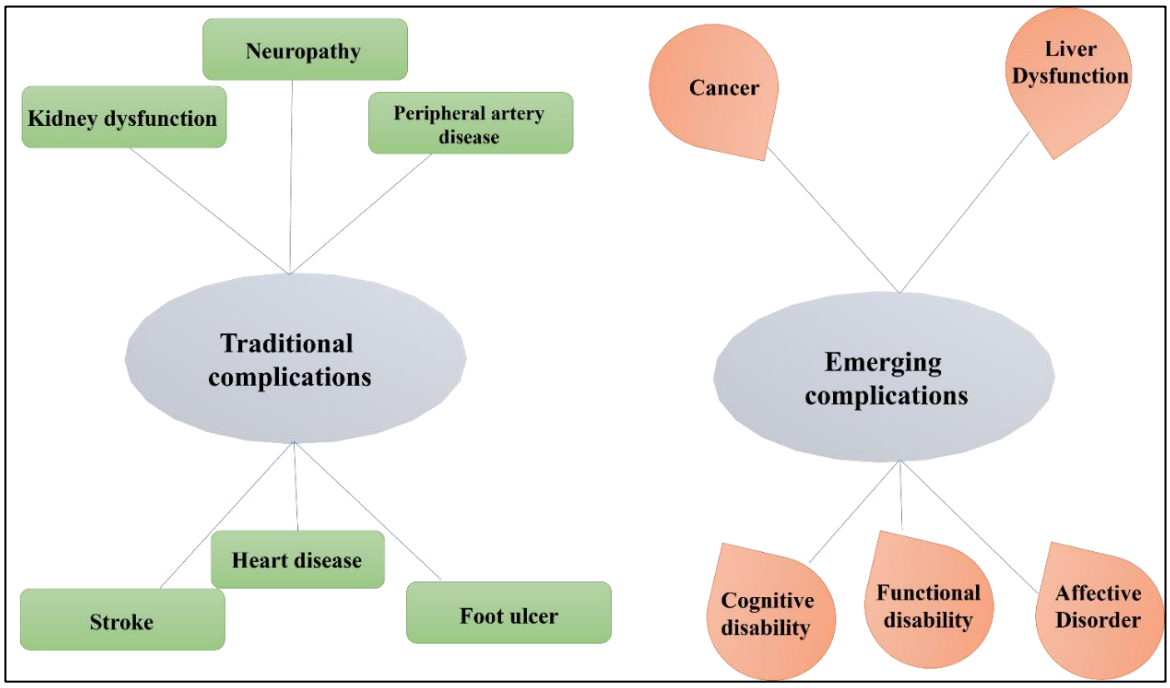


Figure 2.4: Graphical representation of major traditional complications and emerging complications of diabetes mellitus (DM) Source: (Tomic *et al.,* 2022a)

c. *Gestational diabetes:* A variable degree of glucose imbalance observed during the onset of pregnancy is termed gestational diabetes mellitus (GDM). In these conditions, the function of insulin is affected by the hormone secreted by the placenta. Insulin resistance (IR) begins during the middle of pregnancy and develops up to the third trimester. The affected hormones and adipokines of IR are TNF-α, human placental lactogen, and placental growth hormone, all these response hormones are secreted from the placenta. Besides this, some other factors like increased cortisol, progesterone and estrogen are responsible for an imbalance in glucose and insulin concentration. The major risk factors associated with the development of GDM are obesity, family history, prediabetes, number of previous pregnancies, and persistent glucosuria. The overall incidence of GDM is about 1-20% and is still continuously in rising state, which is similar to the rising rate of type-II diabetes which is now a major headache for the healthcare system. GDM has a higher risk of other incidence like gestational hypertension, eclampsia, and pre-eclampsia (Alfadhli, 2015; Buchanan & Xiang, 2005; Maraschin, 2013).

2.5. Exocytosis of β-cells and its active role in the development of DM

The insulin secretions from the pancreatic β-cells are stimulated by two primary mechanisms i.e., by activating and strengthening of different pathways. Glucose and other essential nutrients depolarize the β-cells by closing the ATP-sensitive K^+ ion channel in the plasma membrane increasing the intracellular ca^{2+} concentration by ca^{2+} ion influx. Concurrently, the eukaryotic actin-based motor protein Myosin gets attached to the cell surface of β-cells (initiation of exocytosis process) and by triggering signals it transports towards the final stage of exocytosis of the insulin granules and finally released to the bloodstream (Figure 2.6). The stimulatory hormones and neurotransmitters enhance the initial triggering signals and significantly activate the amplifying pathways which are biochemically different from those activated by nutrients. There are mainly six different active sites/pathways such as I) stimulation of metabolism, II) action on membrane receptors, III) increase of $[Ca^{2+}]$ by the closure of K^+_{ATP} channels, IV) stimulation of amplifying pathways, V) increase of $[Ca^{2+}]$ ion by other means, and VI) action on nuclear receptors, are mainly responsible for the secretions of insulin from the pancreatic β-cells. To monitor the nutritional state, β-cells are clustered within islets that are strategically connected to the vasculature. The islets are surrounded by capillaries containing numerous small pores known as fenestrae which enhance nutrient exchange between the surrounding exocrine tissue. This fenestration process facilitates the rapid diffusion of insulin granules

to the bloodstream. In addition to this, many amino acids and fatty acids also play a crucial role in insulin secretions (J. C. Henquin, 2000; J.-C. Henquin, 2004; Seino *et al.,* 2000).

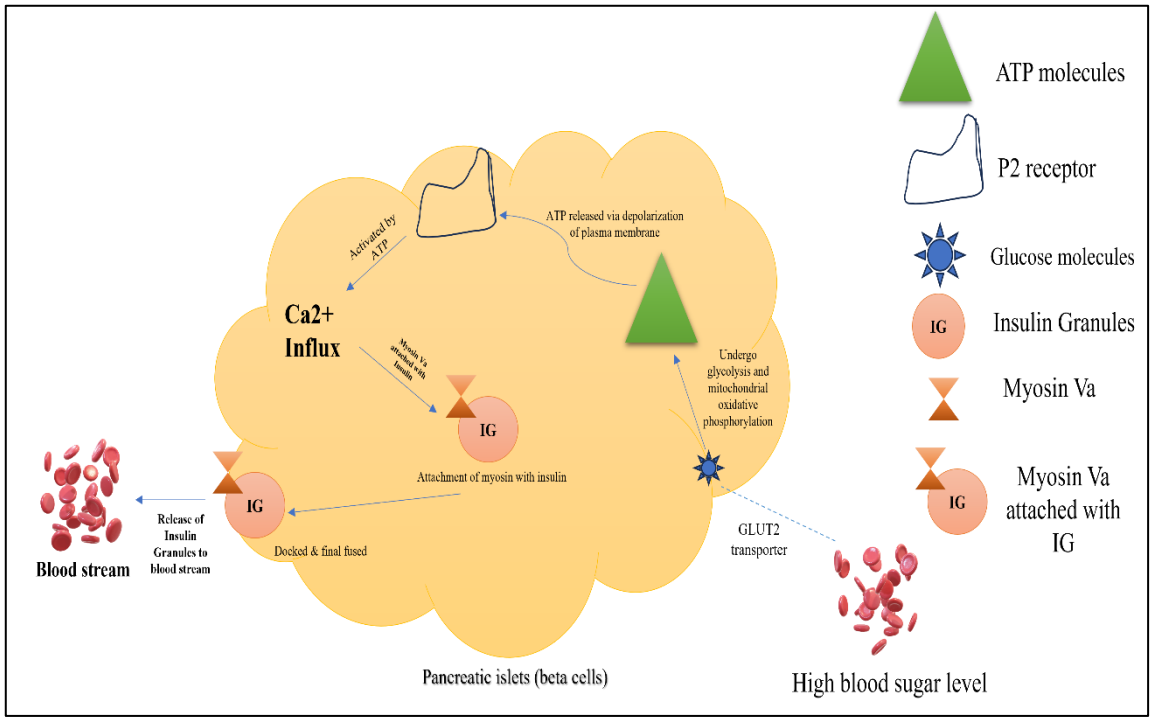


Figure 2.5: Illustrating the mechanism actions of exocytosis of insulin granules from the islets of Langerhans to the bloodstream.

Both T1DM and T2DM share a common cellular signalling pathway which selectively destroyed the pancreatic β-cells mainly through apoptosis. In T1DM, the β-cells are destroyed due to autoimmune reactions whereas in T2DM it is due to excessive nutrients. The destruction of pancreatic β-cells in T1DM involves the driving of inflammatory cells like T helper type 1 (Th1) cells and macrophages, into the islets, leading to insulitis. These cells release the proinflammatory cytokines like interleukin-1 (IL-1), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), which are key mediators for the destruction process. T2DM is mainly developed due to chronic insulin resistance and loss of β-cells mass due to increased apoptosis of the pancreatic β-cells. The conditions like gestational diabetes, obesity, impaired glucose tolerance, and polycystic ovarian syndrome are the key factors associated with chronic insulin resistance, which leads to increased β-cell apoptosis and a significant reduction in β-cell mass. Long-term exposure to conditions like glucotoxicity (hyperglycemia) and lipotoxicity (increased level of fatty acid) resulting in β-cells damage/dysfunction. This chronic exposure disrupts the normal insulin synthesis and secretions finally contributing to the pathogenesis of T2DM.

In addition to the above pathogenesis of T1DM and T2DM, oxidative stress and hypoxia conditions also play a crucial role in β-cells dysfunctions. The aerobic, anaerobic

glycolysis, production of reactive oxygen species (ROS), and oxidative phosphorylation are associated with the normal function of these pancreatic β-cells including insulin secretions and glucose sensing. Over the last two decades, hypoxia and ROS pathway plays a crucial role in the regulation of β-cells functions. The misleading activations of this involved pathway directly led to the dysfunction/destruction of pancreatic β-cells (Fu *et al.,* 2012; Gerber & Rutter, 2017; Page & Reisman, 2013).

2.6. Experimental Diabetes

Diabetes mellitus (DM) is often instigated in animal models because these models play a crucial role in understanding the disease's pathogenesis and the mode of action of administered drugs for effective treatment. Even in the current scenario *in vitro* and *in silico* strategies are mostly implemented still *in vivo* model remains an effective one for understanding the complexity of the disease. The diabetic model is developed by selectively destroying the pancreatic β-cells of the islets of Langerhans, which is responsible for insulin secretions to maintain glucose homeostasis. There are several well-established methods for the development of a diabetic model i.e., chemical inductions and genetic mutation. Both the method for the development of DM is significant as they illustrate the mechanism of disease development which helps correlate it with humans (Arndt *et al.,* 2013; Graham & Schuurman, 2015; Heydemann, 2016; Roep & Atkinson, 2004).

2.6.1. Genetic mutation

A number of mice models have been developed by genetic manipulation i.e., AKITA, Goto-Kakizaki (GK) rats, Zucker Diabetic Fatty (ZDF) rats, Obese Spontaneously Hypertensive (SHR) model, ESS rats, etc. Among these models, AKITA mice model is the most commonly used, genetically characterized by a mutation in Ins2 gene (Ins2+/C96Y), which causes improper secretions of insulin due to inappropriate insulin folding and high stress in the ER of pancreatic β-cells. This alternation in specific genes leads to β-cells apoptosis and resulting the development of diabetes. This model is crucial for understanding the molecular mechanism of the administrated drug for protecting the pancreatic β-cells and managing the disease progression (Heydemann, 2016; Portha, 2005; Portha *et al.,* 2009, 2012).

2.6.2. Chemical induction

There are some diabetogenic agents available (like alloxan, streptozotocin) which are used to induce diabetes in animal models. Depending upon the species of animals the

doses and route of administration vary. The common mechanism of these diabetogenic agents is selectively destroy the pancreatic β-cells what is it selectively effect the endocrine cell subtype (i.e., pancreatic β-cells) and leads to hyperglycemic conditions (Lekan *et al.,* 2020; Lenzen, 2008). The current study implies alloxan for the development of diabetes in the experimental animals. Alloxan selectively destroys pancreatic β-cells by enhancing the production of reactive oxygen species (ROS) through a redox cycle involving alloxan and its byproduct, dialuric acid, which subsequently increased the oxidative stress and leads to dysfunction of pancreatic β-cells.

2.6.3. Alloxan: Mechanism of action

Alloxan (5,5-dihydroxyl pyrimi-dine-2,4,6-trione) an organic chemical is a cytotoxic glucose analogue, commonly used to induce diabetes in experimental animals. This diabetogenic agent is selectively uptake by the pancreatic β-cells via glucose transporter 2 (GLUT2) and leads to the destruction of β-cells specifically rather than other cells. In addition to this Alloxan undergoes a redox cycle facilitated by reducing agents such as reduced glutathione (GSH), cysteine, ascorbate, and protein-bound sulfhydryl (-SH) groups. This redox cycle generates reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals. These ROS cause significant damage to cellular components such as DNA, proteins, and lipids. The superoxide radicals produced during this process undergo dismutation in the presence of superoxide dismutase, forming hydrogen peroxide (H_2O_2). Subsequently, in the presence of ferrous ions, hydrogen peroxide participates in the Fenton reaction, leading to the formation of highly reactive hydroxyl radicals. These hydroxyl radicals further exacerbate cellular damage, contributing to the destruction of pancreatic beta cells and the development of diabetes. This oxidative stress significantly affects mitochondrial functions and further contributes in the destruction of pancreatic β-cells by blighting the ATP productions and persuading apoptosis pathway (Katoh *et al.,* 2002; King, 2012; Kottaisamy *et al.,* 2021; Lekan *et al.,* 2020; Lenzen, 2008).

2.7. Complications and Risk factor of DM

Over the past few decades, DM has become the most prevalent disorder constituting a serious public health challenge in the twenty-first century because of other disease related complications. The long-term traditional complications like macrovascular conditions (coronary heart disease, peripheral arterial disease, and stroke), and microvascular conditions (peripheral neuropathy, diabetic kidney disease, and retinopathy) are mostly seen. For the management and treatment of this life-threatening disease and its adverse

side effects a number of synthetic drugs have been established. Still, a heavy burden of mortality is directly associated with these traditional complications. The severity of these complications directly relied upon the type of diabetes and the duration of diabetes. Recent studies have highlighted a comparatively high mortality rate among individuals having both cancer and diabetes in different regions worldwide underscoring the complex interplay between these two conditions. Furthermore, it is also linked with an increased risk of other several new emerging complications including liver disease (non-alcoholic fatty liver, non-alcoholic steatohepatitis & fatty liver), functional disability, cognitive disability (dementia and cognitive impairment), depressions, anxiety, etc. (Gregg *et al.,* 2018; Harding *et al.,* 2019; Pearson-Stuttard *et al.,* 2021; Tomic *et al.,* 2022b).

2.8. Diagnosis and Treatment of Diabetes

Diabetes trends for a major cause of morbidity among non-communicable diseases and contributes to about 80% of deaths globally. Maintenance of a proper healthy lifestyle and metabolic dysfunction leads to diabetes. By utilizing synthetic drugs and modulating lifestyle the complications and symptoms of DM can be managed rather than its complete cure. Diabetes is mainly diagnosed based on the fasting blood sugar levels, and HbA1C percentage criteria. Among all, HbA1C and C-peptide levels are more appropriate criterion for the screening of early diabetes purpose (R Paul Robertson, 2023).

For the management of DM, a number of different classes of synthetic drugs are used i.e., biguanides (ex. metformin), meglitinides (ex. repaglinide and nateglinides), thiazolidinediones (ex. pioglitazone), and sulfonylureas (ex. glyburide and glipizide) (V. Mishra *et al.,* 2021; Sen *et al.,* 2015).

2.8.1. Biguanides

Biguanides are a class of oral antihyperglycemic drugs regarded as the first-line drug for the management of diabetes. Metformin belonging to this class is the most commonly prescribed drug for the treatment of diabetes. This class of drugs exerts multifaceted mechanisms in managing diabetes. They effectively lower elevated blood glucose levels by enhancing glucose uptake through the activation of insulin receptors and glucose transporters in target cells such as skeletal muscles and the liver. It also decreased the triglyceride and serum LDL cholesterol levels upon administration. It has also been studied that metformin didn't have any impact on pancreatic β-cells. This drug activates the AMPK enzyme which helps in the regulation of hepatic gluconeogenesis gene expression and it inhibits the mitochondrial complex 1 and glycerophosphate dehydrogenase (GPDH). This collective mode of action of metformin helps in declining the high blood glucose and

HbA1c level (Khursheed *et al.,* 2019; V. Mishra *et al.,* 2021; R Paul Robertson, 2023; Tomic *et al.,* 2022a).

2.8.2. Meglitinides

Meglitinides, another class of oral anti-diabetic drugs used for the management and treatment of T2DM. They bind directly to specific receptors on pancreatic β-cells, causing the closure of ATP-sensitive potassium channels. This leads to the depolarization of the cell membrane resulting in the influx of calcium. Increased intracellular calcium induces insulin secretion. It has been studied that this class of drugs are more helpful for managing the high postprandial blood sugar level within a shorter duration of time. Meglitinide and its derivatives are used as an adjunct to diet and exercise to improve glycemic control with T2DM either as monotherapy or with other antidiabetic drugs (Black *et al.,* 2007; Le *et al.,* 2020; Melander, 2004).

2.8.3. Thiazolidinediones

Thiazolidinediones are widely used as an antihyperglycemic drug. Its mode of action is activation of the peroxisome proliferator-enacted receptor γ (PPR- γ) transcription factor that leads to insulin susceptibility in the liver adipocytes and heart muscles. The previous study regarding this class of drugs suggests that they enhance insulin sensitivity (Lebovitz, 2019), decreasing plasma triglycerides by elevating adiponectin levels, plasma-free fatty acids, and increasing HDL cholesterol levels. This class significantly controls the fasting and postprandial blood glucose levels. Further, increases the insulin secretions which leads to a reduction in glycated hemoglobin (HbA1c) levels in DM individuals. Also, regarding the progression study this class (rosiglitazone and pioglitazone) plays an extent role in reversing the prediabetic stage to normal over a limited time of treatment illustrating the preservation of the beta cells insulin secretory functions (Davidson *et al.,* 2018; Lebovitz, 2019; Yasmin & Jayaprakash, 2017).

2.8.4. Sulfonylureas

Sulfonylurea is the first oral antidiabetic drug commonly known as insulin secretagogues drug that works in several different pathways. By stimulating insulin secretions from the pancreatic β-cells it reduces the blood glucose levels. Upon long-term used, this drug exerts an insulin-independent mode of action (insulin-sensitizing and insulin-mimetic activities) for blood glucose reduction in muscles and adipocyte tissue via increased productions of diacylglycerol which activates protein kinase C. These activations enhance the glucose uptake by enhancing the expression of glucose transporter isoforms, such as GLUT4, on the cell surface. This class of drugs directly interacts with the sulfonylurea receptor protein, SURX, on pancreatic β-cells, which closes the ATP-

sensitive potassium channel (KATP) and finally results in depolarization of the β-cells membrane, subsequently opens voltage-gated calcium channels. The continuous flow of the calcium ion towards the β-cells accelerates the exocytosis of the insulin granules and finally consequential in increased the insulin secretions (Müller, 2000; Qian *et al.,* 2018; Thulé & Umpierrez, 2014; Volke *et al.,* 2022).

The mode of action of these first-line treatment drugs and their mode of action are represented below (Figure 2.5).

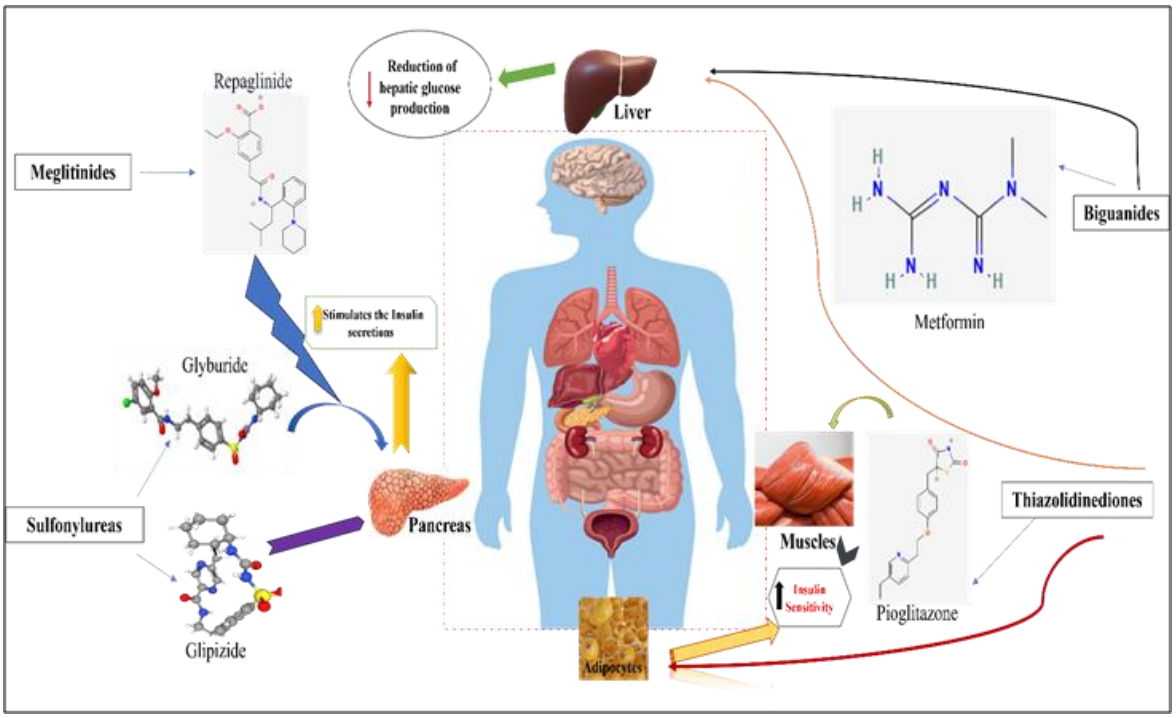


Figure 2.6: Representation of the different synthetic drugs used for the management of DM and their mode of actions.

2.9. Complications of DM due to synthetic drugs

The adverse effects of the currently used synthetic drugs vary on the class of medications which can significantly impact the life style and quality of life. Metformin is regarded as the 1st line drug for the management of DM showing gastrointestinal side effects, including nausea, diarrhea, vomiting, and abdominal discomfort, etc. Besides this, it is also associated with severe complications like lactic acidosis, but these complications are rarely seen in patients with renal impairment, hepatic dysfunction, or other conditions predisposing to hypoxia. Thiazolidinediones another class of antidiabetic agents have the therapeutic potential by activating peroxisome proliferator-activated receptor gamma (PPAR-γ), which enhances adipocyte differentiation and lipid storage, resulting in weight gain. Also, these drugs have side effects like heart failure because of fluid retention, and bone fracture due to their effects on bone metabolism. Meglitinides (like repaglinide and

nateglinide) can cause hypoglycemia side effects due to their rapid and short-acting stimulations of insulin release by binding at the different active sites of the sulfonylurea receptor. Sulfonylureas, the first oral antidiabetic drugs are commonly associated with hypoglycemia, particularly in patients with renal insufficiency. Similar to meglitinides this drug also has the side effects of weight gain, but the rate of weight gain is higher in sulfonylurea treated patients compared to meglitinides treated. This is because sulfonylureas promote the anabolic process during the insulin secretions which directly leads to a significant weight gain. In addition to this common side effect, this drug has been directly linked with an increased rate of cardiovascular events, potentially due to their impact on myocardial ischemic preconditioning (Banerjee *et al.,* 2019; Bodmer *et al.,* 2008; Kimmel & Inzucchi, 2005; Prabhakar *et al.,* 2014; Qian *et al.,* 2018; Safavi *et al.,* 2013).

2.10. Alternative medications

Revising the adverse side effects associated with the currently used synthetic antidiabetic drugs, most developing countries are increasingly turning to alternative sources of medication i.e., natural or herbal products. Evidence suggests that around 30-57% of individuals with diabetes globally, mostly rely on alternative medications (AM). An estimated of around 80% of the total population of many developing countries depend upon these AM for their healthcare. The developed countries like USA, Australia, and United Kingdom, around 46% of those having diabetes have reported the use of AM. The increasing rate of interest in the use of these herbal medications is mainly due to the higher efficacy, less or no side effects than these pure allopathic therapies. Further, AM declined the hyperglycemia without using pharmaceutical drugs or insulin injections. Over the last decades, the United States reported the use of these AM (herbal remedies) by 380% for the management and treatment of DM (Chang *et al.,* 2013; D. Kumar *et al.,* 2006; Oubré *et al.,* 1997; Ranasinghe *et al.,* 2012).

India being the major flora of natural products from ancient reported a higher (67%) use of these AM among the diabetic populations. It has also been suggested that among the diabetic population around 97% directly rely on herbalism. The ethnobotanical society reported that around 800 plants are found in the flora which are reported for their antidiabetic properties, out of which 410 plants are experimentally proven. Among 410 plants about 100 plants are reported with their mechanism of action for the antihyperglycemic effects. The common plants traditionally used for the management of DM are *Gymnema sylvestre, Acacia arabica, Momordica charantia, Trigonella foenum*

graceum, Bacopa manneri, Tinospora cardifolia, Caseria esculenta, *Enicostema littorale, Mangifera indica*, *Curcuma longa* etc.

In the current study, we developed a polyherbal formulation by mixing an equal proportion of 16 medicinal plants traditionally used for the treatment of diabetes in the Gandhamardan hill range area, Bargarh district, Odisha. The selected plants for the preparation of the polyherbal formulations are *Tinospora cordifolia* (Willd.) (stem), *Mangifera indica* L. (seed), *Syzygium cumini* (L.) (bark), *Terminalia arjuna (Roxb. ex DC.)* (bark), *Curcuma longa* L. (rhizome), *Alianthus excelsa* Roxb. (leaf), *Caesalpinia bonduc* L. (Roxb.) (seed), *Swertia chirayita* (Roxb.) (stem), *Holarrhena pubescens* Wall. ex G.Don (root), *Azadirachta indica* A.Juss. (leaf), *Murraya koenigii* (L.) Spreng. (leaf), *Withania coagulans* (flower), *Salacia oblonga Wall. ex Wight & Arn.* (root), *Cedrus deodara (Roxb. ex D. Don)* (bark), *Picrorhiza kurroa* Royle ex Benth. (root), and *Pterocarpus marsupium* Roxb. (bark).

A. Tinospora cordifolia:

Tinospora cordifolia commonly known as Giloy/Guduchi, an important medicinal plant described in the traditional system (like Unani, Ayurveda and Siddha) regarding their diverse therapeutic properties such as antioxidant, anti-inflammatory adaptogenic, immunomodulatory, and antidiabetic effects. This plant is native to India, a perennial climbing shrub traditionally used for the management of many diseases over many decades. It is also regarded as a magical rejuvenating herb due to its prominent therapeutic effects. The stem of this plant is rich in nutrients and digestive components. The therapeutic effects are due to the presence of secondary metabolites and active constituents like magnoflorine, tinosporin, berberine, palmatine, tembetarine, tinocordifolin present in the stem of the plant. In addition to this stem also has a higher concentration of alkaloid content which supports its medicinal usage. Research evidence suggests that this plant extract has the potential to regulate high blood glucose levels, enhance insulin secretions, recover oxidative stress, inhibit gluconeogenesis & glycogenolysis, and offer promising therapeutic in the management of DM. The preclinical study in the animal model highlighted the therapeutic potential of *T cordifolia* in regulating glucose metabolism and modulating oxidative stress pathways demonstrating its prominent antidiabetic effect both in *in vivo* and *in vitro* evidence (Ahmad Dar *et al.,* 2023; A. Gupta *et al.,* 2024; S. Gupta *et al.,* 2023; Roy *et al.,* 2015).

B. Mangifera indica:

Mangifera indica, commonly referred to as the mango tree, is recognized as a tropical fruit-bearing tree renowned for its potential medicinal properties, particularly in the management of diabetes. Originating from South Asia, notably India, this plant has been well known due to its therapeutic effects in managing many diseases. Different plant parts including leaves, bark, flowers, and seeds, have been utilized in traditional medicine systems. Traditional medicinal practices, such as Ayurveda and traditional Chinese medicine, have employed mango-derived remedies to address a spectrum of ailments, from gastrointestinal issues to inflammatory conditions (Patwardhan *et al.,* 2005). Recent scientific inquiries have unveiled the potential health benefits of mangoes, elucidating their rich nutritional profile abundant in vitamins, minerals, antioxidants, and dietary fibre (Lebaka *et al.,* 2021).

Furthermore, investigations into mango bioactive compounds, such as polyphenols, carotenoids, and terpenoids, have uncovered their diverse therapeutic properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (M. Kumar *et al.,* 2021). Of particular interest is emerging evidence suggesting potential antidiabetic effects of certain bioactive compounds found in mangoes. However, further research, including well-designed clinical trials, must validate these findings and fully elucidate underlying mechanisms (Chaudhury *et al.,* 2017). Mangifera indica has a rich history of traditional use, with indigenous populations across Asia, South America, India, and East Africa utilizing its fruit in treating diabetes and associated ailments. Exploring its therapeutic potential in diabetes management represents a promising avenue in modern medicine.

C. Syzygium cumini

Syzygium cumini, commonly known as Jamun, Black Plum, or Indian Blackberry, has garnered considerable attention in the field of diabetic research due to its potential therapeutic activity (Ayyanar & Subash-Babu, 2012; Chagas *et al.,* 2015). This tropical evergreen tree, belonging to the *Myrtaceae* family, is native to the Indian subcontinent and other regions of Southeast Asia. Various parts of this plant, including seeds, bark, leaves, and fruits, have traditionally been used in Ayurvedic and Unani medicine for the treatment of various ailments, particularly diabetes (Ayyanar & Subash-Babu, 2012). This is an excellent source of bioactive components such as flavonoids, polyphenols, antioxidants, iron, and vitamin C (Rizvi *et al.,* 2022). *S cumini*, in particular, has been extensively studied for its antidiabetic properties (Prabakaran & Shanmugave, 2018). The seeds are especially notable for their high content of phenolic compounds, flavonoids, and other phytochemicals that are believed to contribute to its hypoglycaemic activity (Ayyanar &

Subash-Babu, 2012). These compounds have been shown to enhance insulin secretion, improve insulin sensitivity, and inhibit enzymes involved in carbohydrate metabolism (Chagas *et al.,* 2015). Furthermore, the antioxidant properties of this plant play a crucial role in mitigating oxidative stress, which is a key factor in the pathogenesis of diabetes and its complications (Caturano *et al.,* 2023). Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, leading to cellular damage and inflammation. By scavenging free radicals and boosting antioxidant defenses, it helps to protect the pancreatic β-cells and other tissues from oxidative damage, thereby improving overall glycaemic control (Pizzino *et al.,* 2017; Vona *et al.,* 2021).

A growing body of scientific evidence supports the pharmacological potential of *Syzygium cumini* bark in diabetes management (Qamar *et al.,* 2022). Numerous studies have highlighted the hypoglycaemic, antioxidant, and anti-inflammatory properties of different extracts derived from this plant. These bioactive compounds have been shown to exert beneficial effects on glucose metabolism, insulin sensitivity, and pancreatic function (Amir Rawa *et al.,* 2022; Moreira *et al.,* 2021).

D. Terminalia arjuna

From ancient India has a rich traditional culture of the use of medicinal plants for alignment including diabetes. Among the various plants *Terminalia arjuna* commonly known as Arjuna plant, is one of them having a potent therapeutic activity. This plant belongs to Combretaceae family and is native to southeast Asia and India subcontinent (predominantly located in the riverbank and dry riverbeds). Evidence has suggested its various therapeutic domains including antioxidant, anti-inflammatory, anti-diabetic, hypercholesterolemia, hepatoprotective, respiratory disorder, cardiovascular, Gastrointestinal disorder, UTI, anti-cancer, and blood thinning. The previous study suggests the enzymatic inhibitory activity of the methanolic and aqueous extract of this plant. The chemical profiling exhibits the presence of a number of chemical compounds including arjunetin, arjungenin, ellagic acid, gallic acid, and quercetin. These isolated compounds have been reported to excerpts therapeutic activity including antidiabetic, anti-inflammatory, antiviral, anticancer, antioxidant, antimicrobial, and cardioprotective activity (M. M. Alam & Begum, 2011; Ragavan & Krishnakumari, 2006a; Shengule *et al.,* 2018).

Emerging scientific evidence underscores the variable pharmacological potential of *Terminalia arjuna* in the management of diabetes. The bioactive components actively influence the glucose metabolism, insulin sensitivity, increase the pancreatic functions.

The treatment with this extract significantly promotes the recovery of pancreatic β-cells through the exocrine cells of the pancreas, highlighting its active role in antidiabetic potential (M. M. Alam & Begum, 2011; Biswas *et al.,* 2011; Ragavan & Krishnakumari, 2006b).

E. Curcuma longa

Zingiberaceae family is a well-known species in India for their potent therapeutic activity. Turmeric botanically renamed as *Curcuma longa* has been most commonly used as a spice, food preservative, dye, herbal medicine and colouring material over the last few decades. Besides these miscellaneous uses, this plant exhibits a number of therapeutic activities including anticancer, antioxidant, anti-inflammatory, anti-clotting, antimicrobial activity. Due to its antioxidant activity, this plant is used as a food preservative. Previous published evidence suggests that the aqueous and alcoholic extract of this plant have the potential of lowering the blood glucose level, cytoprotecting against oxidative stress, competitive inhibitor of an α-amylase, and inhibiting starch breakdown significantly reduces the postprandial blood glucose. The chemical profiling suggests the presence of compounds like curcumin, desmethoxycurcumin, bisdemethoxycurcumin, ar-turmerone. The expelled biological activity by these reported compounds includes antimicrobial, neuroprotective, anti-inflammatory, anticancer, antioxidant activities (Kalaycıoğlu *et al.,* 2017; Kuroda *et al.,* 2005; Sabir *et al.,* 2020; Widowati *et al.,* 2018).

The preclinical study in the animal model highlighted the therapeutic potential of the *C longa* in regulating glucose metabolism and modulating oxidative stress pathways demonstrating its prominent antidiabetic effect both in *in vivo* and *in vitro* evidence (Butala *et al.,* 2018).

F. Alianthus excelsa

Over the past decades, traditional medicinal plants have gained a lot of interest in the field of multi-disciplinary research. Among the diverse array of medicinal plants *Alianthus excelsa* commonly known as “Mahanim/tree of heaven” belongs to family Simaroubaceae, indigenous to central and southern India. The different plant parts are used to treat a variety of diseases in different regions worldwide by traditional healers. The traditional use of this plant as a drug includes panic, diarrhoea, bronchitis, and dysenteries. Moreover, this plant is also used for bronchodilatory, antiasthmatic, antiallergic, and fever. In addition to this in China, the bark of the plant is used as an alternative medication to control the release of blood in stool, in Asia and Australia bark used to treat bark has been used for worms, excess vaginal discharge, malaria, and asthma. This plant is also used to treat gonorrhoea, epilepsy, tapeworm infection, and high blood pressure. The isolated

compounds from the plant are apigenin, luteolin, kaempferol-3-O-α-arabinopyranoside, kaempferol-3-Oβ-galactopyranoside, quercetin-3-O-α-arabinopyranoside, and luteolin-7-O-β-glucopyranoside. These compounds not only showed hypoglycaemic activity but also showed antifertility, antifungal, antimalarial, antibacterial, anti-amoebic, antipyretic, leishmanicidal, hepatoprotective, and Antiasthmatic activity (Cabrera *et al.,* 2008; D. Kumar *et al.,* 2010, 2011; Mohammed *et al.,* 2023). The chemical constituents found in the extract of this plant showed a promising therapeutic activity. By revising the diverse pharmacological effects this plant holds substantial potential as a natural alternative medication for treating a variety of diseases. Continued research and clinical studies are essential to fully harness its medicinal potential and ensure its safe and effective use in contemporary medicine.

G. Caesalpinia bonduc

Caesalpinia bonduc, known locally in Odisha as Gill and commonly referred to as "Nata Karanja," has garnered significant attention as a botanical of interest. This shrub, belonging to the *Caesalpiniaceae* family, is native to the hotter regions of India, Myanmar, Sri Lanka, and Bangladesh (Choudhury *et al.,* 2017). Its widespread distribution, particularly in coastal areas of India, underscores its cultural and medicinal significance. Renowned for its diverse pharmacological properties, this plant has been a cornerstone of various traditional medicinal practices (Sasidharan *et al.,* 2021). Research has documented its effectiveness in various therapeutic domains, encompassing antipyretic, antidiuretic, anthelmintic, antibacterial, anti-anaphylactic, antidiarrhoeal, antiviral, antiasthmatic, antiemetic, antiestrogenic, anticancer, hepatoprotective, antioxidant, antimalarial, antimicrobial, antifertility, and anti-inflammatory properties (Billah *et al.,* 2013; Sasidharan *et al.,* 2021; Shukla *et al.,* 2010; Subbiah *et al.,* 2019). Chemical analysis has revealed the presence of cassane diterpenoids like caesalpinins and caesalmins, as well as norcassane diterpenoids such as norcaesalpinins, in C. bonduc seeds (Fei *et al.,* 2022). Additionally, stems, roots, and seeds contain cassane diterpenoids (e.g., taepeenins A-L) and norcassane diterpenoids (e.g., nortaepeenins A & B), while leaves contain phenolic acids like caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, and gallic acid (Sasidharan *et al.,* 2021; Wu *et al.,* 2014). Traditional use of various parts of the *Caesalpinia bonduc* plant, including its seeds, roots, and leaves, has been documented for their therapeutic benefits (Dongre *et al.,* 2012; Sindete *et al.,* 2021). Of particular interest is its traditional application in managing diabetes mellitus, where indigenous tribes, notably those in the Andaman and Nicobar Islands, have employed aqueous decoctions *of Caesalpinia bonduc* seeds to alleviate symptoms (Sasidharan *et al.,* 2021).

H. Swertia chirayita

Among the diverse array of medicinal plants with antidiabetic properties, *Swertia chirayita* stands out as a promising candidate. *S. chirayita*, also known as "Chirata" or "Chiretta," is an herbaceous plant belonging to the Gentianaceae family (Kumar and Staden, 2016). It is native to the Himalayan regions of India, Nepal, and Bhutan, where it has been traditionally used for its medicinal properties (Raghav *et al.,* 2022). The therapeutic potential of this plant in the management of diabetes has attracted scientific interest, leading to preclinical trials using animal models (Dey *et al.,* 2020). These studies have provided valuable insights into the antidiabetic effects and mechanisms of action of *S. chirayita*, paving the way for further research and potential clinical applications. *Swertia chirayita* has a long history of use in traditional medicine systems such as Ayurveda and Unani for its bitter taste and medicinal properties. It contains a variety of bioactive compounds, including amarogentin, swerchirin, swertiamarin, mangiferin, and oleanolic acid, among others. These compounds have been reported to possess antidiabetic, hepatoprotective, anti-inflammatory, and antioxidant activities (Kshirsagar *et al.,* 2019). Several preclinical studies have explored the antidiabetic effects of this plant using experimental models (S. Alam *et al.,* 2022; Choudhury *et al.,* 2017; Salehi *et al.,* 2019). This study yielded a significant insight into the antidiabetic properties of *S. chirayita*, encompassing several key aspects. One such aspect is its ability to regulate blood glucose levels, as evidenced by studies demonstrating a reduction in blood glucose levels by stimulating insulin secretion from pancreatic β-cells, improved glucose uptake in peripheral tissues, and inhibition of gluconeogenesis in the liver, in diabetic rats treated with *S. chirayita* extracts (Manjunatha *et al.,* 2023). Moreover, S. chirayita has shown promise in improving insulin sensitivity, particularly in insulin-resistant Wistar rat models (Dey *et al.,* 2020). This improvement is associated with enhanced insulin signaling pathways, increased translocation of GLUT4 (glucose transporter 4) to cell membranes, and enhanced glucose uptake in skeletal muscle and adipose tissue (Wang *et al.,* 2020). Additionally, preclinical trials have indicated that *S. chirayita* extracts offer protection against diabetic complications such as nephropathy, neuropathy, and retinopathy (Raghav *et al.,* 2022). These protective effects are attributed to the plant's antioxidant, anti-inflammatory, and anti-apoptotic properties, which help mitigate the damage caused by chronic hyperglycemia (Ansari *et al.,* 2023). Overall, these preclinical findings highlight the multifaceted therapeutic potential of *S. chirayita* in addressing various aspects of diabetes, including blood glucose regulation, insulin sensitivity improvement, protection against complications, and safety considerations (Dey *et al.,* 2020; Raghav *et al.,* 2022).

Additional studies are needed to elucidate the underlying mechanisms of action, optimize dosage regimens, and evaluate long-term efficacy and safety of this plant extract.

I. Holarrhena pubescens

Among the myriads of natural resources, medicinal plants have emerged as a promising frontier, offering a treasure trove of bioactive compounds with therapeutic potential. *Holarrhena pubescens*, also known as *Holarrhena antidysenterica*, is native to Africa, as well as tropical and subtropical regions of Asia, including India and Pakistan is an important medicinal plant (Zahara *et al.,* 2020). In Indian medicine, *Holarrhena pubescens* is commonly used to treat conditions such as bleeding piles, diarrhoea, amoebic dysentery, liver disorders, and irritable bowel syndrome, owing to its astringent and bitter properties (Zahara *et al.,* 2020). The stem bark, locally in western Odisha is known as Indrajav and commonly called Kurchi, contains various medicinal properties such as being astringent, antidiarrheal, antidysentery, anti-anthelmintic, stomachic, febrifugal, digestive, and tonic (Srivastava, 2023). Its therapeutic benefits have been supported by pharmacological and clinical investigations, highlighting its efficacy in managing cutaneous, intestinal, and diabetic conditions (Sena *et al.,* 2010). Due to its effectiveness and minimal side effects, this plant is considered a highly beneficial medicinal plant in traditional medicine practices. The exploration into its potential as an antidiabetic agent goes beyond the conventional boundaries of medical practices, venturing into the realm of preclinical research to reveal its therapeutic properties (Alyahya *et al.,* 2023). This undertaking signifies a preclinical journey, a scientific expedition merging ancient insights with contemporary scientific approaches to stimulate discovery and reveal innovative treatments for diabetes mellitus.

J. Azadirachta indica

Azadirachta indica belonging to Meliacae family commonly known as “neem” is an important plant found in the flora of Asian subregion, Nigeria and other parts of Africa. This plant possesses a number of pharmacological activities including cardiovascular, antimicrobial, antihelminthic, hypolipidemic, antipyretic, anti-inflammatory, depressant, antifungal, antiviral, antifertility, contraceptive and sedative activities. The seeds and leaves of the plant show the anti-hyperglycemia effect. Besides this neem oil is also beneficial for hair, improves liver functions, detoxifies the blood, and balances blood sugar levels. The chemical profile of the plant excreted a total of 135 chemical constituents and most of the compounds showed hypoglycaemic activity. The scientific mechanistic evidence suggests, that by improving the insulin signaling molecules and GLUT4 protein to enhance oxidation in the skeletal muscle this plant controls the hyperglycemia. The

isolated compounds from the plant are nimbolite, nimbinin, nimbidin, nimbolide, quercetine, nimbin, azadirachtin, which exhibit a variety of pharmacological activity including hepatoprotective, antioxidant, and antimalarial activity. Besides this, the leaf extract exerts the regenerative capacity of pancreatic β-cells as well as the weight gain and normalized the high blood glucose level in a diabetic rodent model (Atangwho *et al.,* 2012; McCalla *et al.,* 2015; P. Patil *et al.,* 2013; Revankar, 2014; Satyanarayana *et al.,* 2015).

K. Murraya koenigii

Murraya koenigii, commonly known as curry leaf, is a plant deeply rooted in traditional medicine, especially within the Indian Ayurvedic system. It belongs to the Rutaceae family, which includes over 150 genera and 1600 species. This plant is highly valued for its distinctive aroma and medicinal properties, making it a significant export commodity for India due to its substantial foreign revenue potential (Balakrishnan *et al.,* 2020; Palwankar *et al.,* 2020). Various chemical compounds have been extracted from every part of the plant, including P-gurjunene, P-caryophyllene, P-elemene, and O-phellandrene being the key constituents responsible for its characteristic aroma (Mankar *et al.,* 2021). For centuries, this plant has been a staple in Indian cuisine and traditional medicine. The plant is known for its tonic and stomachic properties (Reddy *et al.,* 2018). Its bark and roots are used as stimulants and for treating eruptions and bites from poisonous animals (Balakrishnan *et al.,* 2020). The green leaves are consumed raw to treat dysentery, diarrhoea, and vomiting (Acharya & Acharya, 2009). Additionally, the leaves and roots are traditionally used for their bitter, anthelmintic, analgesic properties and for treating piles, inflammation, itching, leukoderma, and blood disorders (Lakshmikandhan, 2020). Several scientific studies have been conducted to explore the efficacy of the whole plant or its parts in various extract forms for treating different diseases (Balakrishnan *et al.,* 2020; Varghese *et al.,* 2018). *M. koenigii* contains numerous chemical constituents that interact in complex ways to produce their pharmacodynamic effects (Maheswari *et al.,* 2024). Many active constituents responsible for its medicinal properties have been isolated and characterized. This plant has demonstrated antioxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic, and cholesterol-lowering activities (Suthar *et al.,* 2022). The leaves, beyond their culinary use, have long been esteemed for their medicinal qualities. Scientific research has identified several bioactive compounds in *M. koenigii*, including carbazole alkaloids, flavonoids, and tannins, which exhibit a variety of therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial, and notably, antidiabetic effects. These compounds help regenerate pancreatic β-cells and lower blood sugar levels. This scientific validation of traditional uses has spurred further investigation into how this

plant can help manage diabetes (Bhupatiraju *et al.,* 2023). The antidiabetic properties of *M. koenigii* are largely due to its rich array of bioactive compounds (Salehi *et al.,* 2019). Carbazole alkaloids, such as mahanine and mahanimbine, have shown promise in lowering blood glucose levels (Salehi *et al.,* 2019). These compounds are thought to promote the regeneration of pancreatic β-cells, which are essential for insulin production. Additionally, the flavonoids and tannins in the leaves have potent antioxidant properties that help reduce oxidative stress, a key factor in diabetes-related complications. One significant way this plant helps to manage diabetes is by inhibiting α-glucosidase, an enzyme responsible for breaking down carbohydrates. By blocking this enzyme, compounds like mahanine can slow the digestion and absorption of carbohydrates, preventing spikes in blood sugar after meals. Moreover, the anti-inflammatory properties of these compounds aid in reducing inflammation-induced insulin resistance, which further supports better blood sugar control (Behl *et al.,* 2022).

L. Withania coagulans

Withania coagulans, commonly known as Indian cheese maker or Paneer Dodi, locally in Western Odisha it is known as Paneer Phul, belongs to the Solanaceae family and has been used in traditional medicine for centuries (Kherade *et al.,* 2021). The plant is native to regions of India, Pakistan, Afghanistan, and Iran. This plant is often referred to as "paneer" in Punjab, due to its milk-coagulating properties found in its fruits and leaves. The coagulating ability is attributed to the pulp and husk of the berries, which contain an enzyme responsible for this activity. The leaves of this plant, commonly known as Paneer Dodi, measure between 2.5-5.7 cm in length and 1-2.2 cm in width. They are lanceolate-oblong, entire, obtuse, and covered with a persistent, greyish tomentum that is difficult to remove (S. Gupta *et al.,* 2023). In ethnomedicine, *W. coagulans* is known for its diverse therapeutic properties, including antidiabetic, anti-inflammatory, hepatoprotective, and immunomodulatory effects (Banerjee *et al.,* 2019). The Fruits possess sedative, emetic, and diuretic properties. They are used to treat various conditions, including diabetes, nervous exhaustion, debility, insomnia, wasting diseases, failure to thrive in children, impotence, chronic liver disease, dyspepsia, flatulent colic, asthma, biliousness, and other gastrointestinal infections. The berries are also utilized for blood purification. Chewing the plant's twigs is a traditional method for cleaning teeth, and inhaling the smoke is said to relieve toothaches. In the northwestern region of India, traditional healers use the dried fruits of this species to treat diabetic patients, although its antihyperglycemic activity has not been systematically evaluated. Traditional healers have long utilized various parts of the plant, such as fruits, leaves, and roots, to treat a wide range of ailments. Various studies

have shown that this plant has garnered significant attention due to its reported hypoglycaemic and antidiabetic effects (Khan *et al.,* 2021). The fruit of the plant, in particular, has been extensively used to manage diabetes mellitus in traditional Indian and Unani systems of medicine (Sampathkumar *et al.,* 2019).

M. Salacia oblonga

Numerous reviews on medicinal plants used for diabetes mellitus (DM) treatment exist, but only a few have undergone rigorous scientific evaluation. While many plants exhibit hypolipidemic activity, only a select few demonstrate both hypoglycaemic and hypolipidemic effects. *Salacia oblonga*, belonging to Hippocrateaceae family, is a large woody climber found in the forests of Sri Lanka and southern India. Traditionally this plant is used in gonorrhoea, rheumatism, and skin diseases. Despite its extensive use as a traditional remedy, the biochemical mechanisms underlying its effects on physiological and pathophysiological functions remain largely unexplored. *S. oblonga* has been noted for its ability to reduce postprandial glycemia. The stem and root of the plant contain a number of active constituents like salacinol, kotalanol, and kotalgenin-16-acetate showing pharmacological activity including α-glucosidase inhibitions, enhancing the insulin sensitivity and promoting the regeneration of pancreatic β-cells which is important for maintaining the glucose homeostasis in diabetes. Beside this *S oblonga* also shows antilipidemic, antimalarial, anti-inflammatory, antimicrobial, antiperoxidative, antileukemic, and astringent activities. Additionally, studies have shown that the root bark extract can prevent hyperglycemia and reduce lipid peroxidation, which ultimately helps in managing diabetes and its associated neuropathy (Bhat *et al.,* 2012; Chelladurai & Chinnachamy, 2018; Deepak *et al.,* 2020; Krishnakumar *et al.,* 2000; Matsuda *et al.,* 1999; Surekha *et al.,* 2015).

N. Cedrus deodara

Medicinal plants gain a valuable interest and are recognized as a natural source of discovering potential medicines. The traditional knowledge about the use of medicinal plants passed through generations is now the main focus of many industries for the development of new potent antimicrobials, antibiotics, antidiabetics, and antioxidant medications. Among a variety class/species of medicinal plants *Cedrus deodara* commonly known as devdaar belonging to the family Pinaceae, stands out due to its wide range of ethnomedicinal activity. The gum and oil extracted from this plant are used to treat hiccoughs, fever, dyspepsia, insomnia, and inflammation, however, the leaves and the wood of the plant are used for tuberculosis and carminative, diaphoretic, & diuretic, respectively. The plant contains a variety of active constituents including

dihydromyricetin, himachald, isocentdarol, dewarene, himadarol, α-himachalene, β-himachalene centdard, dewardiol, dewarenol, taxifolin, cetrin, allohimachalol, cedeodarin, and cedrinoside excreting the pharmacological activity like anti-inflammatory, analgesic, antidiabetic, antiproliferative activities. The antidiabetic mechanisms of this plant encompass the enhancement of insulin secretion, inhibition of carbohydrate-digesting enzymes, anti-inflammatory properties, and the regenerative capacity of pancreatic β-cells. Revising this pharmacological importance of the plant it may regarded as a promising natural source for developing new potent medications for various diseases including Diabetes (Bisht *et al.,* 2021; S. Patil *et al.,* 2011; Pradhan & Mahapatra, 2016; Sharma & Parashar, 2021; Singh *et al.,* 2013).

O. Picrorhiza kurroa

Picrorhiza kurroa commonly known as katuki, is a small herb located in India, mostly found in the Alpine Himalayas from Kashmir to Sikkim. This plant is well known in the ayurveda system and traditionally being used for the treatment of liver disorders, upper respiratory tract infections, fever, dyspepsia, and chronic diarrhea. The traditional healers used roots & rhizomes found in the Himalayan regions to treat fever, hepatitis, respiratory tract diseases, allergies, and other inflammatory conditions. Besides this, *P kurroa* exhibits a wide range of biological activities such as antimicrobial, hepatoprotective, antioxidant, anticancer, antiarthritic, antidiabetic, antimutagenic, cardioprotective, antimalarial, anti-inflammatory, anti-ulcer, antiasthmatic, immunomodulatory, hypolipemic, and nephroprotective activities. The active constituents isolated from the plant are apocynin, drosin, and nine cucurbitacin glycosides. Apocynin is a catechol, that inhibits neutrophil oxidative burst and acts as a powerful anti-inflammatory agent, while cucurbitacins are highly cytotoxic and possess antitumor effects. The antidiabetic activity of these constituents shows a significant reduction of the fasting blood sugar (FBS) level and controls dyslipidemia. In addition to this, the scientific evidence suggests the hydroalcoholic extract of the plant demonstrated a strong β-cell regeneration potential, antihyperglycemic effects, enhanced insulin expression, and improvements in hepatic and renal functions (Almeleebia *et al.,* 2022; Husain *et al.,* 2014; Joy & Kuttan, 1999; S. Kumar *et al.,* 2017; Nisar *et al.,* 2022; Salma *et al.,* 2017).

P. Pterocarpus marsupium

Pterocarpus marsupium (Leguminosae), also known as Indian kino or Bijasa, a large tree mostly found in the mixed forests of central and Peninsular India, as well as Sri Lanka. The ayurvedic medicine system this plant gained a major focus due to its curative and lenitive properties. Traditionally, the gum and bark are used for the treatment of heartburn

and diabetes, while the leaves are applied to boils, sores, and various skin diseases. In addition to this, another traditional technique that has been employed for the management of diabetes is storing water overnight in tumblers made from the heartwood of *P. marsupium* and the next morning applied in the body for the prevention of chest pain and diabetes. The flower is used to treat fever, depurative, hemostatic, and rejuvenating agent, and its wood for chest and body pain as well as indigestion. The bark is effective in preventing cataract formation and reducing hyperglycemia in alloxanized diabetic rats, while the heartwood serves as a hypoglycemic agent. This plant is rich in various phytoconstituents like flavonoids, terpenoids, tannins, glycosides, sterols, phenols, and saponins, showing biological activity such as antidiabetic, antihyperlipidemic, and antioxidant effects. The scientific evidence on flavonoids and phenols suggests the ability of the regeneration of pancreatic beta cells in alloxan-induced diabetic models and antihyperglycemic effects, respectively. The major active compounds detected from these plants are pterosupin, pterostilbene, liquirtigenin, isoliquiritigenin, epicatechin, kinoin, kinotannic acid, kino-red, beta-eudesmol, carsupin, marsupol, and marsupinol, shown to increase the insulin secretion, and enhances the islets number in pancreas (Devgun *et al.,* 2009; Dhanabal *et al.,* 2006; Katiyar *et al.,* 2016; Maruthupandian *et al.,* 2011; A. Mishra *et al.,* 2013; Pant *et al.,* 2017; Vijayan, 2019).

2.11. Identified compound from these Plants and their mechanism of action

Sl No	Plant Name	Active Phyto-constituents	Mechanism of action	Reference
1.	*Tinospora cordifolia*	Magnoflorine	By inhibiting the α-amylase and α – glucosidase enzyme, enhanced insulin secretions.	(Durmaz *et al.,* 2022; Okon *et al.,* 2020)
		Tinosporin	Enhanced insulin sensitivity, inhibitions of the α-amylase and α – glucosidase.	(Kumar Roy *et al.,* 2020b; N. M. Reddy & Reddy, 2015)
		Berberine	Enhanced insulin secretions and sensitivity.	(Lee *et al.,* 2006)

			Regeneration of pancreatic β-cells.	
		Palmatine	Enhanced the glucose uptake, insulin secretions and reduced the oxidative stress.	(Ekeuku *et al.,* 2020; Nwabueze *et al.,* 2022)
		Tembetarine	Enhanced insulin sensitivity and reduce the oxidative stress.	(Chandramohan *et al.,* 2023)
2.	*Mangifera indica*	Mangiferin	Inhibitions of α-amylase and glucosidase activity and enhanced insulin sensitivity.	(da Silva Lopes *et al.,* 2024; Du *et al.,* 2018)
		Gallic acid	Restoration of β-cells, reduce oxidative stress and inhibitions of enzyme (α-amylase and glucosidase).	(Adefegha *et al.,* 2015; Khodeer *et al.,* 2023; Kimani *et al.,* 2023)
		Ascorbic acid	Enhanced insulin sensitivity, reduction of glycations products, enhanced the endothelial function, reduce oxidative stress by	(Abdelrahim *et al.,* 2023; Murtaza *et al.,* 2022; Won *et al.,* 2021)

			neutralizing the free radicals.	
		Quercetin	Inhibitions of DPP-IV enzyme & carbohydrate digestive enzyme (α-glucosidase), enhance the function of pancreatic β-cells to improved insulin secretions, improved glucose utilization in skeletal and adipose tissue.	(Bule *et al.,* 2019)
		Linolenic acid	Improving the lipid profiles (reduce TG and increased HDL), reduced insulin resistance and increased insulin sensitivity.	(Jackson *et al.,* 2024; Mousavi *et al.,* 2021; Prada *et al.,* 2023)
		β-Sitosterol	Increases fasting plasma insulin level, enhanced glucose uptake in adipocytes and stimulate adipogenesis in preadipocytes cells.	(Ambavade *et al.,* 2014)

		β -Carotene	Inhibitions of the proinflammatory cytokines subsequently enhanced the insulin sensitivity and glucose metabolism, significantly reduce the oxidative stress.	(S. Alam *et al.,* 2022; J. M. Castellano *et al.,* 2013b)
		Catechin	Enhanced the insulin sensitivity, maintaining the mitochondrial function, reduce the ER stress (which subsequently enhanced the β-cells function), enhanced the regulation of intestinal function.	(Baranwal *et al.,* 2021; Wen *et al.,* 2022)
		Ellagic Acid	Enhanced the glucose uptake by the peripheral skeletal tissue along with the stimulation of pancreatic β-cells for increased insulin secretions, enhanced the hexokinase activity	(Fatima *et al.,* 2017; Ghazaee *et al.,* 2024; Jadhav & Puchchakayala, 2012; Malini *et al.,* 2011)

			in liver, which subsequently decreased the glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney.	
3.	*Syzygium cumini*	Jamboline	Hypolipidemic activity, enhanced glucose uptake and improve the overall glucose metabolism, significantly reduce the oxidative stress, inhibits the carbohydrate breaking enzyme (α-amylase and glucosidase).	(S. Srivastava & Chandra, 2013; Tyagi *et al.,* 2019)
		Anthocyanins	Inhibition of α-amylase and glucosidase enzyme, enhanced the serum insulin level and oxidative cell damage	(D. Kaur *et al.,* 2024; Y. Singh & Bhatnagar, 2019)
		Myricetin	Inhibits the alpha amylase enzyme, enhanced the insulin sensitivity	(Baldissera *et al.,* 2016; Franco *et al.,* 2020; Lalitha

			and glucose uptake & utilization. In addition to this it also inhibits DPP-4 enzyme which subsequently increased the level of GLP-1. Preservation of pancreatic β-cells.	*et al.,* 2020; Li *et al.,* 2017; Manaharan *et al.,* 2012; Niisato & Marunaka, 2023; Syama *et al.,* 2018a)
		Kaempferol	Inhibits the DPP-IV enzyme, prevent the β-cells apoptosis deaths followed by the enhanced insulin secretions, α-glucosidase inhibitory activity.	(Mahmud *et al.,* 2023; Rashid *et al.,* 2022; Syama *et al.,* 2018b)
		Betulinic acid	Inhibition of α-amylase enzyme, Stimulates the expression of GLT-4 and activating AMPK it enhanced the glucose uptake by the muscle cells.	(Song *et al.,* 2021; Vellingiri *et al.,* 2016)
		Mycaminose	Enhanced the insulin secretions by regeneration of pancreatic β-cells and improved the glucose tolerance.	(Y. Singh & Bhatnagar, 2019)

4.	*Terminalia arjuna*	Arjunetin	Reduced lipotoxicity, improved insulin sensitivity, preserving the pancreatic β-cells damage and their function.	(Chaudhury *et al.,* 2017; Kimmel & Inzucchi, 2005; Nie & Cooper, 2021)
		Arjungenin	Inhibitions of α-amylase and glucosidase enzyme. Enhanced insulin sensitivity, glucose uptake & promotes adipogenesis. It also helps in protecting pancreatic β-cells from oxidative stress and inflammation.	(Ansari *et al.,* 2022; A. Mohammed *et al.,* 2015; Paarakh, 2010; Sivaraman *et al.,* 2015)
5.	*Curcuma longa*	Curcumin	Regeneration of pancreatic β-cells, Enhanced the glucose regulation by inhibiting the DPP-4, that subsequently increase the GLP-1 secretions. It increases insulin sensitivity and lipid metabolism. In addition to this	(Mohammadi *et al.,* 2021)

			it helps in reducing the FBS and HbA1C levels. Inhibition of carbohydrate breakdown enzyme (α-amylase & glucosidase).	
		Desmethoxycurcumin	Scavenging free radicals, reduce oxidative stress, enhanced insulin secretions & glucose uptake in muscles cells, protecting pancreatic β-cells from apoptosis.	(Chuengsamarn *et al.,* 2012; Mohammadi *et al.,* 2021; Vaithiyalingam *et al.,* 2023)
		Bisdemethoxycurcumin	Protects the pancreatic β-cells from oxidative damages, enhanced the insulin sensitivity, reduced the hepatic glucose production.	(Vaithiyalingam *et al.,* 2023)
		Ar-turmerone	Inhibition of carbohydrate break enzyme, improved glucose uptake	(Zhang & Kitts, 2021b)
6.	*Alianthus excelsa*	Apigenin	Enhance glucose metabolism,	(Jiang *et al.,* 2022)

			insulin sensitivity, reduced oxidative stress and regeneration of pancreatic β-cells.	
		Luteolin	Inhibition of α-glucosidase and protein tyrosine phosphate 1B (PTP1B), reduce the oxidative stress and protective activity of the pancreatic β-cells.	(Choi *et al.,* 2014; Han *et al.,* 2023)
		luteolin-7-O-β-glucopyranoside	Inhibition of α-glucosidase. Additionally, a strong antioxidant & anti-inflammatory subsequently reduce the oxidative stress and protect the pancreatic β-cells.	(Borges *et al.,* 2021; Neamah *et al.,* 2018)
7.	*Caesalpinia bonduc*	Caesalpinins	Insulin mimetic activity, enhanced insulin secretions from β-cells, increased glucose uptake in the peripheral tissue, inhibition of	(Chan *et al.,* 2018; Chu & Wu, 2023a; N. Gupta *et al.,* 2013)

			carbohydrate digestion enzymes.	
		Caesalmins	Enhanced insulin sensitivity, inhibition of the glucosidase enzyme.	(Chu & Wu, 2023b)
		Norcaesalpinins	Mitigating the chronic inflammation linked with insulin resistance, enhanced insulin sensitivity and inhibition of carbohydrate digestion enzyme.	(Sasidharan *et al.,* 2021b)
		Nortaepeenins A & B	Enhance insulin sensitivity, inhibition of carbohydrate digestion enzyme, β-cells protection, and enhanced insulin secretions.	(Chan *et al.,* 2018; Pournaghi *et al.,* 2020; Sasidharan *et al.,* 2021b)
		Caffeic acid	Inhibitions of carbohydrate digestion enzyme, enhance insulin sensitivity, improved glucose metabolism, promotes insulin release from β-	(Aijaz *et al.,* 2023; Al-Hussaini *et al.,* 2015; Chiou *et al.,* 2017; Eid *et al.,* 2017; Xu *et al.,* 2020; Yusuf *et al.,*

			cells, enhanced GLUT4 translocation, suppress hepatic glucose output inhibition of gluconeogenesis & adipogenesis.	2019; Zhao *et al.,* 2022)
		Chlorogenic acid	Stimulates the glucose metabolism, inhibits the carbohydrate digestion enzyme, regulates the lipid metabolism, acts on liver enzyme regulation	(Savych *et al.,* 2021; A. K. Singh *et al.,* 2021)
		p-coumaric acid	Inhibitions of gluconeogenesis enzyme, improves lipid profile via GLUT2 activation, minimize oxidative stress, and enhanced insulin sensitivity.	(Abdel-Moneim *et al.,* 2022; Amalan *et al.,* 2016)
		Ferulic acid	Inhibits the carbohydrate digestive enzyme, prevent the glycation of protein, enhanced the insulin	(Nankar *et al.,* 2017; Narasimhan *et al.,* 2015; Salau *et al.,* 2023;

			secretions, restores the pancreatic health and β-cells, improved lipid metabolism,	Zduńska *et al.,* 2018)
8.	*Swertia chirayita*	Amarogentin	Improved the GLUT 4 expression in skeletal muscles, decreased the phosphoenolpyruvate carboxykinase enzyme of liver, which subsequently enhanced glucose homoeostasis. Enhanced the insulin sensitivity, inhibition of carbohydrate digestion enzyme.	(Nag *et al.,* 2015; Niu *et al.,* 2016; Potunuru *et al.,* 2019)
		Swerchirin	Enhanced insulin secretions, improves the glucose metabolism, inhibits protein glycations,	(Dey *et al.,* 2020b; V. Kumar & Van Staden, 2016b)
		Swertiamarin	Regenerations of pancreatic β-cells, enhanced insulin sensitivity, Inhibition of	(Fadzil *et al.,* 2021)

			carbohydrate digestion enzyme (α-amylase), enhanced glucose & lipid metabolism.	
		Oleanolic acid	Enhanced insulin response, preserves the functionality & survival of pancreatic β-cells, modulates the enzyme directly linked with insulin biosynthesis.	(J. M. Castellano *et al.,* 2013a; J. M. ; Castellano *et al.,* 2022)
9.	*Holarrhena pubescens*	Conessine	Enhanced insulin sensitivity, protects the pancreatic β-cells from damages, inhibits the carbohydrate digestion enzyme (α-glucosidase),	(Bala *et al.,* 2022; D. Kumar *et al.,* 2023)
		Iso-conessine	Improves insulin secretions & inhibition of α-Glucosidase enzyme.	(Jamadagni *et al.,* 2017)
		Conimine	Enhanced insulin sensitivity, reduce oxidative stress, inhibition of α-Glucosidase	(Singh Chouhan *et al.,* 2017; Zahara *et al.,* 2020c)

			enzyme, improved the altered biochemical parameter (i.e., serum cholesterol, triglycerides, and liver enzymes).	
		Holarrhimine	Enhanced insulin sensitivity and inhibits the carbohydrate digestion enzyme (α-glucosidase).	(Sinha *et al.,* 2013; Zahara *et al.,* 2020c)
		Conarrhimine	Inhibition of α-glucosidase enzyme, enhanced insulin secretions and reduced the oxidative stress & insulin resistance.	(Thawkar *et al.,* 2024; ZHOU *et al.,* 2017)
10.	*Azadirachta indica*	Nimbidin	Inhibitions of α-amylase & glucosidase enzymes, enhanced insulin secretions and insulin sensitivity.	(Ganorkar *et al.,* 2023)
		Nimbinin	Improved insulin sensitivity, and secretions, inhibits the carbohydrate digestion enzyme (α-amylase & glucosidase),	(Padarthi *et al.,* 2024; Palshikar & Pandiyan, 2021; Rafe, 2017)

			hypolipidemic activity, reduce the oxidative stress.	
		Azadirachtin	Inhibits the carbohydrate digestion enzyme (α-amylase & glucosidase) in mix mode i.e., it can bind to both the enzyme and the enzyme-substrate complex. Improves the elevated lipid profile, insulin sensitivity and secretions.	(Nkeng-Asong *et al.,* 2019; Ponnusamy *et al.,* 2015)
		Nimbolide	Inhibitions of both the carbohydrate digestion enzymes. Enhanced insulin secretions and sensitivity, significantly reduce the oxidative stress. Inhibitions of lipid accumulation (reduce the intracellular cholesterol, free fatty acid and triglycerides).	(Alshammari *et al.,* 2017; Rajendran *et al.,* 2024)

11.	*Murraya koenigii*	Mahanine	Enhanced the glucose uptake in skeletal muscles and adipocytes cells, subsequently improved the insulin sensitivity & reduce the insulin resistance. Enhanced GLUT4 translocation in myotubes. Inhibitions of α-amylase and α-glucosidase, enzymes. Anti-adipogenic property,	(Nooron *et al.,* 2017; Parthasarathy *et al.,* 2018; Samanta *et al.,* 2018)
		Mahanimbine	Hypoglycaemic activity (inhibits the α-amylase and α-glucosidase enzymes), hypolipidemic activity (lowers the triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels while increasing high-density lipoprotein (HDL) levels), improved	(Nooron *et al.,* 2017; Parthasarathy *et al.,* 2018)

			insulin sensitivity and secretions. Enhanced glucose clearance, & lowered dietary fat absorption, leading to increased fat excretion.	
		Bismurrayafoline E	stimulate insulin secretion from pancreatic β-cells, improving glucose uptake. Inhibit enzymes like α-glucosidase and α-amylase, slowing carbohydrate digestion and glucose absorption.	(Ajay S *et al.,* 2011; Kaloni *et al.,* 2020; Phatak *et al.,* 2019)
12.	*Withania coagulans*	Withanolides	α-Glucosidase & amylase enzyme inhibitions, enhanced insulin sensitivity.	(Das, 2023; Maher *et al.,* 2020)
13.	*Salacia oblonga*	Salacinol	Inhibition of α-Glucosidase enzyme	(Morikawa *et al.,* 2015, 2021)
		Kotalanol	α-Glucosidase inhibitors in small intestine.	(Tanabe *et al.,* 2012)
		Kotalgenin-16-acetate	Aldose reductase inhibitors, inhibitions of α-	(Deepak *et al.,* 2020; S.

			glucosidase and reduce the oxidative stress nby exhibiting antioxidant properties.	Gupta *et al.,* 2022)
14.	*Cedrus deodara*	Himadarol	Improves the glucose uptake & insulin secretions. Reduce the oxidative stress. inhibits β-cells apoptosis, inhibits the amylin aggregation which is responsible for the protection of β-cells.	(S. Gupta *et al.,* 2011; Pathak *et al.,* 2023)
		Atlantone	inhibiting pro-inflammatory cytokines, which can improve insulin sensitivity, enhancing insulin secretion and action, protecting pancreatic β-cells from damage and inhibit enzymes like α-glucosidase and α-amylase enzymes.	(S. Kumar *et al.,* 2022; Sintos & Cabrera, 2024)
		Cedrin	Inhibition of amylin aggregation	(Bisht *et al.,* 2021, 2023;

			which subsequently causing prevent of β-cell disruption. Protecting β-cells from apoptosis. Enhanced insulin sensitivity. Reduce oxidative stress.	Somuah-Asante, 2016)
15.	*Picrorhiza kurroa*	Apocynin	Inhibits the NADPH oxidation which results in the inhibitions of ROS production. Enhances the activity of antioxidant enzymes, protecting cells from oxidative damage. improves mitochondrial function by enhancing the activity of electron transport chain complexes, reducing mitochondrial oxidative stress. enhances insulin sensitivity and glucose uptake. reducing lipid accumulation and	(Bharadwaja *et al.,* 2021; Bravo-Sánchez *et al.,* 2021; Ekozin *et al.,* 2022)

			adipocyte differentiation and mimics insulin by enhancing the expression of key markers like IRTK, IRS-1, PI3K, and GLUT-4.	
		Drosin	Inhibits enzymes like α-amylase and α-glucosidase. Mimics the action of insulin, enhancing glucose uptake by cells and improving insulin sensitivity. Modulates inflammatory pathways, reducing inflammation that can impair insulin signalling. Reduction of oxidative stress.	(Rahimi *et al.,* 2023; Salma *et al.,* 2017; B. Singh *et al.,* 2021)
16.	*Pterocarpus marsupium*	Pterosupin	α-amylase & glucosidase inhibition, exhibits anti-inflammatory properties subsequently protects the pancreatic β-cells. Enhanced insulin	(Chinni & Jyothsna, 2023; Perera, 2016)

			sensitivity and glucose uptake.	
		Pterostilbene	improves insulin sensitivity, secretions and glucose utilization. in adipose tissue, enhancing glucose uptake and metabolism by promoting the translocation of GLUT4 to the cell membrane. Reduction of oxidative stress and subsequently protects the pancreatic β-cells. Normalizes the levels of key glucose metabolism enzymes.	(Elango *et al.,* 2016; McCormack & McFadden, 2013; Sun *et al.,* 2019)
		Liquirtigenin	Reduce the insulin resistance and improved the secretions, enhanced the viability and functions of pancreatic β-cells.	(Alzahrani *et al.,* 2022; Danao *et al.,* 2023)

		Epicatechin	Regeneration of pancreatic β-cells, enhanced insulin sensitivity and secretions, inhibitions of digestive enzymes (α-glucosidase).	(Mechchate *et al.,* 2021)
		Kinotannic acid	Inhibitions of both the digestive enzymes, reducing the oxidative stress & modulate the inflammatory pathway subsequently protects the pancreatic β-cells. enhanced insulin sensitivity and secretions, improved the glucose uptake by the cells.	(Ajayi *et al.,* 2017)
		β-eudesmol	Enhanced insulin sensitivity, reduce oxidative stress, improved peripheral glucose utilizations, inhibits the carbohydrate digestive enzymes.	(Ghazouani *et al.,* 2016; Kumar Bharti & Bioanal Biomed, 2013; Miyazawa *et al.,* 1996)

		Carsupin	Stimulates the insulin secretions, protects the β-cells from damage, enhanced the glucose uptake	(Chinni & Jyothsna, 2023)
		Marsupol	Inhibitions of digestive enzymes, improved insulin secretions and helps in reduce the oxidative stress.	(Anshika *et al.,* 2022; Danao *et al.,* 2023)

Chapter-3

Exploring the Therapeutic Potential of the Polyherbal Formulation for Diabetes Management

Abstract

Diabetes mellitus is a global pandemic characterized by the progressive loss of pancreatic β-cells. Preliminary evidence suggests the favourable impact of ethnopharmacological agents in the control of diabetes. This study aimed to validate the antidiabetic activity of an aqueous polyherbal extract (APE) via in silico, in vitro, and in vivo models.

UHPLC–Q-TOF-MS analysis of APE was performed to identify bioactive secondary plant metabolites. In silico approaches implemented to predict the binding efficacy of the active phytoconstituents. Biochemical estimation (total phenolic and flavonoid content), free radical scavenging activity, and in vitro and in vivo antidiabetic activities of APE were performed. Histomorphological and immunohistological studies of the pancreatic islets were carried out in diabetic animals for microarchitectural study.

UHPLC-Q-TOF-MS identified a total of 60 compounds in APE, of which only 39 were reported to have antidiabetic activity. In silico study revealed a strong interaction of verbacoside B with the target proteins. APE is characterized by high flavonoid and phenolic contents with strong antioxidant properties. In an in vitro enzymatic assay, APE inhibited α-amylase and α-glucosidase, and the calculated IC50 values were 54.26 ± 0.14 and 26.47 ± 0.12 µg/ml, respectively. The in vitro glucose uptake assay revealed significantly increased uptake of APE in a dose-dependent manner. At 500 mg/kg b.w., APE significantly decreased blood glucose levels and HbA1c levels and had no side effects on liver or kidney function, as measured by blood parameters. Immunohistological observation revealed 47% regeneration of pancreatic β-cells with APE treatment in diabetic animals.

The polyherbal formulation has prominent antidiabetic activity, as evidenced by in silico, in vitro and in vivo studies, by modulating the cellular composition and regenerating β-cells of the pancreatic islet.

3.1. Introduction

Diabetes mellitus (DM) is a group of metabolic ailments clinically characterized by hyperglycemic (elevated blood glucose) conditions. India has become the capital of diabetes, contributing approximately 77 million cases annually (Anjana *et al.,*2023). The islets of Langerhans play an important role in glucose homeostasis (Cabrera *et al.,*2008; Steiner *et al.,*2010) and include 60–70% β-cells (insulin-producing), 15–20% α-cells (glucagon-producing), and the remaining 10–20% are pancreatic polypeptide pp-cells, δ cells, and ε-cells (Bosco *et al.,*2010; Huang *et al.,*2018). The distribution pattern of α- and β-cells varies depending on the size of the islet; maintaining an optimal size is important for their functional activity. The mantle core architecture, i.e., α-cells at the periphery and β-cells at the center position within the islet, enhances insulin secretion via the paracrine effect (Kilimnik *et al.,*2012; Mishra *et al.,*2024). Dysfunction/destruction of pancreatic β-cells leads to an imbalance in glucose homeostasis and the development of diabetes (Ighodaro *et al.,*2017). It is a major noncommunicable metabolic disorder that disturbs postprandial blood glucose and HbA1c levels and is a crucial parameter to be considered in diabetes management. Despite the introduction of new antidiabetic medications, prolonged use leads to several adverse side effects, including neuropathy, retinopathy, cardiovascular complications, and gastrointestinal complications (nausea, diarrhea, and abdominal pain). These drugs address only the primary clinical symptoms rather than the pathophysiological mechanism of DM (Kimani *et al.,*2023; Susilawati *et al.,*2023).

Recent research on DM revealed a chance of endogenous pancreatic β-cell regeneration to mitigate diabetes symptoms (Zhong & Jiang, 2019). In addition, several other approaches, including the differentiation of induced pluripotent stem cells (iPSCs) into new pancreatic β-cells, islet transplantation and the modification of other pancreatic cell subtypes into β-cells, are available, but most of these treatment strategies are unsuccessful in animal models and human trials. The data collected from ethnological sources, such as folklore (as little scientific evidence is present), and traditional healers of Gandhamardan Hill (Bargarh district) have given hope for mitigating diabetic complications in this unventured area. An alternative antidiabetic medication with high efficacy and minimal or no side effects is needed to overcome the current challenges. Therefore, in the present study, we developed a polyherbal formulation comprising 16 selected medicinal plants, such as *Tinospora cordifolia* (Willd.) (stem), *Mangifera indica* L. (seed), *Syzygium cumini* (L.) (bark), *Terminalia arjuna (Roxb. ex DC.)* (bark), *Curcuma longa* L. (rhizome), *Alianthus excelsa* Roxb. (leaf), *Caesalpinia bonduc* L. (Roxb.) (seed),

Swertia chirayita (Roxb.) (stem), *Holarrhena pubescens* Wall. ex G.Don (root), *Azadirachta indica* A.Juss. (leaf), *Murraya koenigii* (L.) Spreng. (leaf), *Withania coagulans* (flower), *Salacia oblonga Wall. ex Wight & Arn.* (root), *Cedrus deodara (Roxb. ex D. Don)* (bark), *Picrorhiza kurroa* Royle ex Benth. (root), and *Pterocarpus marsupium* Roxb. (bark) of Odisha. On the basis of the literature review and documented traditional uses, these edible plant parts (leaves, flowers, bark, seeds, stems, roots, and rhizomes) were selected for inclusion in the development of a formulation to achieve improved therapeutic effects via an alternative mechanism to combat diabetes without any adverse effects (Bahadur, 2016; Das *et al.,*2023; Rafe, 2017; Thirumalai *et al.,*2012). This study highlights a folk medicine (a polyherbal formulation) with *in vitro* antioxidant and antidiabetic activities and β-cell regeneration ability in *in silico*, *in vitro,* and *in vivo* antidiabetic models.

3.2. Materials & Methods

3.2.1. Chemicals and reagents

The chemicals used in the present study, including α-amylase, α-glucosidase, alloxan, metformin, acarbose, formaldehyde, TPTZ, ABTS, acetate buffer, xylene, propanol, acetone, DMEM, penicillin–streptomycin, and trypsin-EDTA solutions, were purchased from Sigma Aldrich, USA. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, sodium chloride, ferric chloride, potassium persulphate, potassium phosphate monobasic, and potassium phosphate dibasic were purchased from Himedia, India. LC–MS-grade solvents such as acetonitrile and water were obtained from Avantor, J.T. Baker, Germany. LC–MS grade reagents such as formic acid, culture media (DMEM), DMSO, MTT, and 2-NBDG were procured from Thermo Fisher Scientific, USA, and ammonium formate was obtained from VWR, Germany. Deionized water was purified with a Milli-Q system (Millipore, USA).

3.2.2. Collections and preparation of the polyherbal formulation

The different parts of the selected plants were collected from the Gandhamardan Hill range of Bargarh District, Odisha. The collected plant parts were washed adequately with distilled water and shade-dried. The dried materials were powdered with a versatile pulverizer (B.D. instruments) and passed through a sieve of 10 mesh for uniformity. The polyherbal formulation was prepared by mixing an equal proportion of each plant material, followed by aqueous extraction via the Soxhlet extraction system and lyophilization (Svl,

SVFD 501 M). The lyophilized aqueous polyherbal extract (APE) was stored for the antidiabetic study.

3.2.3. Ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS) analysis of APE

UHPLC–MS analysis of APE was performed on a Xevo G3 QToF Waters Corporation (M.A., USA) equipped with Acquity UPLC I Class Plus and MassLynx software (Waters Corporation, USA). Progenesis Q.I. software (Waters Corporation, USA) was used to analyse the separated compounds. Different compounds were separated via an Acquity UPLC HSS T3 column (100×2.1 mm×1.8 µm) (Waters Corporation, USA). The column and sample temperature were maintained at 40°C and 15°C, respectively. The instrumental parameters were set as ionization type of ESI (mode-MSE), an acquisition time of 25 min with a collision energy of 6 eV (low) and high collision energies of 10–40 eV (ramp) and 10–30 eV for the +ve and –ve modes, respectively. The capillary voltages were set to 3.0 kV and 2.5 kV for the +ve and –ve modes, respectively, whereas the cone voltages were set to 40 V and 30 V for the +ve and –ve modes, respectively. The source and desolvation temperatures were set at 130°C and 500°C, respectively. The cone and desolvation gas flows were maintained at rates of 50 L/h and 750 L/h, respectively. A solution of leucine enkephalin (200 pg/mL, Waters, USA) was infused at a flow rate of 10 µl/min to generate reference ions of m/z 556.2771 (M+H, +ve mode) and m/z 554.2615 (M-H, –ve mode). The solvent elution was performed at a flow rate of 0.4 ml/min via gradient mobile phase (solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in acetonitrile) for +ve analysis, whereas in –ve mode of analysis, the gradient mobile phase was 1 mM ammonium formate in water (solvent A) and acetonitrile (solvent B). In both modes of analysis, the volume ratios of solvent B were as follows: 5% (0–1 min), 5–25% (1–5 min), 25–35% (5–8 min), 35–45% (8–11 min), 45–55% (11–14 min), 55–90% (14–20 min), 90–95% (20–20.1 min), and 100% (20.1–25 min). The test solution (5 µl) was injected, and the chromatographs were recorded for 25 min.

3.2.4. Biochemical estimation of APE

3.2.4.1. Total flavonoid content (TFC)

To estimate the total flavonoid content (TFC) of APE, a reaction mixture of 200 µl of $AlCl_3$ (2%) and 200 µl of APE (1 mg/ml) was mixed at a 1:1 ratio, followed by incubation at 37°C for 1 hr. To the reaction mixture, 400 µl of distilled water was added, and the absorbance was measured at 420 nm via a spectrophotometer (Shimadzu UV-

3600i). Quercetin (1 mg/ml) was used as a control, and a calibration curve was obtained. The TFC was expressed as the dry weight of quercetin (mg/g) (Salih *et al.,*2021).

3.2.4.2. Total phenol content (TPC)

The Folin–Ciocalteu reagent method was used to quantify the total phenolic content (TPC) of APE, as reported previously with slight modifications (Salih *et al.,*2021). APE (0.2 mg/ml) and Folin-Ciocalteu reagent (50 µl) were mixed at a 1:1 ratio and incubated for 10 mins. Then, 50 µl of sodium carbonate solution (20%) was added to neutralize the reaction mixture, followed by incubation for 30 min at room temperature. The absorbance was measured at 765 nm via a UV–Vis spectrophotometer (Shimadzu UV–3600i). Gallic acid (0.1 mg/ml) was used as a standard control. The TPC was expressed as dry weight gallic acid (GAE).

3.2.5. In vitro antioxidant analysis of APE

3.2.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity (free radical scavenging assay) of APE was determined as described previously (Baliyan *et al.,*2022). DPPH solution (1 mg/ml) and APE at various concentrations (20, 40, 60, 80, 100 µg/ml) were prepared. In a test tube, 3.0 ml of DPPH and 100 µl of APE were mixed properly, and the reaction mixture was incubated at room temperature for 30 min in the dark. The absorbance was then measured at 517 nm via a spectrophotometer (Shimadzu UV-3600i). Ascorbic acid (1 mg/ml) was used as a control. The experiments were carried out in duplicate, and the IC_{50} values were obtained. The following formula was used to calculate % inhibition:

$$\% \text{ Inhibition} = [(A_{Con} - A_S)/A_{Con}] \times 100$$

where A_{Con} = absorbance of the control and A_S = absorbance of the sample.

3.3.5.2. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

The ABTS assay of APE was carried out as described previously with certain modifications (Adebiyi *et al.,* 2017). Briefly, the ABTS solution was obtained by adding ABTS (7.4 mM) and potassium persulphate (2.6 Mm) at a 1:1 ratio and incubating it for 12–16 hr in the dark before use. Various concentrations of APE were prepared (20, 40, 60, 80, and 100 µg/ml). In a test tube, 1.90 ml of ABTS solution was mixed with 0.10 ml of APE, and the reaction mixture was incubated in the dark for 2 hr. The absorbance was then measured at 734 nm via a UV–Vis spectrophotometer (Shimadzu UV–3600i). Ascorbic acid (1 mg/ml) was used as a control. The experiments were performed in duplicate, and

the results are expressed as the $IC_{50\ values}$. The following formula was used to calculate % inhibition:

$$\% \text{ Inhibition} = [(A_{Con} - A_S)/A_{Con}] \times 100$$

where A_{Con} = absorbance of the control and A_S = absorbance of the sample.

3.2.5.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay of APE was carried out as described previously with slight modifications (Naik *et al.,*2023). Briefly, various concentrations of APE (20, 40, 60, 80, 100 µg/ml) and FRAP solution (0.1 M acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride (10:1:1, v/v/v)) were prepared. To 1.90 ml of FRAP solution, 0.10 ml of APE was added, the reaction mixture was placed in a water bath (37°C) for 30 min, and the absorbance was measured at 593 nm via a UV–Vis spectrophotometer (Shimadzu UV-3600i). Ascorbic acid (1 mg/ml) was used as a standard control. The experiments were run in duplicate, and the results are expressed as the $IC_{50\ values}$. The following formula was used to calculate % inhibition:

$$\% \text{ Inhibition} = [(A_{Con} - A_S)/A_{Con}] \times 100$$

where A_{Con} = absorbance of the control and A_S = absorbance of the sample.

3.2.6. In silico study of APE

3.2.6.1. Protein preparation

A higher resolution protein (PDB ID: 1K3A and 7WSM) was obtained from the protein data bank (PDB). The protein was subsequently prepared via the Protein Preparation Wizard and Prime tools (Schrödinger, Inc., NY), followed by energy minimization via Macro model (Schrödinger, Inc., NY) (Pragyandipta *et al.,*2023).

3.2.6.2. Ligand preparation

The molecular structures of verbascoside B, apigenin 7-apiosyl-glucoside, curcucomosin A, articolic acid, withanolide F, terminolic acid, andrographic acid, ricinoleic acid, mangiferic acid, and gymnemic acid I were obtained via chem draw. The prepared ligands were then imported into the Maestro environment, followed by energy minimization via a macromodel (Schrödinger, Inc., NY) with the OPLS 2005 force field and PRCG algorithm (energy gradient of 0.001). Geometric optimization was performed via Jaguar (Schrödinger, Inc., NY), and various conformations of the structures were generated via LigPrep (Schrödinger, Inc., NY).

3.2.6.3. Molecular docking

The prepared ligands were docked with the insulin-like growth factor 1 receptor kinase protein to understand the structural basis of the specificity of this target protein via the glide algorithm (Schrödinger, Inc., NY). An inner grid box (20 Å × 20 Å × 20 Å) was created via the Glide grid-receptor generation algorithm (Schrödinger, Inc., NY). An outer grid box (20 Å × 20 Å × 20 Å) was created to ensure that each ligand atom of a valid pose was located within it. Subsequently, Glide XP (extra precision) was utilized for docking, and all the docked poses were analysed on the basis of the Glide XP score (Pragyandipta *et al.,* 2023).

3.2.7. In vitro antidiabetic activity of APE

3.2.7.1. α-Amylase activity

The α-amylase inhibitory activity of APE was determined as described previously (Rath *et al.,*2020). APE (2.5 mg/ml stock concentration and 20, 40, 60, 80, or 100 µg/ml working concentration) was mixed with 1 ml of α-amylase (0.02 mg/ml) and incubated at 25°C for 3 min, followed by the addition of 1 ml of color reagent (51.961 mol/L) and placement in a water bath (B.D. instruments, LS WB-10P) at 85°C for 15 min. After the incubation period, the reaction mixture was cooled and diluted with 9 ml of distilled water, and the absorbance was measured at 540 nm via a spectrophotometer (Shimadzu UV-3600i). Acarbose solution (500 µg/ml stock solution, 20, 40, 60, 80, or 100 µg/ml working concentration) was used as the standard control. The % inhibition of α-amylase was calculated via the following formula:

$$\% \text{ inhibition} = 100 \times [(A_{Con} - A_{Sm})/A_{Con}]$$

where A_{Con} = absorbance of the control and A_S = absorbance of the sample.

3.2.7.2. α-Glucosidase activity

APE (100 µl, 1 mg/ml) was added to 100 µl of phosphate buffer (0.1 mol/l, pH 6.9) and 100 µl of α-glucosidase solution (2.5 mg/ml stock concentration, 20, 40, 60, 80, 100 µg/ml working concentration), followed by incubation at 25°C for 5 min. After incubation, 100 µl of p-nitrophenyl-α-D-glucopyranoside solution (5 mmol/l) was added, and the mixture was further incubated at 25°C for 10 min. Subsequently, the absorbance was recorded at 405 nm via a spectrophotometer (Shimadzu UV-3600i). Acarbose solution (500 µg/ml stock concentration; 20, 40, 60, 80, and 100 µg/ml working concentrations) was used as a standard control. The % inhibition of α-glucosidase was calculated via the following formula, and the results are expressed as the IC_{50}. (Rath *et al.,*2020)

$$\% \text{ inhibition} = 100 \times [(A_{Con} - A_{Sm})/A_{Control}]$$

where A_{Con} = absorbance of the control and A_S = absorbance of the sample.

3.2.7.3. Cell culture

The 3T3L1 and MIN6 cell lines were purchased from NCCS Pune and were cultured in DMEM (Thermo Fisher) supplemented with 10% FBS (Gibco) and 1% penicillin–streptomycin antibiotics (Sigma) at 37°C and 5% CO_2 in a humidifier incubator (Eppendorf).

3.2.7.4. Cytotoxicity assay

A 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was carried out to assess cell cytotoxicity in the 3T3L1 cell line as previously published, with slight modifications (Soeng *et al.,*2015). Briefly, the cells were seeded in 96-well culture plates (5*10^3 per well) and treated with varying concentrations of APE (20, 40, 60, 80, 100, 400, 800 & 1000 µg/ml) at 37°C and 5% CO_2 in a humidifier incubator for 48 hr. After the incubation period, the media was replaced with 20 µl of MTT and incubated for another 4 hr at 37°C in the dark. The absorbance was measured at 490 nm using a microplate reader. A cell cytotoxicity assay was performed to determine the safe concentration for further study.

3.2.7.5. Glucose uptake assay

The glucose uptake assay in the MIN6 cell line was conducted via the fluorescent glucose analogue 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose (2-NBDG) as previously described (Alsawalha *et al.,*2019; Dubey *et al.,*2018), with slight modifications. Briefly, the cells were seeded at a density of $^{5\times103}$ per well in a 96-well culture plate with complete media. The complete media was replaced with glucose-free media for 3 hrs, followed by treatment with the standard drug (100 µg/ml) and APE at various concentrations (100, 200, 500, 700 & 1000 µg/ml) for 48 hrs. After the incubation period was complete, the treated media was replaced with glucose-free media supplemented with 100 µM 2-NBDG for 2 hrs, followed by washing the cells in chilled PBS. The absorbance was measured at 535 nm via a microplate reader (Bio-Rad).

3.2.8. In vivo antidiabetic study

3.2.8.1. Animal housing and maintenance

Wistar rats of both sexes (195 ± 15 g) were kept under standard laboratory conditions at room temperature (25 ± 2°C) with a 12 h L:D cycle for two weeks prior to the start of

the experiment. The animals were fed *ad libitum* with a standard pellet diet and allowed free access to water. After the institutional animal ethical clearance (IEAC) of the Department of Biotechnology & Bioinformatics, Sambalpur University, Burla, Odisha vide no. SU/BTBI/IAEC/2023/02 was obtained, the experiment was conducted.

3.2.8.2. Acute toxicity

Oral acute toxicity studies were performed as per the Organization for Economic Cooperation and Development guidelines (Oecd, 2008). Before dosing, the animals were segregated into five groups (n=6) and fasted overnight. APE was given orally at single doses of 1000, 2000, 3000, 4000, and 5000 mg/kg b.w. in groups I-V, respectively. After dosing, the animals were observed for an initial 30 min, followed by 4 h, 24 h, and finally 72 hr. Several parameters include alterations in the skin, fur, eyes, mucous membranes, and body weight, as well as changes in respiratory, circulatory, and behavioural patterns. Additionally, the mortality rate was monitored to assess the toxicity of APE (Kanhar & Sahoo, 2019).

3.2.8.3. Subacute toxicity

The oral subacute toxicity study was conducted as per OECD guidelines. Briefly, the animals were fasted overnight prior to the dosing of APE. At a dose of 500 mg/kg b.w., the animals were given APE orally for four weeks, followed by observation of any clinical symptoms of mortality or changes in the behavioural and physiochemical parameters. Upon completion of the experiment, vital organs such as the brain, liver, kidney, pancreas, lungs, and heart were dissected for histopathological observation.

3.2.8.4. Induction of diabetes mellitus

The chemical induction of diabetes in the animals was carried out following a previously described method, with certain modifications (Hamadjida *et al.,*2024). The animals were fasted overnight, and the blood glucose level was determined via a glucometer (Accu Check, India) before the chemical induction of D.M. Freshly prepared alloxan (140 mg/kg b.w.) was intraperitoneally (i.p.) induced, and the blood glucose level was checked every other day. Patients with blood glucose levels ≥ 250 mg/dl were included in the current study (Mollica *et al.,*2017).

3.2.8.5. Antidiabetic study of APE

Diabetic rats were randomly divided into four groups (n=6). All the treatment groups were administered their respective drugs for 28 days.

Group-I (normal control): fed 0.9% normal saline

Group II (diabetic control, alloxan-induced): fed 0.9% normal saline

Group III (positive control): fed metformin (100 mg/kg b.w., p.o.)

Group IV (treatment control): fed APE (500 mg/kg b.w., p.o.)

Every other week, body weight, blood sugar, and glycated haemoglobin (HbA1C) levels were measured on the 7^{th}, 14^{th}, 21^{st} and 28^{th} days. At the end of the experiment (on the 29^{th} day), the animals were anaesthetized by mild anaesthesia (isoflurane) followed by cervical dislocation, and blood was collected via cardiac puncture. The serum was separated from the blood (at 5000 rpm for 10 min) to study biochemical parameters, including urea, creatinine (CREA), alanine transaminase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), albumin (ALB), alkaline phosphatase (ALP), cholesterol (CHOL), triglycerides (T.G.), total protein (T.P.), high-density lipoprotein (HDL), and insulin, via an automated biochemical analyser (Biovet, Smart-5DX) with their respective standard kits. The pancreas tissue was collected in 10% neutral buffered formalin (NBF) for histopathological and immunohistochemistry studies.

3.2.8.6. Statistical analysis

The data are presented as the mean ± SEM (n=6). Statistical analysis was carried out at $p<0.05$ between the experimental groups via one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test via GraphPad (Prism 9, Graph PAD, USA).

3.2.8.7. Histopathological and immunohistochemistry studies

For the histopathological study, the collected pancreas was fixed with 10% NBF. Then, the samples were subjected to dehydration and embedded in paraffin (Medimas, India). A 3.5 µm thick paraffin section was cut and stained with hematoxylin & eosin (El-Desouki *et al.,*2015) to look over the autolytic changes in the tissue. The tissue with prominent microarchitecture was further stained for immunohistochemistry.

Immunohistochemistry (IHC) of 3.5 µM thick paraffin sections was performed with primary antibodies such as anti-synaptophysin, anti-insulin (Pathnitu, Livermore, California), and anti-glucagon (Bioss Inc., USA). The primary antibody was detected by a secondary antibody labelled with polyexcel horseradish peroxidase (HRP) and 3,3'-diaminobenzidine chromogens (DAB) (Pathnsitu, Livermore, California). The stained slides were examined under a bright field inverted microscope to observe the

morphological features and the status of insulin-secreting β-cells in the pancreatic islets (Mishra *et al.,*2024).

3.3. Results

3.3.1. UHPLC-Q-TOF-MS analysis of APE

The marker components of APE were identified and validated by utilizing the respective mass ions, online and offline spectral databases, fragmentation patterns, and relevant literature. With the use of a full-spectrum scan, data acquisition was carried out in both positive (+ve) and negative (-ve) ionization modes. The chromatogram revealed a total of 60 compounds (37 from the +ve spectrum and 23 from the -ve spectrum) represented by their peak number, R.T., molecular formula, compound name, m/z ratio, fragment number, and molecular mass (Table 3.1). The compounds identified from positive mode ionization were (1) gallic acid (RT-1.55, m/z-171.0281), (2) mangiferin (RT-4.18, m/z-423.0936), (3) naringenin (RT-4.28, m/z-193.0488), (4) litseglutine B (RT-4.43, m/z-342.1709), (5) apigenin 7-apiosyl-glucoside (RT-4.68, m/z-565.1561), (6) resokaempferol 7-glucoside (RT- 5.26, m/z-433.1133), (7) ellagic acid (RT-5.28, m/z-303.0139), (8) ajmaline (RT-5.50, m/z-327.2073), (9) yohimbine (RT-6.02, m/z-355.2022), (10) apigenin 7-glucuronide (RT-6.04, m/z-447.0930), (11) 7-hydroxyeucommic acid (RT-6.14, m/z-241.0683), (12) andrographolactone (RT-7.58, m/z-297.1852), (13) 23-hydroxybetulinic acid (RT-7.66, m/z- 473.3624), (14) 7α-hydroxycampesterol (RT-7.74, m/z-439.3560), (15) turmerone (RT-7.76, m/z-217.1586), (16) bonducellpin D, (RT-7.94, m/z-427.1729), (17) α-curcumene (RT-8.01, m/z-197.1305), (18) caesalpinolide D (RT-8.01, m/z-335.2208), (19) liquiritigenin (RT-8.08, m/z-257.0815), (20) 14-deoxy-11,12-didehydroandrographiside (RT-8.09, m/z-517.2413), (21) norcaesalpinin E (RT-8.32, m/z-399.1788), (22) isoliquiritigenin 4,4'-diglucoside (RT- 8.54, m/z-581.1894), (23) arjunolic acid (RT-9.09, m/z-489.3580), (24) verbascoside B (RT- 9.75, m/z-549.1797), (25) 2,3-dihydrowithanolide E (RT-9.92, m/z-511.2674), (26) arjungenin (RT-10.45, m/z-527.3350), (27) curcucomosin A (RT-10.73, m/z-315.1960), (28) 3-O-coumaroylarjunolic

acid (RT-10.82, m/z-673.3527), (29) β-hydrojuglone (RT-12.07, m/z-177.0539), (30) 9,10,18-trihydroxyoctadecanoic acid (RT-12.25, m/z-355.2458), (31) curcumenone (RT-12.98, m/z-257.1512), (32) gymnemic acid I (RT-13.05, m/z-807.4521), (33) stigmasterol glucoside (RT-13.66, m/z-597.4148), (34) gymnemic acid ii (RT-13.66, m/z-809.4681), (35) 9,10-epoxyoctadecanoic acid (RT-14.32, m/z-337.2352), (36) mangiferic acid (RT-17.95, m/z-281.2476), and (37) ricinoleic acid (RT-17.95, m/z-299.2583) (Figure 3.1. A, Table 1). Similarly, the compounds identified from -ve mode of ionization were (1) ellagic acid 2-rhamnoside (RT-2.49, m/z-447.0569), (2) 5-feruloylquinic acid (RT-3.30, m/z-367.1032), (3) andrographic acid (RT-3.53, m/z-363.1808), (4) isovitexin 6"-O-glucoside (RT-3.55, m/z-593.1513), (5) lucidin 3-O-β-primeveroside (RT-3.82, m/z-563.1400), (6) apigenin 7-glucoside-(2",3")-diacetate (RT-4.20, m/z-515.1189), (7) luteolin-5-O-glucoside (RT-4.32, m/z-447.0929), (8) apigenin 4'-glucuronide (RT-4.43, m/z-445.0777), (9) 14-Acetylandrographolide (RT-4.48, m/z-391.2127), (10) δ-caesalpin (RT-4.78, m/z-411.2015), (11) deacylgymnemic acid (RT-4.78, m/z-681.3857), (12) norcaesalpinin F (RT-5.24, m/z-435.1661), (13) terminolic acid (RT-7.61, m/z-503.3380), (14) gymnemic acid iv (R.T.- 7.69, m/z-763.4277), (15) gymnemic acid xiii (RT-8.06, m/z-765.4427), (16) neoandrographolide (R.T.- 9.65, m/z-525.2707), (17) withanolide A (RT-11.02, m/z-469.2603), (18) withanolide F (RT-11.57, m/z-505.2342), (19) withanone (RT-11.64, m/z-469.2603), (20) 6-keto stearic acid (RT-11.98, m/z- 297.2433), (21) azadirachtin I (RT-14.34, m/z-617.2582), (22) myristic acid (RT-14.98, m/z- 227.2011), and (23) neocaesalpin C (RT-15.55, m/z-465.2151) (Figure 3.1.B, Table 1).

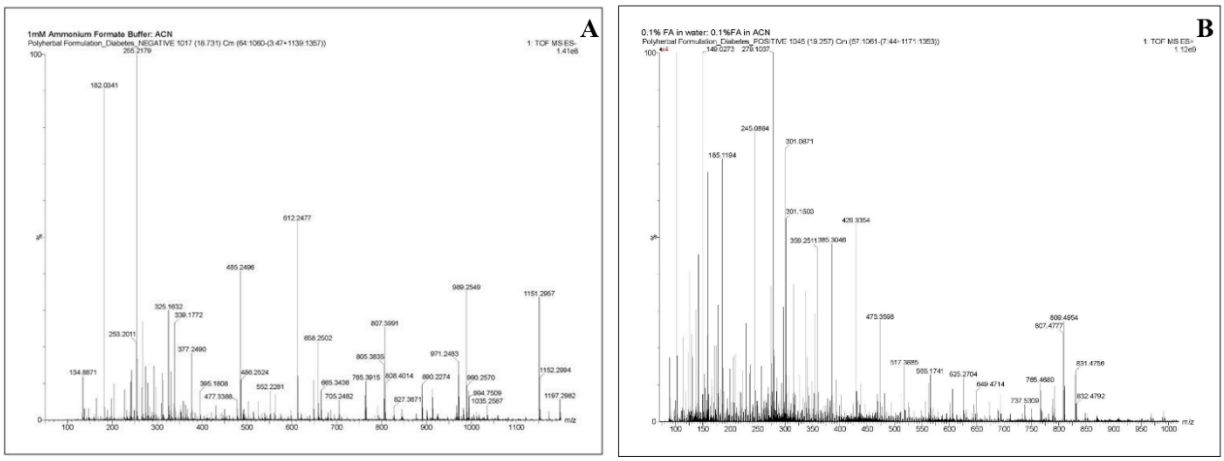


Figure 3.1. A. UHPLC-MS chromatogram of APE in positive mode, **B.** UHPLC-MS chromatogram of APE in negative mode.

Table 3.1. Ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS) analysis of APE indicated the presence of different marker compounds.

(A) Positive ionization mode analysis of APE									
Sl. No.	RT	Compound	Mol. formula	Mol. wt.	m/z	Fragments	Nature of compound	Biological activity	References
1	1.55	Gallic acid	$C_7H_6O_5$	170.02	171.0281	153.0183, 37.0233, 135.0077, 27.0390, 125.0234, 09.0284, 107.0128	Phenolic	Antidiabetic	Xu *et al.,* 2021
2	4.18	Mangiferin	$C_{19}H_{18}O_{11}$	422.09	423.0936	405.0817, 95.0973, 387.0711, 57.0605, 339.0500, 27.0500, 303.0475, 73.0394, 265.0343, 53.0183, 134.0574, 27.0365, 122.0574	Flavonoid	Antidiabetic Anticancer	Ganogpichayagrai *et al.,*2017
3	4.28	Noreugenin	$C_{10}H_8O_4$	192.0415	193.0488	177.0546, 63.0390, 153.0183, 49.0234, 147.0441, 45.0285, 125.0233	Chromone	Antidiabetic	Hartogh & Tsiani, 2019
4	4.43	Litseglutine B	$C_{20}H_{23}NO_4$	341.1636	342.1709	297.1122, 265.0860	Alkaloid	Antidiabetic	Jamaddar *et al.,*2023

5	4.68	Apigenin 7-apiosyl-glucoside	$C_{26}H_{28}O_{14}$	564.1488	565.1561	547.1447, 29.1341, 481.1130, 27.1024, 409.0918, 49.0707, 325.0707, 95.0601, 145.0284	Flavonoid	Antidiabetic	Assefa *et al.,*2021
6	5.26	Resokaempferol 7-glucoside	$C_{21}H_{20}O_{10}$	432.1060	433.1133	415.1024, 97.0918, 367.0813, 51.0863, 349.0707, 37.0707, 313.0707, 95.0601, 283.0601, 71.0601, 163.0390, 117.0335	Flavonoid		
7	5.28	Ellagic acid	$C_{14}H_6O_8$	302.0066	303.0139	275.0187, 257.0081	Polyphenol	Antioxidant Antidiabetic	Fatima *et al.,*2017
8	5.50	Ajmaline	$C_{20}H_{26}N_2O_2$	326.2000	327.2073	309.1962, 91.1856, 262.1465, 48.1308, 237.1386	Alkaloid	Antidiabetic	Guasch *et al.,*2012
9	6.02	Yohimbine	$C_{21}H_{26}N_2O_3$	354.1949	355.2022	337.1911, 09.1598, 224.1308, 12.1281, 194.1176, 70.0938, 158.0938, 44.0808, 134.0965, 130.0651	Alkaloid	Antidiabetic	Abdel-Zaher *et al.,*2001
10	6.04	Apigenin 7-glucuronide	$C_{21}H_{18}O_{11}$	446.0857	447.0930	293.0445, 71.0601, 163.0390	Flavonoid	Antidiabetic	Assefa *et al.,*2021
11	6.14	7-hydroxyeucommic acid	$C_9H_{14}O_6$	218.0791	241.0683	201.0758, 81.0471, 163.0365, 59.0652, 137.0598, 31.0467, 123.0441, 13.0234, 111.0441	Carboxylic acid		
12	7.58	Andrographolactone	$C_{20}H_{24}O_2$	296.1779	297.1852	285.1849, 57.1536, 241.1224, 29.1223,	Diterpenoid	Antidiabetic	Wang *et al.,*2009

						227.1067, 15.1067, 187.1482, 81.0699			
13	7.66	23-hydroxybetulinic acid	$C_{30}H_{48}O_4$	472.3551	473.3624	437.3415, 25.3414, 407.3309, 19.1744, 207.1744, 03.1795, 189.1638, 177.1638	Triterpenoid		
14	7.74	7α-hydroxycampesterol	$C_{28}H_{48}O_2$	416.3668	439.3560	421.3441, 89.1614, 161.0937, 119.0831	Steroid		
15	7.76	ar-Turmerone	$C_{15}H_{20}O$	216.1513	217.1586	199.1482, 75.1118, 161.0961, 35.1169, 121.1012, 19.0856, 93.0699, 91.0543, 83.0492	Sesquiterpenoid	Antidiabetic	Lekshmi *et al.,*2012
16	7.94	Bonducellpin D	$C_{22}H_{28}O_7$	404.1837	427.1729	301.0707, 77.0547, 161.0937, 35.0805, 124.0883, 05.0675, 93.0675	Diterpenoid		
17	8.01	(-)-α-curcumene	$C_{13}H_{18}$	174.1413	197.1305	183.1144, 75.1482, 173.1325, 57.0988, 147.1169, 42.0753, 133.1012, 19.0856, 115.0518, 07.0831, 105.0675, 91.0543, 79.0518	Sesquiterpene		
18	8.01	Caesalpinolide D	$C_{20}H_{30}O_4$	334.2135	335.2208	303.1955, 91.1591, 285.1849, 57.1900, 32.1094, 127.0390	Diterpene		
19	8.08	Liquiritigenin	$C_{15}H_{12}O_4$	256.0742	257.0815	149.0234, 137.0597	Flavonoid	Antidiabetic	Bae *et al.,*2018
20	8.09	14-deoxy-11,12-didehydroa	$C_{26}H_{38}O_9$	494.2521	517.2413	315.1955, 97.1850, 285.1849, 57.1537,	Diterpenoid	Antihyperglycemic	Kamaraj *et al.,*2023

		ndrographiside				221.0784, 95.0628, 177.0522			
21	8.32	Norcaesalpinin E	$C_{21}H_{28}O_6$	376.1896	399.1788	361.1646, 41.1150, 335.1853, 26.0551, 301.1410, 93.1748, 287.1254, 90.1173, 178.0625, 65.0911, 147.0441, 134.0363	Diterpenoid	Antimalarial	
22	8.54	Isoliquiritigenin 4,4'-diglucoside	$C_{27}H_{32}O_{14}$	580.1821	581.1894	177.0547, 49.0234, 133.0648, 19.0492, 103.0543	Flavonoid		
23	9.09	Arjunolic acid	$C_{30}H_{48}O_5$	488.3507	489.3580	453.3364, 07.3309, 217.1587, 65.1587, 187.1482, 74.1251, 133.1012, 19.0856, 105.0699	Triterpenoid	Antidiabetic	Aamir *et al.,*2022
24	9.75	Verbascoside B	$C_{21}H_{34}O_{15}$	526.1905	549.1797	534.1919, 149.0209	Polyphenol	Antidiabetic	(Galli *et al.,*2020)
25	9.92	2,3-dihydrowithanolide E	$C_{28}H_{40}O_7$	488.2782	511.2674	493.2580, 71.2742, 375.2166, 51.2166, 325.1201, 85.1850, 273.1486, 69.0860, 149.0961, 123.0441	Steroid		
26	10.45	Arjungenin	$C_{30}H_{48}O_6$	504.3458	527.3350	487.3418, 69.3313, 433.3077, 23.3258, 322.1903, 49.1486, 232.1458, 01.1638, 187.1482, 175.1482	Triterpenoid	Antidiabetic	Mohammed *et al.,*2015
27	10.73	Curcucomosin A	$C_{20}H_{26}O_3$	314.1887	315.1960	297.1850, 85.1849, 257.1537, 41.1224, 227.1067, 15.1067,	Diterpenoid		

						173.1325, 59.1169, 133.1012, 19.0856, 105.0699			
28	10.82	3-O-coumaroyl arjunolic acid	$C_{39}H_{54}O_7$	634.3896	673.3527	593.3473, 71.3469, 453.3364, 95.2949, 247.1693, 233.1537	Triterpenoid		
29	12.07	β-hydrojuglone	$C_{10}H_8O_3$	176.0466	177.0539	149.0233, 27.0284, 93.0335	Phenolic		
30	12.25	9,10,18-trihydroxy octadecanoic acid	$C_{18}H_{36}O_5$	332.2566	355.2458	297.2425, 79.2294, 163.1093, 35.1144, 127.1118, 21.0988, 113.0967, 05.0311, 91.0518, 79.0518, 65.0362	Hydrocarbon		
31	12.98	Curcumenone	$C_{15}H_{22}O_2$	234.1620	257.1512	235.1693, 79.1067, 165.0911, 151.01118, 125.0961, 94.0567	Sesquiterpenoid	Antidiabetic	Zhang & Kitts, 2021
32	13.03	Gymnemic acid I	$C_{43}H_{66}O_{14}$	806.4448	807.4521	789.4420, 771.4315, 729.4209, 689.3896, 671.3790, 653.4024, 513.3575, 495.3464, 453.3364, 269.1900, 215.1431, 189.1274, 141.0158	Triterpenoid	Antidiabetic	Abbas *et al.,*2019
33	13.66	Stigmasterol glucoside	$C_{35}H_{58}O_6$	574.4256	597.4148	537.3914, 495.3469, 175.1457, 133.0988, 121.0988, 107.0831	Steroid	Antidiabetic	Bakrim *et al.,*2022
34	13.66	Gymnemic acid ii	$C_{43}H_{68}O_{14}$	808.4608	809.4681	791.4577, 773.4471, 731.4365, 713.4260, 689.3896, 671.3790,	Triterpenoid	Antidiabetic	Abbas *et al.,*2019

						655.4180, 615.4256, 597.4150, 477.3390, 453.3364, 217.1587, 189.1274, 135.0805			
35	14.32	9,10-epoxyoctadecanoic acid	$C_{18}H_{34}O_4$	314.2460	337.2352	297.2425, 279.2319, 261.2189, 181.1199, 163.1093, 147.1144, 135.0780, 119.0467, 105.0675, 91.0518	Hydrocarbon		
36	17.95	Mangiferic acid	$C_{18}H_{32}O_2$	280.2403	281.2476	263.2370, 245.2264, 219.1744, 207.1744, 193.1587, 179.1431, 165.1274, 137.1325, 123.1169, 111.1169, 97.1012, 83.0856, 69.0699	Fatty acid	Antidiabetic	Mohammed, A. H. *et al.,*2016
37	17.95	Ricinoleic acid	$C_{18}H_{34}O_3$	298.2510	299.2583	281.2476, 263.2370, 245.2240, 219.1719, 203.1770, 193.1563, 189.1614, 175.1457, 161.1301, 147.1144, 135.1144, 121.0988, 111.0805, 109.0988, 95.0831, 81.0675, 67.0518	Fatty acid	Antidiabetic	Yoshida *et al.,*2020
(B) Negative ionization mode analysis of APE									
1	2.49	Ellagic acid 2-rhamnoside	$C_{20}H_{16}O_{12}$	448.0642	447.0569	433.0412, 300.9989, 283.9962, 145.0506	Polyphenol	Antidiabetic	(Jayakumar *et al.,* 2013a)
2	3.30	5-Feruloylquinic acid	$C_{17}H_{20}O_9$	368.1105	367.1032	303.0874, 292.0952, 193.0506,	Ester	Antidiabetic	(Meng *et al.,* 2013)

						191.0561, 173.0455, 162.0533, 155.0350, 149.0455, 147.0299, 134.0373, 117.0346, 113.0244, 103.0400, 71.0138			
3	3.53	Andrographic acid	$C_{20}H_{28}O_6$	364.1881	363.1808	363.1813, 333.1707, 319.1914, 275.1285, 217.1598, 83.0502	Carboxylic acid	Antidiabetic	(Jayakumar *et al.,*2013a)
4	3.55	Isovitexin 6"-O-glucoside	$C_{27}H_{30}O_{15}$	594.1586	593.1513	564.1484, 503.1195, 473.1089, 461.1300, 455.0983, 431.1195, 425.0878, 413.0878, 395.0772, 383.0772, 365.0667, 353.0666, 189.0768, 135.0298, 116.0478	Flavonoid	Antidiabetic	(Abdulai *et al.,*2021)
5	3.82	Lucidin 3-O-β-primeveroside	$C_{26}H_{28}O_{14}$	564.1473	563.1400	545.1300, 503.1195, 473.1089, 445.0983, 425.0878, 415.1034, 395.0772, 383.0772, 365.0667, 353.066, 323.0561, 311.0561, 295.0611, 221.0667	Anthraquinone		
6	4.20	Apigenin 7-glucoside-(2",3")-diacetate	$C_{25}H_{24}O_{12}$	516.1262	515.1189	503.1195, 473.1089, 465.0827, 455.0983, 443.0983, 413.0878, 383.0772, 371.0983, 353.0667, 325.0717, 263.0772,	Flavonoid		

						243.0510, 197.6455, 173.0455, 161.0244, 143.0349, 135.0451			
7	4.32	Luteolin-5-O-glucoside	$C_{21}H_{20}O_{11}$	448.1002	447.0929	429.0827, 369.0616, 357.0616, 339.0510, 327.0510, 313.0565, 299.0561, 285.0404, 179.0349, 161.0344, 135.0451, 109.0295	Flavonoid	Antidiabetic	(Zang *et al.,* 2016)
8	4.43	Apigenin 4'-glucuronide	$C_{21}H_{18}O_{11}$	446.0850	445.0777	327.0510, 269.0455, 175.0248, 157.0242, 155.0037, 131.0349, 125.0244, 113.0244, 99.0087, 89.0244, 71.0138	Flavonoid	Antioxidant	(Salehi, Venditti, *et al.,* 2019)
9	4.48	14-Acetylandrographolide	$C_{22}H_{32}O_6$	392.2200	391.2127	347.2228, 275.1289, 131.0849, 101.0244, 85.0295	Diterpenoid	Antidiabetic	(Suemanotham *et al.,*2023)
10	4.78	δ-caesalpin	$C_{20}H_{30}O_6$	366.2033	411.2015	383.2075, 390.1891, 321.1344, 299.1863, 279.0874, 137.0244	Diterpenoid		
11	4.78	Deacylgymnemic acid	$C_{36}H_{58}O_{12}$	682.3930	681.3857	665.3906, 149.0455, 113.0244	Triterpenoid		(Shenoy *et al.,* 2018)
12	5.24	Norcaesalpinin F	$C_{21}H_{26}O_7$	390.1679	435.1661	419.1711, 404.1477, 361.1656, 337.0929, 305.1394, 268.0952, 224.1054, 163.0400, 145.0295, 111.0088, 101.0244, 89.0244	Diterpenoid		(Al-Snafi & Al, 2015)
13	7.61	Terminolic acid	$C_{30}H_{48}O_6$	504.3452	503.3380	485.3272, 453.3010,	Triterpenoid		(Trang *et al.,* 2024)

						441.3374, 425.3061, 409.3112, 221.1547, 145.0506, 119.0350, 89.0244			
14	7.69	Gymnemic acid iv	$C_{41}H_{64}O_{13}$	764.4350	763.4277	171.0662, 85.0295	Triterpenoid	Anti-diabetic	(Abbas *et al.,*2019)
15	8.06	Gymnemic acid xiii	$C_{41}H_{66}O_{13}$	766.4500	765.4427	331.1915, 165.0404, 113.0244, 101.2444,	Triterpenoid	Anti-diabetic	(Abbas *et al.,*2019)
16	9.65	Neoandrographolide	$C_{26}H_{40}O_{8}$	480.2725	525.2707	479.2650, 349.2020, 317.2122, 275.1136, 257.1031, 221.0667, 179.0561, 171.0662, 161.0455, 113.0244, 103.0400, 85.0295	Diterpenoid	Anti-inflamatory	(Price & Fischer, 2014)
17	11.02	Withanolide A	$C_{28}H_{38}O_{6}$	470.2675	469.2603	451.2490, 409.2020, 401.2333, 343.1995, 331.1915, 305.1758, 226.0999, 187.0764, 171.0663, 143.0350, 127.0407	Steroid	Anti-diabetic	(Jonathan *et al.,* 2015)
18	11.57	Withanolide F	$C_{28}H_{38}O_{6}$	470.2648	505.2342	265.1445	Steroid	Anti-diabetic	(Jonathan *et al.,* 2015)
19	11.64	Withanone	$C_{28}H_{38}O_{6}$	470.2676	469.2603	451.2489, 433.2384, 355.1915, 327.1965, 297.1496, 171.0663, 127.0401	Triterpenoid		
20	11.98	6-keto stearic acid	$C_{18}H_{34}O_{3}$	298.2506	297.2433	279.2329, 171.1026, 85.0295	Fatty acid		
21	14.34	Azadirachtin I	$C_{32}H_{42}O_{12}$	618.2655	617.2582	544.1952	Hydrocarbon	Antidiabetic	(Dallaqua *et al.,*2012)
22	14.98	Myristic acid	$C_{14}H_{28}O_{2}$	228.2084	227.2011	99.0451, 85.0295	Fatty acid	Antidiabetic	(Un *et al.,* 2022)
23	15.55	Neocaesalpin C	$C_{24}H_{34}O_{9}$	466.2224	465.2151	337.2020, 145.0506,	Triterpenoid		

						127.0400, 121.0295, 113.0244, 108.0217, 85.0295			

3.3.2. Biochemical estimation APE

3.3.2.1. Total flavonoid content

In the field of drug discovery, the flavonoid content of medicinal plants is a crucial part of interest. Some well-known mechanisms of flavonoids include the inhibition of oxidative enzymes, hydrolytic free radical scavenging activity, and anti-inflammatory action. The total flavonoid content (TFC) of APE was calculated to be 6.465 mg/Q.E. g D.W. (Figure 3.2. A).

3.3.2.2. Total phenol content

Phenol is an abundant secondary metabolite that is found mainly in plants and comprises many biologically active ingredients. These compounds have a variety of biological activities, including anticarcinogenic, antioxidant, and antimutagenic activities. The total phenol content of APE was calculated to be 10.089 mg/GAE g D.W. (Figure 3.2. B).

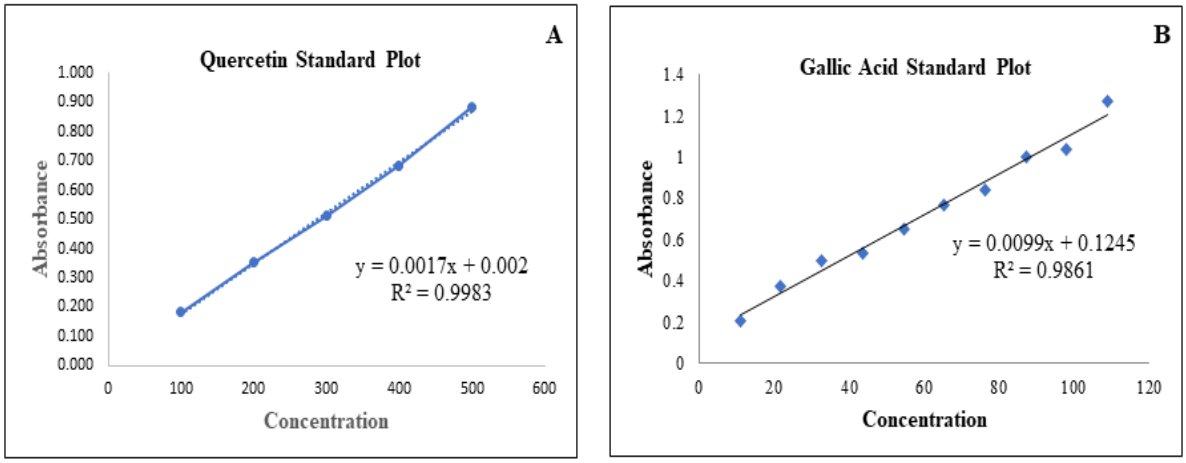


Figure 3.2. A & B. Standard curves for TFC and TPC, respectively.

3.3.3. In vitro antioxidant analysis of APE

3.3.3.1. DPPH free radical scavenging assay

DPPH radical scavenging activity provides a reliable, quantitative, and comparative measure of antioxidant activity and is widely used to evaluate the antioxidant

activity of medicinal plants. The antioxidant activity (DPPH) of APE was calculated and expressed as an IC_{50} value of 24.15 µg, which was compared with the IC_{50} of ascorbic acid (38.42 µg) (Figure 3.3. A-B).

3.3.3.2. ABTS radical scavenging assay

ABTS is a reliable, versatile, and quantitative method for assessing the antioxidant capacity of plant extracts. This assay helps to identify and standardize the therapeutic potential of the extract. Hence, in the present study, the antioxidant potential of APE was also identified via the ABTS assay. The calculated IC_{50} of APE was 25.485 µg, which was comparable with that of the standard ascorbic acid (29.702 µg) (Figure 3.3 C-D).

3.3.3.3. Ferric reducing antioxidant power (FRAP) assay

Antioxidants are multifaceted processes that are mediated by several mechanisms and are influenced by many factors. Hence, in the present study, we used a reducing power assay (FRAP) to understand the different mechanisms of APE. The IC_{50} value for APE was calculated to be 39.95 µg, and the IC_{50} value for the standard was calculated to be 44.87 µg (Fig. 3.3. E-F).

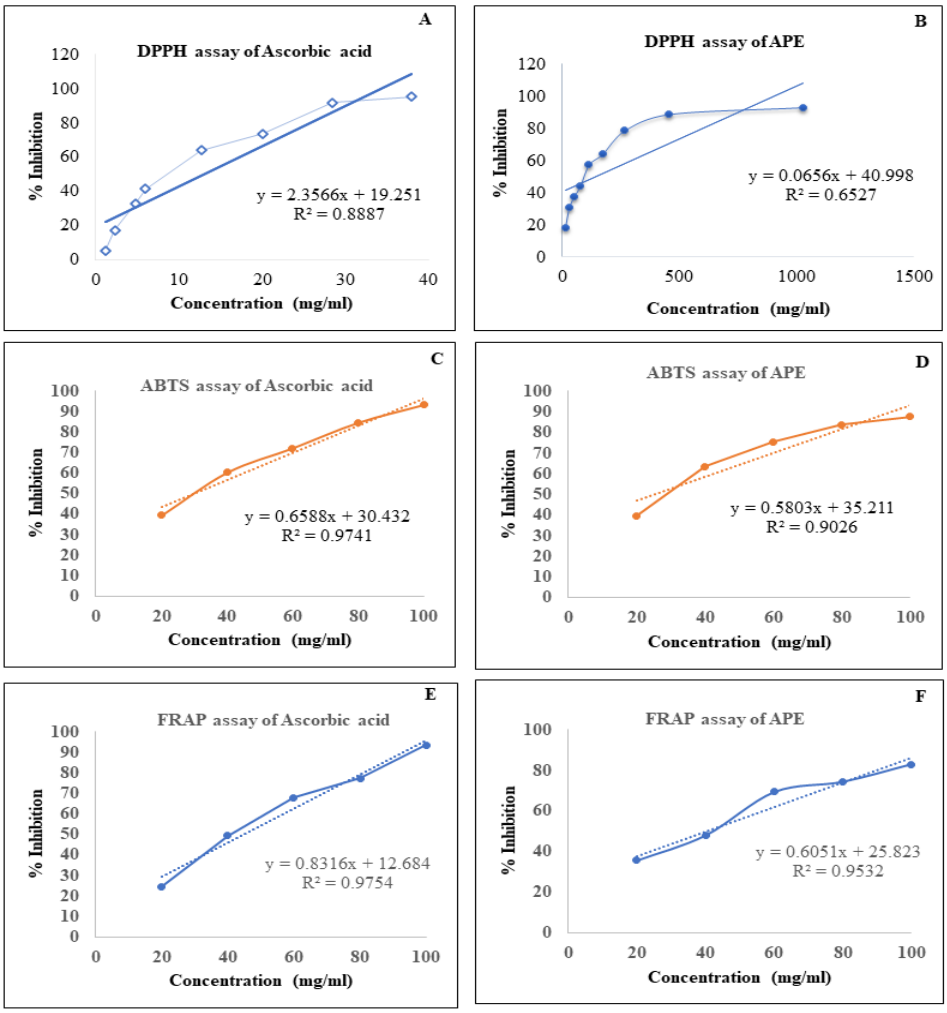


Figure 3.3. A & B. DPPH radical scavenging assay of ascorbic acid and APE, respectively. **C & D.** ABTS radical scavenging activity of ascorbic acid and APE, respectively, and **E & F.** FRAP assay of ascorbic acid and APE, respectively.

3.3.4. In vitro enzymatic assay

APE showed better biological activity in different *in vitro* antidiabetic assays. A dose-dependent increase in the percentage of the inhibitory activity against α-amylase and α-glucosidase was observed. The calculated IC_{50} values of APE for the α-amylase and α-glucosidase assays were 54.26 ± 0.14 and 26.47 ± 0.12 mg, respectively, whereas those of acarbose were 54.08 and 61.93 mg for α-amylase and α-glucosidase, respectively (Figure 3.4).

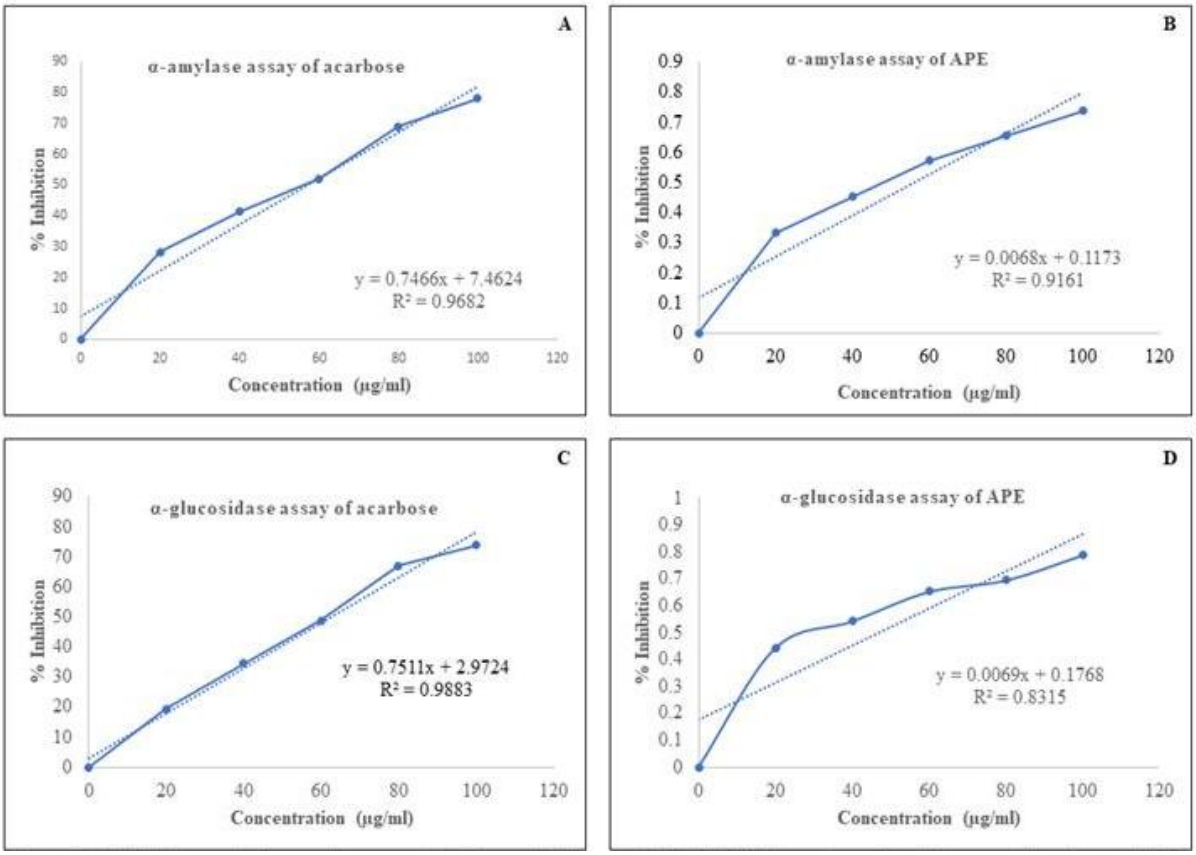


Figure 3.4. A & B. α-amylase assay of acarbose and APE, respectively. **C & D.** α-Glucosidase assay of acarbose and APE.

3.3.5. In vitro cell culture

3.3.5.1. Cytotoxicity assay

3T3-L1 cells were treated with different concentrations of APE (20–800 µg/ml), resulting in a noncytotoxic effect on the cells. The variable concentration of the extract and the cell viability are plotted in the graph below (Figure 3.5). Notably, even at a relatively

high concentration (800 µg/ml) of APE, the cell viability was 56.10% after 24 hrs. of exposure, and the calculated IC_{50} value was 1025 ± 3.011 µg/ml. These findings suggest that APE can be utilized for further *in vitro* antidiabetic studies without compromising cell viability.

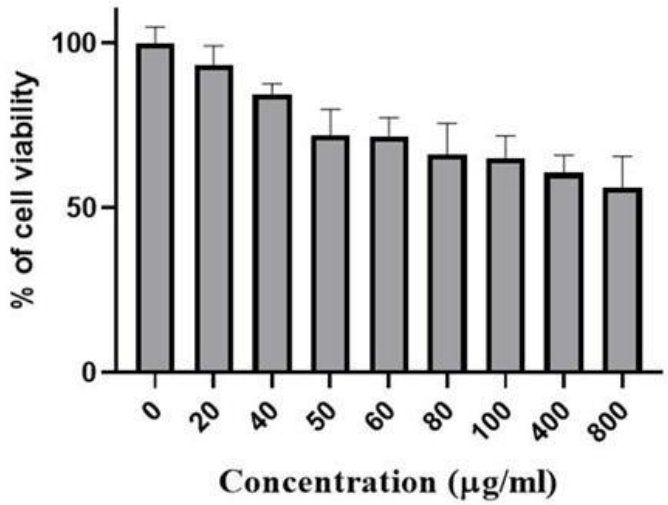


Fig 3.5. Cell viability assay of the APE extract. Even at a relatively high concentration (800 µg/ml) of APE, the cell viability was 56.10% after 24 hrs. of exposure, and the calculated IC_{50} value was 1025 ± 3.011 µg.

3.3.5.2. Glucose uptake assay

Glucose-starved MIN6 cells were treated with different concentrations of APE followed by a 2-NBDG assay. Compared with the control cells, the cells treated with the lower concentration exhibited an uptake of 30%; however, the higher concentration resulted in 80% glucose uptake (Figure 3.6). This result suggests a dose-dependent increase in glucose uptake by the cells. Higher glucose uptake indicates insulin mimic activity, improved glucose homeostasis, and the upregulation of the GLT4 transporter, which enhances insulin secretion, with APE treatment.

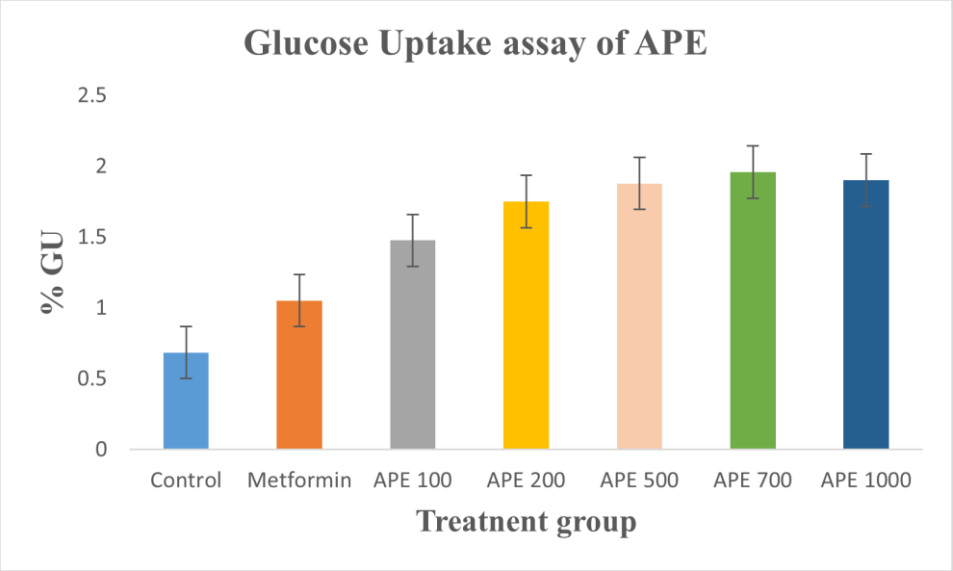


Figure 3.6: Glucose uptake assay of APE. These results suggest a dose-dependent increase in glucose uptake by the cells.

3.3.6. In silico study

3.3.6.1. Molecular docking study

The docking of the compounds identified from APE and the standard drug against the insulin-like growth factor 1 receptor kinase protein and GLUT 4 protein was performed via the blind docking approach to identify the most potent binding region, and their binding affinity was evaluated via the Glide XPScore function. Docking scores are collated in Tables 3.2 & 3.3.

Among all the selected compounds, verbascoside B exhibited the highest binding affinity for both the insulin-like growth factor 1 receptor kinase protein and the GLUT 4 protein, with docking scores of -12.433 and -17.825 kcal/mol, respectively, while the standard drugs subsequently had binding affinities of -2.605 and -3.332 kcal/mol. These sites were considered potential binding sites for the above compounds. Furthermore, the binding modes of these compounds were visualized with Ligplot (Figures 3.7 A & B). The binding of verbascoside B with the insulin-like growth factor 1 receptor kinase protein involves four hydrogen bonds with Gly A976, Met A1052, Thr A1053 and Asp A1123, and the binding with GLUT 4 also involves four hydrogen bonds with Ser A153, Trp A404,

Trp A428 and Asn A431. The standard reference drug gliclazide bound to the insulin-like growth factor 1 receptor kinase protein and GLUT4 protein and exhibited only one hydrogen bond with Gly A1112 and Ser A153, respectively. Moreover, the interactions of these compounds with the binding site residues revealed substantial hydrophobic interactions.

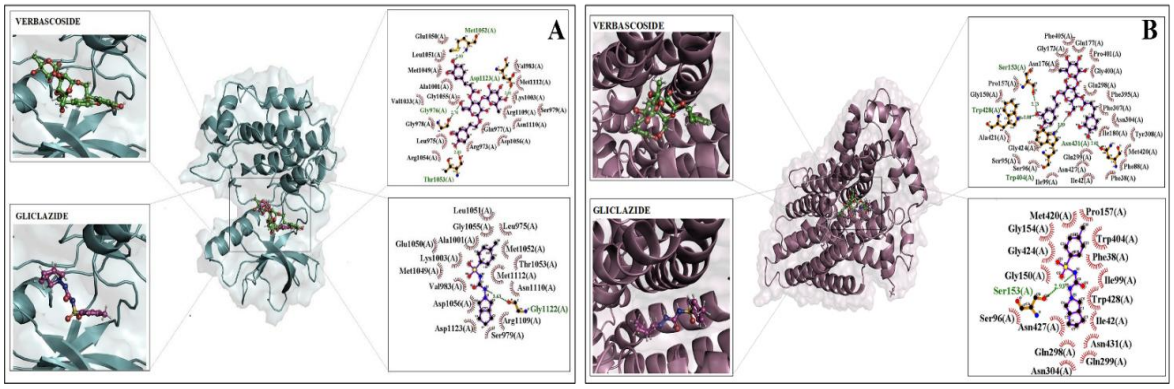


Figure 3.7: The verbascoside B compound identified from APE was found to be well accommodated inside the active binding site of the insulin-like growth factor 1 receptor kinase (IGF-I) (A) and GLUT4 (B) protein. The binding site is represented as a macro model surfaces cyan color (IGF-I) and purple colour (GLUT 4). Ligplot analysis revealed the interaction of amino acid binding sites with verbascoside B and gliclazide. The binding site residues involved in the interactions are slightly different, mainly because of the variation in functional groups. The hydrogen bonds formed (if any) are represented as dotted lines.

Table 3.2: Docking results (Glide XP) of identified compounds from APE with respect to different binding sites of the insulin-like growth factor 1 receptor kinase (1K3A) protein by sitemap (Schrodinger package, 2023-3).

Verbascoside B				
Site ID	**Site score**	**Volume (Å)^3**	**Dscore (Å)^3**	**Glide XP score (Kcal/mol)**
1	1.029	479.257	1.075	-12.433

2	0.846	184.191	0.834	-9.133
3	0.628	131.069	0.599	-9.022
4	0.693	133.298	0.628	-10.602
5	0.650	124.466	0.599	-10.439
Apigenin 7-apiosyl-glucoside				
1	1.029	479.257	1.075	-11.704
2	0.846	184.191	0.834	-6.810
3	0.628	131.069	0.599	-8.996
4	0.693	133.298	0.628	-8.863
5	0.650	124.466	0.599	-8.334
Curcucomosin A				
1	1.029	479.257	1.075	-6.235
2	0.846	184.191	0.834	-2.409
3	0.628	131.069	0.599	-3.742
4	0.693	133.298	0.628	-5.023
5	0.650	124.466	0.599	-3.576
Arjunolic acid				
1	1.029	479.257	1.075	-4.631
2	0.846	184.191	0.834	-3.966
3	0.628	131.069	0.599	-3.850
4	0.693	133.298	0.628	-4.906
5	0.650	124.466	0.599	-5.884
Withanolide F				
1	1.029	479.257	1.075	-5.344
2	0.846	184.191	0.834	-3.747
3	0.628	131.069	0.599	-4.488
4	0.693	133.298	0.628	-5.182
5	0.650	124.466	0.599	-5.520
Terminolic acid				
1	1.029	479.257	1.075	-5.413
2	0.846	184.191	0.834	-3.521
3	0.628	131.069	0.599	-5.245
4	0.693	133.298	0.628	-5.438
5	0.650	124.466	0.599	-6.032

Andrographic acid				
1	1.029	479.257	1.075	-5.712
2	0.846	184.191	0.834	-4.106
3	0.628	131.069	0.599	-4.010
4	0.693	133.298	0.628	-5.198
5	0.650	124.466	0.599	-5.335
Ricinoleic acid				
1	1.029	479.257	1.075	-6.190
2	0.846	184.191	0.834	-1.473
3	0.628	131.069	0.599	-1.846
4	0.693	133.298	0.628	-1.858
5	0.650	124.466	0.599	-4.348
Mangiferic acid				
1	1.029	479.257	1.075	-1.078
2	0.846	184.191	0.834	-1.318
3	0.628	131.069	0.599	-1.548
4	0.693	133.298	0.628	-0.393
5	0.650	124.466	0.599	-1.165
Gymnemic acid I				
1	1.029	479.257	1.075	-7.682
2	0.846	184.191	0.834	-6.610
3	0.628	131.069	0.599	-6.403
4	0.693	133.298	0.628	-7.831
5	0.650	124.466	0.599	-8.637
Gliclazide (Standard)				
1	1.029	479.257	1.075	-2.605
2	0.846	184.191	0.834	-2.048
3	0.628	131.069	0.599	-0.791
4	0.693	133.298	0.628	-2.067
5	0.650	124.466	0.599	-1.301

Table 3.3: Docking results (Glide XP) of identified compounds from APE with respect to different binding sites of the GLUT4 protein by sitemap (Schrodinger package, 2023-3).

Verbascoside B				
Site ID	**Site score**	**Volume (Å)^3**	**Dscore (Å)^3**	**Glide XP score (Kcal/mol)**
1	1.091	1402.827	1.131	-17.825
2	1.023	456.276	1.093	-12.033
3	1.037	217.376	1.019	-10.841
4	0.834	160.524	0.877	-9.788
5	0.899	73.316	0.954	-3.254
Apigenin 7-apiosyl-glucoside				
1	1.091	1402.827	1.131	-15.042
2	1.023	456.276	1.093	-10.676
3	1.037	217.376	1.019	-7.587
4	0.834	160.524	0.877	-6.749
5	0.899	73.316	0.954	-3.347
Curcucomosin A				
1	1.091	1402.827	1.131	-8.017
2	1.023	456.276	1.093	-4.808
3	1.037	217.376	1.019	-3.786
4	0.834	160.524	0.877	-4.330
5	0.899	73.316	0.954	-3.607
Arjunolic acid				
1	1.091	1402.827	1.131	-10.034
2	1.023	456.276	1.093	-5.980
3	1.037	217.376	1.019	-2.607
4	0.834	160.524	0.877	-3.929
5	0.899	73.316	0.954	-4.357
Withanolide F				
1	1.091	1402.827	1.131	-10.215
2	1.023	456.276	1.093	-5.617
3	1.037	217.376	1.019	-3.858
4	0.834	160.524	0.877	-3.828

5	0.899	73.316	0.954	-4.616
Terminolic acid				
1	1.091	1402.827	1.131	-9.274
2	1.023	456.276	1.093	-6.155
3	1.037	217.376	1.019	-3.556
4	0.834	160.524	0.877	-4.684
5	0.899	73.316	0.954	-5.437
Andrographic acid				
1	1.091	1402.827	1.131	-7.367
2	1.023	456.276	1.093	-4.505
3	1.037	217.376	1.019	-5.806
4	0.834	160.524	0.877	-3.447
5	0.899	73.316	0.954	-4.930
Ricinoleic acid				
1	1.091	1402.827	1.131	-5.420
2	1.023	456.276	1.093	-3.841
3	1.037	217.376	1.019	-2.416
4	0.834	160.524	0.877	-3.590
5	0.899	73.316	0.954	-1.494
Mangiferic acid				
1	1.091	1402.827	1.131	-3.866
2	1.023	456.276	1.093	-2.496
3	1.037	217.376	1.019	-2.552
4	0.834	160.524	0.877	-3.109
5	0.899	73.316	0.954	-1.496
Gymnemic acid I				
1	1.091	1402.827	1.131	-12.090
2	1.023	456.276	1.093	-6.674
3	1.037	217.376	1.019	-8.132
4	0.834	160.524	0.877	-6.326
5	0.899	73.316	0.954	
Gliclazide (Standard)				
1	1.091	1402.827	1.131	-3.332
2	1.023	456.276	1.093	-2.568

3	1.037	217.376	1.019	-3.219
4	0.834	160.524	0.877	-1.602
5	0.899	73.316	0.954	-2.868

3.3.6.2. ADME property prediction

As previously reported, several parameters were chosen for the ADME calculations (Pragyandipta *et al.*, 2023b). Lipinski's rule for five methods was used to assess the desirability of verbascoside B and standard drugs as vital drugs. The value of verbascoside B was considerable for each characteristic evaluated, and every drug-like capability was validated according to Lipinski's rule of five (Table 4).

Table 3.4: QikProp (Schrodinger package, 2023-3) was used to determine the ADME properties of verbascoside B and the standard gliclazide. These molecules are associated with all the ADME parameters.

	ADME Screening	**Verbascoside B**	**Gliclazide**	**Recommended values**
1	MW.	624.59	323.40	130-725
2	SASA	949.37	657.31	300-1000
3	Accpt HB	20.30	5.00	2.0-20.0
4	QPpolrz	53.15	36.70	13.0-70.0
5	QPlogPoct	42.14	16.11	8.0-35
6	QPlogPw	35.14	9.53	4.0-45.0
7	QPlogPo/w	-1.55	2.64	-2.0-6.5
8	QPlogHERG	-6.91	-4.39	Below -5.0
9	QPPCaco	1.52	512.03	< 25 poor > 500 great
10	QPlogBB	-5.55	-1.02	-3.0-1.2
11	QPPMDCK	0.44	291.70	< 25 poor >500 great
12	QPlogKp	-6.15	-3.10	-8.0- -1.0
13	QPlogKhsa	-1.47	0.13	-1.5-1.5
14	Rule of Five (No. of violations)	3	0	Maximum is 4

3.3.7. In vivo antidiabetic study

3.3.7.1. Acute toxicity effect of APE

In the oral acute toxicity study, the animals in groups I-III did not exhibit any mortality at different doses. However, groups IV and V presented mortality rates of 2/6 and 3/6, respectively. Since a 50% death rate was observed at 5000 mg/kg b.w., the LD_{50} was reported to be 5000 mg/kg b.w. The effective dose of APE was calculated as 500 mg/kg b.w. and was administered to diabetic-induced experimental animals.

3.3.7.2. Subacute effects of APE

The subacute toxicity study of APE in animals revealed no symptoms of mortality or clinical changes throughout the experiment. The body weight, food and water intake (Table 3.5), haematological (Table 3.6 A) and biochemical parameters (Table 3.6 B) did not significantly differ from those of the normal control group.

H&E staining of the organs revealed no pathological alterations. The brain tissue exhibited intact neuronal structures without any inflammation or necrosis. The kidney tissue displayed normal glomerular and tubular architecture, confirming that there was no nephrotoxicity. Liver tissue showed normal architecture of hepatic cells with no hepatocellular damage. Heart tissue appeared normal, without signs of myocardial injury or inflammation. The lung tissue exhibited healthy alveolar structures that were free from inflammation, fibrosis, or cellular necrosis (Figure 3.8).

Table 3.5: Physiochemical parameters of the APE-treated animals and normal controls.

Body Weight (in gm)		
Days	**Control**	**Treated**
1	191.06±3.21	202.1±14.09
7	194.2±4.57	208.1±10.27
14	199.01±8.87	213.5±6.24
21	203.1±4.26	215±13.17
28	206.4±4.4	217.1±12.67
Food intake (in gm)		
1	13.76±1.56	17.03±2.03
7	13.82±1.90	17.09±1.62
14	16.24±2.33	16.09±2.32
21	15.21±1.69	14.05±1.03
28	14.63±1.69	15.76±2.51
Water intake (in ml)		
1	16.78±2.54	18.1± 2.08
7	20.52±1.97	19.34±2.14
14	20.71±1.95	21.34±2.15
21	22.15±1.16	23.42±1.78

28	24.21±3.12	23.91±3.14

Table 3.6: Haematological and biochemical parameters of the APE-treated and normal control groups.

A. Haematological parameter				
	Normal	**Negative**	**Positive**	**Treated**
White blood cell count (WBC (10^3/L))	5.466 ± 0.249	14.8 ±0.653	5.633± 0.169	7.466 ±0.205
Neutrophils (Neu# (10^3/L))	4.466 ± 0.286	12.433 ±0.235	5.5 ± 0.081	6.666±0.262
Lymphocytes (Lym# (10^3/L))	3.466 ± 0.249	8.933 ±0.368	2.633 ± 0.124	3.9 ± 0.163
Monocytes (Mon# (10^3/L))	0.433 ± 0.124	2.766 ±0.286	0.3 ± 0.081	0.6 ± 0.081
Eosinophils (Eos# (10^3/L))	0.123 ± 0.028	0.8 ±0.081	0.033 ± 0.012	0.026 ± 0.4
Basophil (Bas# (10^3/L))	0.05 ± 0.024	0.633 ±0.124	0.03 ± 0.008	0.06 ± 0.06
NLR	1.466 ± 0.169	7.233 ±0.41	1.5 ± 0.081	2.4 ± 0.326
PLR	0.019 ± 0.001	0.06 ±0	0.072 ± 0.076	0.025± 0.02
Red blood cell count (RBC (10^12/L))	5.866 ± 0.286	9.4 ±0.163	5.3 ± 0.163	5.9± 0.163
Haemoglobin (HGB (g/dL))	13.5 ± 0.294	20.533 ±0.205	13.4 ± 0.216	14.166± 0.3
HCT	43.633 ± 0.169	59.433 ±0.169	43.566 ± 0.124	46.766±1.2
MCV (fL)	84.433 ± 0.124	98.666 ±0.205	85.5 ± 0.141	87.066 ± 1.755
MCH (pg)	30.133 ± 0.249	44.566 ±0.286	30.433 ± 0.205	30.866 ± 0.612
MCHC (g/L)	327± 1.632	392.333 2.054	336 ± 1.632	344.333 ± 11.841
RDW-CV	12.7 ± 0.326	20.633 ±0.124	13.333 ± 0.205	14.066 ± 0.385
RDW-SD (fL)	37.5 ± 0.244	65.533 ±0.249	40.466 ± 0.169	45.333 ± 3.633
platelet count (PLT (10^3/L))	2.733 ± 0.124	6.7 ±0.216	3.366 ± 0.124	5 ± 0.216
MPV (fL)	10.5 ± 0.244	18.533 ±0.205	11.266 ± 0.124	11.866 ± 0.169
PDW-CV	50.6 ± 0.163	78.533 ±0.286	53.333 ± 0.205	67.1 ± 0.668
PDW-SD (fL)	10.566 ± 0.205	20.6±0.244	14.433 ± 0.205	14.933 ± 0.449
PCT (mL/L)	0.164 ± 0.003	0.544 ±0.002	0.125 ± 0.001	0.223 ± 0.039

B. Biochemical Parameter					
Parameter	**Normal**	**Negative**	**Positive**	**Treated**	**Range**
GLU (mg/dl)	116.51 ± 5.73	207.53 ± 58.89	90.98 ± 10.36	50.43 ±5.97	74.0 - 143.0
ALB (g/dl)	2.95 ± 0.90	6.816 ± 0.59	2.2 ± 0.36	3.2 ±0.50	2.3 – 4.0
UREA (mg/dl)	29.28 ± 6.31	14.876 ± 2.25	23.11 ± 3.36	33.81 ±13.22	15.0 – 58.0
CREA (mg/fl)	1.375 ± 0.48	25.15 ± 9.09	1.638 ± 0.17	1.63 ±0.16	0.5 – 1.8
CHOL (mg/dl)	157.86 ± 28.42	28.51 ± 11.09	146.1 ± 13.74	169.9 ±13.53	109 - 202
TG (mg/dl)	90.53 ± 45.79	191.44 ± 15.17	96.1 ± 33.69	123.25 ±6.71	40 - 165
ALT (U/L)	84.53 ± 18.40	168.48 ± 13.89	84.53 ± 18.40	54.28 ±1.94	10 - 125
AST (U/L)	32.49 ± 4.40	121.76± 0.94	33.6 ± 3.68	0.4 ±0.18	0 - 50
TP (g/dl)	5.548 ± 0.62	1.02 ± 0.85	6.14 ± 1.35	5.58 ±0.65	5.2 - 8.2
MG (mg/dl)	1.7 ± 0.16	0.316 ± 0.17	1.9 ± 0.35	1.86 ±0.39	1.50 - 2.10
PHOS (mg/dl)	4.85 ± 0.75	33.22 ± 8.38	5.85 ± 1.36	5.383 ±1.11	3.00- 6.20
CA (mg/dl)	9.03 ± 0.36	64.21 ± 13.81	9.033 ± 0.78	9 ±0.69	8.70- 11.80
DBIL (mg/dl)	0.32 ± 0.17	6.2 ± 1.67	0.3803 ± 0.22	0.416 ±0.15	0 - 0.50
TBIL (mg/dl)	0.513 ± 0.29	10.93 ± 0.43	0.61 ± 0.32	0.52 ±0.16	0 - 0.90
HDL (mg/dl)	55.0166 ± 11.18	428.91 ± 33.72	54.88 ± 10.14	124.6 ±1.90	35.0 - 88.0
GGT (U/L)	5.13 ± 2.08	18.8 ± 7.09	6.083 ± 2.06	0.0286 ±0.01	0 - 10.0
ALP (U/L)	122.46 ± 2.68	252.68 ± 21.60	75.23 ± 20.88	91.9 ±12.52	0.1 - 212.0

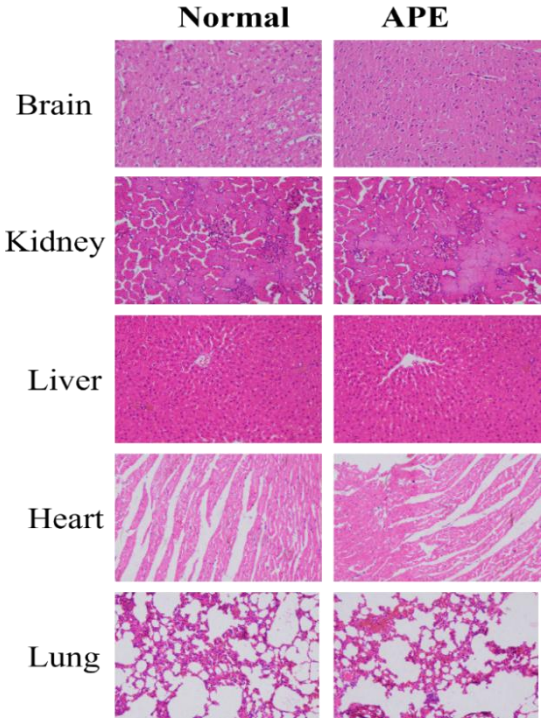


Figure 3.8: H&E staining of vital organs from the toxicity study revealed no observable pathological alterations.

3.3.7.3. Effect of APE on body weight, blood glucose level and HbA1c level

The physical and biochemical parameters, such as body weight, blood glucose level and HbA1c level, of the experimental animals were measured on the 7th, 14th, 21st and 28th days, and the results were compared at the end of the experiment on the 28th day (Table 3.7). The normal control group had a b.w. of 243±2.01 g, but the b.w. of the negative control group significantly decreased ($p<0.001$) to 97±1.98 g. However, the b.w. of the positive control group (metformin-treated) and treatment control group (APE-treated) significantly improved, with values of 132±3.04 g and 290±2.21 g, respectively (Figure 3.9 A). The blood glucose level significantly ($p<0.001$) increased to 467±3.00 mg/dl in the negative control group compared with the normal control group (109±1.56 mg/dl). However, the APE-treated group (treatment control) presented a significant reduction ($p<0.001$) to 130±1.45 mg/dl, which was comparable to that of the positive control group (110±1.45 mg/dl) (Figure 3.9 B). The HbA1c level was reported to be 5.13±0.54 in the normal control group and significantly ($p<0.001$) increased to 15.1±0.02 in the negative control group. However, upon the administration of APE, the value significantly ($p<0.01$) decreased to 4.2 ± 0.01 and was comparable to that of the metformin-treated positive control group (Figure 3.9 C).

Table 3.7. Comparative study of body weight, blood glucose levels and glycated haemoglobin levels in different experimental groups on the 7th, 14th, 21st and 28th days.

A. Body weight (g)				
	Day 7	**Day 14**	**Day 21**	**Day 28**
Normal control	211 ± 1.6	224 ± 1.89	231 ± 0.98	243 ± 2.01
Negative control	192 ± 3.45	122 ± 2.51	103 ± 3.12	97 ± 1.98 ***
Positive control	160 ± 3.01	140 ± 2.45	130 ± 1.67	132 ± 3.04 **
Treatment control	222 ± 4.23	258 ± 2.78	278 ± 1.2	290 ± 2.21 ***

B. Blood glucose level (mg/dl)				
	Day 7	**Day 14**	**Day 21**	**Day 28**
Normal control	95 ± 2.06	88 ± 1.6	104 ± 3.00	109 ± 1.56
Negative control	410 ± 4.72	459 ± 4.35	431 ± 1.00	467 ± 3.00 ***
Positive control	497 ± 2.59	330 ± 2.76	144 ± 1.12	110 ± 1.45 ***
Treatment control	510 ± 1.52	367 ± 2.00	200 ± 2.00	130 ±1.45 ***
C. Glycated haemoglobin (HbA1C) level (mmol/mol)				
Normal control	5.69 ± 0.8	4.89 ± 0.01	5.00 ± 0.56	5.13 ± 0.54
Negative control	15.69 ± 0.20	14.8 ± 0.07	15.9 ± 0.07	15.1 ± 0.02 ***
Positive control	13.7 ± 0.01	9.3 ± 0.3	6.4 ± 0.05	5.56 ± 0.5 ***
Treatment control	12.8 ± 0.08	10.7 ± 0.05	8.8 ± 0.2	6.5 ± 0.01 **

Statistical analysis was carried out via one-way ANOVA followed by the Tukey–Kramer multiple comparisons test. The values are presented as the means ± SEMs (n=6). p* <0.05, p** <0.01, p*** <0.001, and * represent significant differences from the control.

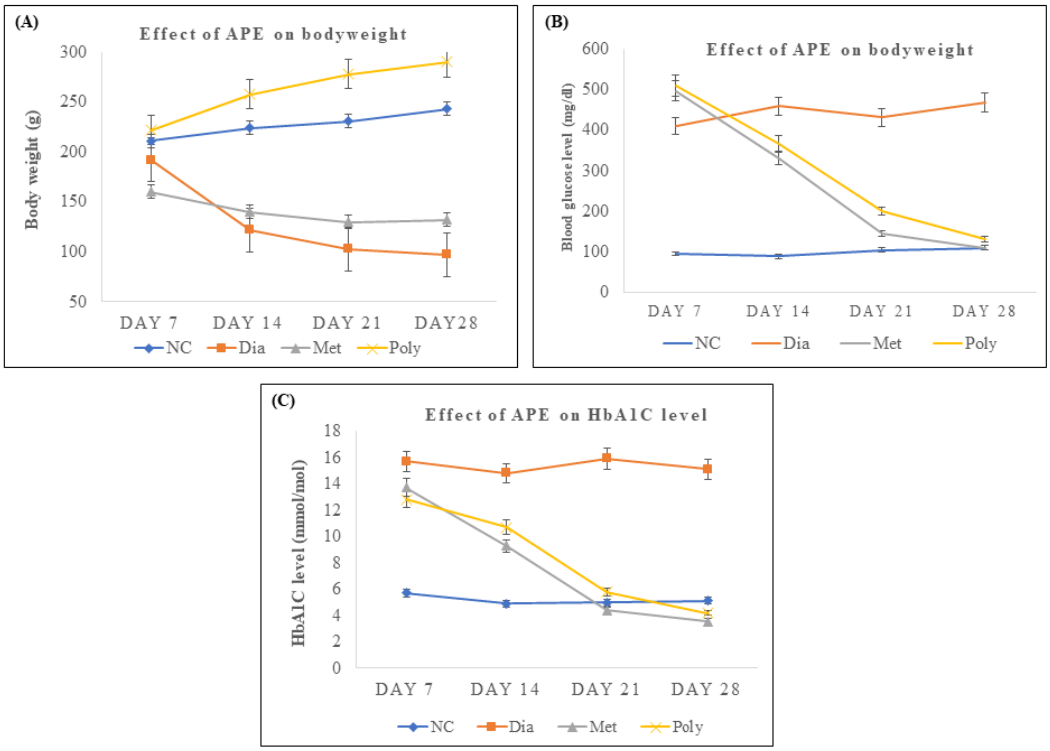


Figure 3.9: A. Increased body weight after the induction of diabetes and the effects of metformin and APE on body weight after 28 days of treatment. **B.** Comparison of preeminent blood sugar levels among the diabetic control, metformin-treated and APE-treated groups and the normal control group. **C.** Effects of metformin and APE on HbA1c levels compared with those of the negative control and normal control groups. (APE-aqueous polyherbal extract, HbA1C-glycated haemoglobin).

3.3.7.4. Effect of APE on biochemical serum markers

To determine the effect of APE on liver and kidney function, which is associated with long-standing diabetes, we measured the following parameters: LFT (alanine aminotransferase, aminotransferase, alkaline phosphatase) and KFT (urea and creatinine). In addition, several other parameters, such as cholesterol, total protein, triglycerides, high-density lipoprotein, and insulin, were measured in the blood serum of all four groups of animals. The levels of serum markers such as insulin and ALB were significantly ($p<0.001$) reduced, and the levels of other serum markers were significantly ($p<0.001$) elevated due to decreased insulin sensitivity and high blood sugar levels. After a treatment period of 28 days, the serum marker levels were very close to those of the normal control group in the animals treated with APE, similar to those of the metformin-treated group (Table 3.3).

Table 3.8. Biochemical estimation of different serum markers in the experimental groups.

Biochemical test	Normal control	Negative control	Positive control	Treatment control
Kidney Function Test (KFT)				
UREA (mg/dl)	49.686±3.22	66.35± 1.75***	46.73±3.97***	44.43±0.25***
CREA (mg/dl)	1.316±0.157	26.42± 1.74***	1.5±0.26***	1.41±1.4***
Liver Function Test/Liver Enzyme (LFT)				
SGPT (U/L)	100.76±5.24	154.95± 2.90***	115.666±3.8***	122.08±3.3***
SGOT (U/L)	32.316±2.07	324.3± 4.57***	43.85±1.31***	43±1.7***
ALB (g/dl)	2.523±0.155	0.81± 0.487***	2.631±0.16***	3.156±0.6***
ALP (U/L)	41.016±4.46	254.45± 10.9***	48.25±2.88***	54±1.2***
Other biochemical parameter				
CHOL (mg/dl)	116.666±3.85	7.316± 4.09***	108.33±3.19***	98±12.4***
TG (mg/dl)	126.316±2.4	229.88± 9.40***	133.2±2.93***	151.40±38.2***
TP (g/dl)	5.583±1.129	2.81± 0.55***	4.691±0.33***	4.1±10.3***
HDL (mg/dl)	69.568±5.201	12.43± 55.4***	70.7±5.66***	84.38±5.4***
Insulin (µU/ml)	2.01 ± 0.01	0.015 ± 0.005***	1.3 ± 0.015***	2.15 ± 0.7***

3.3.7.5. Histopathological and IHC studies

H&E staining of the pancreatic islets revealed a significant structural alternation was observed in the diabetic control group compared to the control. However, APE treated group shows a similar structure with normal control (Figure 3.10 A-D).

Immunohistochemical staining of the normal control pancreas revealed, a strong staining for anti-synaptophysin (Figure 3.10 AS), anti-insulin (Figure 3.10 AI) and anti-glucagon (Figure 3.10 AG) antibodies, indicating intact synaptic function and robust insulin production subsequently maintain a proper glucose homeostasis. In contrast, the diabetic control pancreas represented a reduce intensity of synaptophysin positive (synaptic) cells (Figure 3.10 BS), whereas the insulin stained were negative because of the destruction of β-cells (Figure 3.10 BI). The proportion of glucagon positive cells (α-cells) was increased (46%) compared to the normal, because of the hyperglycemic conditions (Queiroz *et al.,* 2021) (Figure 3.10 BG). This significant pathophysiological alternation was due to diabetes (include impaired insulin secretions). In the metformin-treated group, the anti-synaptophysin stained slide showed a slight increase in intensity compared to diabetic control (Figure 3.10 CS), but the anti-insulin-stained slides represent similar result to the diabetic control (Figure 3.10 CI) suggesting metformin treatment partially restores the synaptic function without modulating the β-cells. The anti-glucagon-stained slides indicates a nearly similar proportion of α-cells compared to the diabetic control group (Figure 3.10 CG). In the APE-treated group, the pancreatic islets stained with anti-synaptophysin antibody revealed the total islet area with a significant increase in intensity suggesting a significant restoration of the synaptic function (Figure 3.10 DS). However, the anti-glucagon antibody-stained slides revealed the normal architecture of the α-cells (reduce proportion of α-cells) (Figure 3.10 DG). The anti-insulin-stained slides of APE treated group revealed a regeneration of the destroyed β-cells. The calculated proportion of β-cell after four weeks of treatment was 47% (Figure 3.10 DI). The mantle core arrangement of α- and β-cells, ensuring an increase in insulin secretion within the endocrine region via paracrine effects.

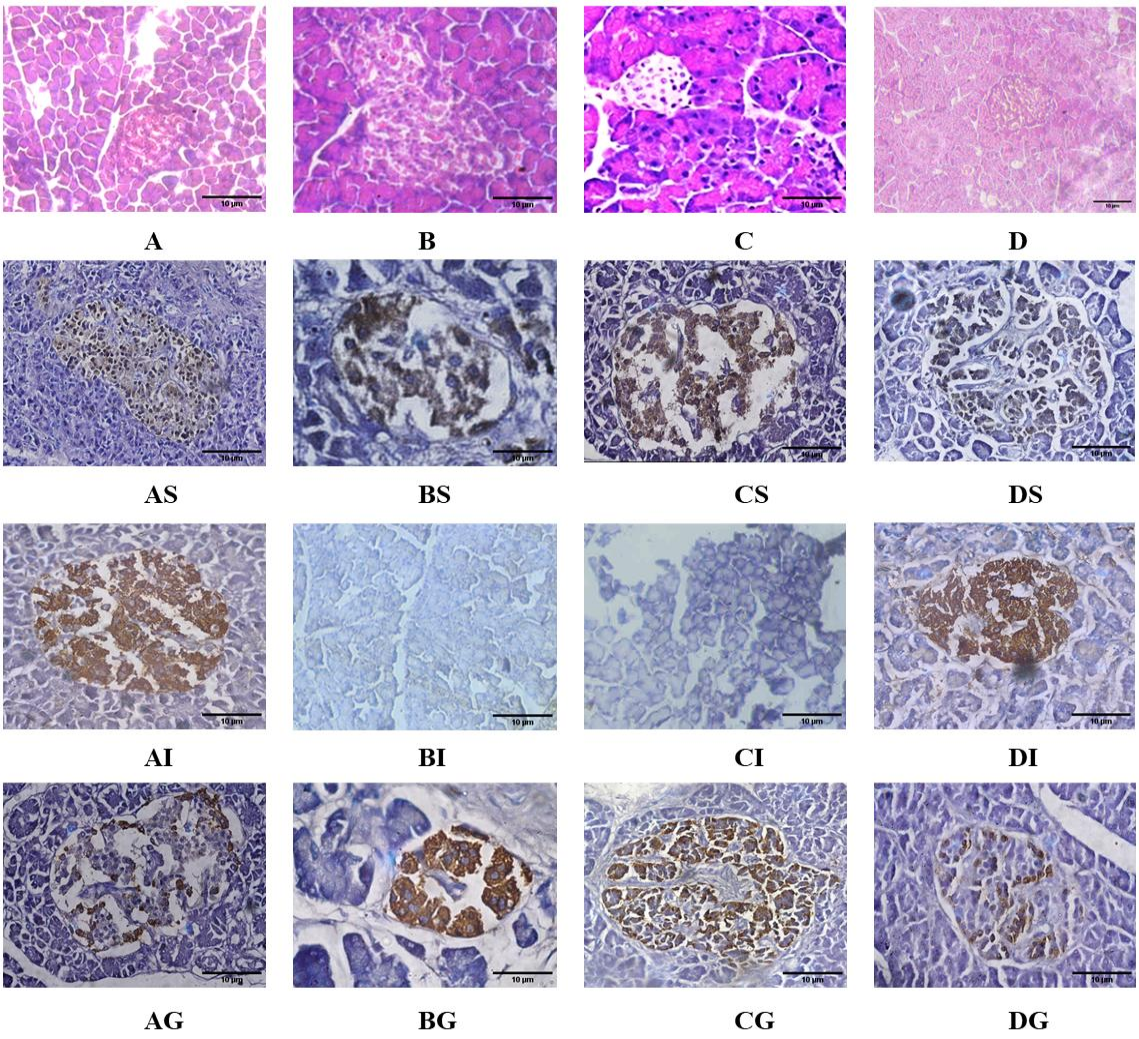


Fig 3.10. Histopathological and immunohistological images of the pancreas in experimental animals. A-D. H&E staining of pancreatic islets from the normal control, negative control, positive control (metformin-treated) and treatment control (APE-treated) groups. AS-DS. IHC staining of the pancreatic islets with an anti-synaptophysin antibody. AI-DI. IHC staining of anti-insulin antibody. AG-DG. IHC staining of anti-glucagon antibody.

3.3.7.6. Analysis of immunohistochemistry images

Immunohistochemical images of all four groups of pancreases were taken via an inverted microscope in the bright field region (Nikon Eclipse TS2R, Japan). The area proportions of the captured images were analysed via ImageJ software by standardizing the microcoding. Synaptophysin-stained slides were used to calculate the total area percentage of the islet. The insulin-stained slides were used to calculate the proportion of β-cells, and the glucagon-stained slides were used to calculate the proportion of α-cells in the islet area. The regeneration of β-cells was calculated for the APE-treated group. The β-cell proportion was calculated to be 64% in the normal control group, whereas 39.80% and

47% regeneration were calculated in the APE-treated group after the 21st and 28th days of treatment, respectively. In contrast, the glucagon proportion in the normal control islets was 20%, whereas that in the diabetic control islets was increased to 46%. However, in the treated group, namely, the metformin- and APE-treated groups, the proportion fell near that in the normal control group. Details of the regeneration β-cells are described in Table 3.4.

Table 3.9. Percentage of pancreatic islet β-cells in normal control and APE-treated rats.

Sl. No.	No of days treated	Group	% of β-cells
1	7	APE treated	9.17
2	14	APE treated	21.88
3	21	APE treated	39.80
4	28	APE treated	47.39
5	28	Normal control	64

3.4. Discussion

Diabetes is a disorder of pancreatic β-cells characterized by dysfunction and apoptosis of these cells. Diabetic agents such as streptozotocin and alloxan selectively destroy pancreatic β-cells via the induction of free radical species such as reactive oxygen species (ROS) and oxidative stress, subsequently resulting in impaired insulin secretion(El-Desouki *et al.,*2015; Taheriazam *et al.,*2023). The key mechanism of alloxan and its metabolic product, dialuric acid, initiates a redox cycle, forming a superoxide radical. This destroys pancreatic β-cells (Abd El Latif *et al.,*2014; El-Tantawy & Haleem, 2014; Szkudelski, 2001). The increasing prevalence of diabetes poses a significant health concern globally (Verma *et al.,*2018). Despite the introduction of new drugs for the management of diabetes, these drugs have adverse side effects, including urinary dysfunction and elevated lipid profiles, during long-term use. In addition, these drugs only target the clinical symptoms; rather, they focus on the pathophysiological causes (Attanayake *et al.,*2019).

The current study focused mainly on developing a polyherbal formulation with antihyperglycemic effects and reviving destroyed pancreatic β-cells. APE was prepared by mixing 16 medicinal plants known for their antidiabetic activity on the basis of traditional methods. An *in vitro* study using the MIN6 cell line revealed increased glucose uptake in APE-treated cells, suggesting increased insulin secretion for the

maintenance of blood APE-treated cells, suggesting increased insulin secretion for the maintenance of blood glucose levels. These results suggest increased glucose uptake in a dose-dependent manner, suggesting that the insulin-mimetic activity of APE improved glucose homeostasis and upregulated the GLUT4 transporter to regulate blood glucose levels. The same result was reported in a previous study on pancreatic β-cell lines (MIN6 and cultured islets), suggesting that flavonoids can increase insulin secretion, inhibit cell apoptosis, and promote the proliferation of pancreatic β-cells (Jamaddar *et al.,*2023).

The contemporary results for blood glucose and HbA1c levels were in line with those of a previous report, which showed that APE significantly decreases blood glucose and glycolate haemoglobin (HbA1C) levels among alloxan-induced diabetic animals. The animals also presented a significant decrease in symptoms related to hyperglycemia, possibly due to the extrapancreatic action of APE, which resulted in a significant decrease in the blood glucose level (Abd El Latif *et al.,*2014; Sukalingam *et al.,*2015). The increasing body weights of the geese in the treatment group indicate the ability of APE to reverse the hyperglycemic effects of the chemical or its treasured phytoconstituents, such as phenols, calcium, amino acids, minerals, and carbohydrates (Abd El Latif *et al.,*2014). The adverse effects of diabetes related to HbA1c are associated primarily with protein glycosylation, which leads to many other complications, including vascular, nerve, and kidney damage; eye complications; and impaired wound healing. Hence, there is a need to attenuate the HbA1c level in diabetes patients (Marmitt *et al.,*2021). A previous study demonstrated that plants, including *Cinnamomum cassia, Catharanthus roseus,* and *Gymnema sylvestre,* decrease the HbA1C level upon treatment (Ihara *et al.,*2000; Semwal *et al.,*2021; Yao *et al.,*2013). The same result was observed in the present study, i.e., the APE-treated and metformin-treated groups presented similar HbA1c levels, which may be due to the presence of phytochemicals such as tannins, sterols, phenolic acids, and phenolic compounds, which are responsible for the antidiabetic properties of these compounds (Sadhu *et al.,*2006). In addition, the LC–MS analysis of APE revealed the presence of phytoconstituents such as neocaesalpin c, luteolin-5-o-glucoside, and tannic acid, isovitexin 6"-o-glucoside, gallic acid, mangiferin, noreugenin, litseglutine b, apigenin 7-apiosyl-glucoside, 14-deoxy-11,12-didehydroandrographiside, azadirachtin I, and δ-caesalpin, which reportedly decrease the blood glucose level by increasing the rate of glucose absorption by the intestine, reducing hepatic glucose production, increasing insulin sensitivity, reducing oxidative stress and inflammation in the body, and inhibiting the enzyme (Abdulai *et al.,*2021; Assefa *et al.,*2021; Ganogpichayagrai *et al.,*2017;

Hartogh & Tsiani, 2019; Jamaddar *et al.,*2023; Jayakumar *et al.,*2013b; Kamaraj *et al.,*2023; Xu *et al.,*2021; Zang *et al.,*2016).

In diabetes, kidney functions are affected, leading to glomerular hyperfiltration and microalbuminuria via increased urea and creatinine levels, as reported previously (Brookes & Power, 2022; Sukalingam *et al.,*2015). These findings align with previous findings; in the diabetic control group, elevated levels of urea and creatinine were observed (66.35±1.75 and 26.42±1.74, respectively) compared with those in the normal control group (49.686±3.227 and 1.316±0.157, respectively). In contrast, the positive control (46.73±3.97, 1.5±0.26) and the APE-treated groups (44.43±0.25, 1.41±1.4) reverted nearly to the normal range. The liver enzyme levels (SGPT, SGOT) were significantly ($p<0.001$) greater in the diabetic control group (154.95± 2.90, 324.3± 4.57) than in the normal control group (100.76±5.24, 32.316±2.070). In contrast, the positive (115.666±3.85, 43.85±1.31) and APE (122.08±3.3, 43±1.7) treatment groups presented stabilized enzyme levels after 28 days of treatment. ALB and ALP levels are also elevated in individuals with diabetes, as observed in the present study. In the diabetic control group, there was a decrease in the ALB level (0.81± 0.487) and an increase in the ALP level (254.45±10.91) compared with those in the normal control group (2.523±0.155 and 41.016±4.460, respectively). After the treatment period, both the positive (2.631±0.16, 48.25±2.88) and APE-treated (3.156±0.6, 54±1.2) groups were close to the normal control group. The current findings on the serum levels were consistent with the results reported previously (Noroozi Karimabad *et al.,*2022)..

The pancreas plays a crucial role in glucose homeostasis (Cabrera *et al.,*2008; Steiner *et al.,*2010), and consists of 60–70% β-cells (insulin-producing), 15–20% α-cells (glucagon-producing), and 10–20% δ cells, ε-cells, and pancreatic polypeptides (Bosco *et al.,*2010; Huang *et al.,*2018). The distribution pattern of α- and β-cells varies depending on the size of the islet; maintaining an optimal size is important for their functional activity. The mantle core architecture of α & β-cells, i.e., α-cells at the periphery and β-cells at the center position within the endocrine region, enhances insulin secretion via the paracrine effect (Kilimnik *et al.,*2012; Mishra *et al.,*2024). Dysfunction/destruction of pancreatic β-cells leads to a hormonal imbalance that subsequently results in diabetes (Ighodaro *et al.,*2017). An increase in postprandial blood glucose levels is another important aspect that should be considered in the management of diabetes (Kimani *et al.,*2023; Susilawati *et al.,*2023).

Recent studies have revealed a chance of endogenous pancreatic β-cell regeneration to mitigate diabetes symptoms, and the findings of the present study align with those reported earlier (Noroozi Karimabad *et al.,*2022), i.e., a disturbed islet architecture was observed in the case of diabetes; however, it was reversed with APE treatment. IHC staining with the primary antibody anti-insulin revealed a significant increase in the number of pancreatic β-cells (Fig. 6), indicating the normal mantle core architecture of the islet. The results of the present study suggest an increase in insulin secretion after treatment with APE. Moreover, ongoing clinical trials are evaluating the safety and efficacy of medicinal plant-based interventions, further contributing to the evidence supporting their use in diabetes care. This research seeks to validate traditional knowledge and potentially integrate these natural remedies into conventional medical practices.

A literature review revealed that medicinal plants promote the regeneration of pancreatic β-cells in diabetic patients. The regeneration of β-cells mainly follows two common mechanisms, i.e., regeneration from nonbeta cells or stem/progenitor cells and, second, through increased proliferation of preexisting β-cells (Kimani *et al.,*2023). The plants that exhibit regeneration activity are *Agaricus bisporus* (Y. Wang *et al.,*2012), *Aralia taibaiensis* via the Wnt, β-catenin, and TCF7L2 pathways (Cui *et al.,*2020), *Ervatamia microphylla* (Kawakami *et al.,*2010), *Glycine max* via the cAMP/PKA signalling pathway (Horiuchi *et al.,*2017), *Rhodiola rosea* by activating Akt/FoxO1 signalling (Ju *et al.,*2017), *Mangifera indica* via STAT3 signalling (H. Wang *et al.,*2018), and *Tinospora cordifolia* by increasing the expression of Pdx-1 mRNA and decreasing the expression of carbonic anhydrase nine mRNAs, *Radix puerariae* via the GLP-1R, Wnt, and STAT signalling pathways (Damame *et al.,*2022). The plants capable of increasing β-cell mass through proliferation include *Angelica sinensis, Hibiscus rosa-sinensis, Woodfordia fruticosa, Cornus officinalis, salidroside,* and *sangguayin* (Tomita, 2016). The above mechanistic study of APE revealed that natural bioactive compounds are responsible for β-cell regeneration and improvement of its function. The compounds identified from APE, such as apigenin 7-apiosyl-glucoside, arjunolic acid, verbascoside B, arjungenin, gymnemic acid I, ii, iv, xiii, and ricinoleic acid, are responsible for the regeneration and proliferation of β-cells, as reported previously (Aamir *et al.,*2022; Abbas *et al.,*2019; Assefa *et al.,*2021; Galli *et al.,*2020; Mohammed *et al.,*2015; Yoshida *et al.,*2020).

The regeneration of pancreatic β-cells can be assessed through histopathological examination of pancreatic tissue, alongside immunohistochemical (IHC) analysis for both

semiquantitative and quantitative measurements of insulin-positive cells and proliferating β-cells (Wickramasinghe *et al.,*2021). The current research demonstrated the regeneration of pancreatic β-cells in alloxan-induced animals after a treatment period of 28 days with APE, and the calculated percentage of β-cell regeneration was 47%. This study suggested that the regeneration of β-cells may be due to the presence of polyphenols and flavonoids. Phytochemical analysis of all the plants revealed a rich source of secondary metabolites, such as alkaloids, terpenoids, saponins, glycosides, flavonoids, tannins, and steroids (Rabizadeh *et al.,*2022). Additionally, the in-silico study revealed a strong binding affinity of the verbascoside B compound (identified from APE) with the insulin-like growth factor I (IGF-I) protein, suggesting a potential role in enhancing insulin secretion. This interaction could modulate the IGF-I signalling pathway, which is known to influence pancreatic β-cell function and glucose homeostasis, thereby offering promising therapeutic benefits for managing diabetes. In addition to these other phytoconstituents, the ability of mangiferin identified from APE has been well studied for its ability to regenerate and enhance β-cell function in *in vivo* models (Ansarullah *et al.,*2012; Attanayake *et al.,*2019).

3.5. Conclusion

The current study mainly focused on the development of an antidiabetic polyherbal formulation comprising sixteen plants that are traditionally well known for their antidiabetic properties. Through a systematic evaluation process based on bioactive compound profiles, pharmacological efficacy, and traditional medicinal use, three plants emerged as the most promising candidates. These plants demonstrated superior potential in terms of glucose regulation, antioxidant activity, and overall safety profile. Thus, these plants were selected for further investigation to assess their synergistic effects on diabetes management. The current findings confirm that APE has prominent antidiabetic activity, as evidenced by *in silico*, *in vitro* and *in vivo* studies, by modulating the cellular composition and regenerating the β-cells of the pancreatic islet, leading to sufficient insulin secretion in alloxan-induced diabetic rats. An *in-silico* study revealed that by modulating the IGF-I signalling pathway, this bioactive compound enhanced insulin secretion and pancreatic β-cell function. More studies are needed to elucidate the exact mechanism of action. In addition, our team has commenced studies at the molecular level to determine the primary mechanism and elucidate the method of action by which APE targets a particular protein that is responsible for diabetes.

Chapter-4

Assessing the Antidiabetic Potential of an Ayurvedic Polyherbal Formulation: β-Cell Restoration and Myosin Va Recovery in the Diabetic Pancreas

Abstract

Diabetes Mellitus is now a significant global health challenge. Though, lifestyle modernization and current medications are beneficial, their long-term use raises concerns due to adverse side effects. Hence, modern research focused on natural products from ancient Ayurvedic literature. This study investigates the antidiabetic efficacy of an ayurvedic polyherbal extract and for the first time we studied the differential expression pattern of myosin Va motor protein in the endocrine pancreas of diabetic animals.

The aqueous extract of the Ayurvedic Polyherbal Formulation (APF) was obtained using the Soxhlet apparatus. The phytochemical analysis (FTIR, GC-MS, TPC, TFC), in-vitro radical scavenging activity (DPPH, FRAP, ABTS), in-vitro and in-vivo antidiabetic activities were performed. Additionally, alternation in the cellular architecture and expression of myosin Va in the normal and diabetic pancreatic islets was also done using immunohistochemistry.

APF exhibits a number of bioactive phyto-constituents in the extract identified from FTIR and GC-MS analysis, quantitative analysis exhibits 8.957 mg/ GAE g TPC and 5.786 mg/Q.E. g D.W. TFC. The APF scavenges the free radical at a minimal concentration, which was comparable with the standard control. The enzymatic assay illustrates that APF inhibits the α-amylase and glucosidase enzymes. The blood glucose level and HbA1C levels were significantly improved with the APF treatment; subsequently, restored of the pancreatic β-cells and enhanced the Myosin Va expression in the diabetic pancreas.

The result concluded that the β-cells and myosin Va expression were lost in diabetes but recovered on APF treatment. Though we have not directly examined the molecular mechanism of the restoration of the myosin Va expression for the exocytosis of insulin granules, the expression of Myosin Va may be inferred as a surrogate marker of the secretary potential of the β-cells of the islets of Langerhans.

4.1. Introduction

Diabetes is a major global health concern, with an incidence rate of approximately 537 million and 2.2 million deaths worldwide, and is among the most complex metabolic syndromes associated with hyperglycemia. It is accompanied by an extensive range of comorbidities, such as insulin resistance, decreased insulin production, and disruption of the metabolism of lipids and proteins (Fasolino *et al.,* 2022; Gerber & Rutter, 2017; Segerstolpe *et al.,* 2016). The major risk factors for morbidity and mortality in patients with diabetes are neurovascular complications, retinopathy, and neuropathy. Several synthetic drugs have been used for the management of diabetes, including biguanides, sulfonylureas, thiazolidinediones, and GLP-1 receptor agonists. However, upon long-term use, these synthetic drugs exhibit many adverse side effects, such as cardiovascular risk (associated with sulfonylureas and thiazolidinediones), gastrointestinal complications, and liver and kidney dysfunctions (associated with biguanides, GLP-1 receptor agonists, and thiazolidinediones), which can significantly impact daily activities (Banday *et al.,* 2020; Dilworth *et al.,* 2021a; Galicia-Garcia *et al.,* 2020a; Sarkar & Rajamani, 2022).

Islets of Langerhans, the highly vascularized microorgan of the pancreas, are scattered throughout the exocrine acinus. A significant loss (~80%) of pancreatic β-cells, including myosin Va motor protein in the islets, was observed in patients with diabetes. Hyperglycemia, the causative agent that triggers the apoptosis of β-cells, is also responsible for the reduced expression of myosin Va in islets (Eguchi *et al.,* 2021). Myosin Va plays crucial roles in organelle trafficking, cell motility, muscle contraction, vision, and hearing. In addition, it plays an active role in the exocytosis of insulin granules (Rodriguez & Cheney, 2002).

According to the WHO report, many developing countries (70–80%) rely on traditional methods of treatment involving the use of medicinal plants to manage diabetes. Compared with modern therapy, the traditional method of treatment is the primary choice of interest for many healthcare patients in developing countries (Ansari *et al.,* 2022).The current study focused on elucidating the possible mechanism of the antidiabetic potential of the developed ayurvedic polyherbal formulation by establishing the status of lost pancreatic β-cells in diabetes and the expression pattern of myosin Va in the endocrine regions of the pancreas via a diabetic animal model.

4.2. Materials and methods

4.2.1. Chemicals and reagents

The chemicals and antibodies used in the present study, such as alloxan, metformin, rabbit polyclonal anti-myosin primary antibody, α-amylase and glucosidase, p-NPG, TPTZ, DPPH, ABTS, and potassium persulphate, were obtained from Sigma Aldrich, USA. The secondary antibody and DAB chromogen were manufactured by Pathnsitu, Livermore, California.

4.2.2. Preparation of the ayurvedic polyherbal formulation and extract preparation

The ayurvedic polyherbal formulation (APF) was prepared by mixing three medicinal plants, namely, *Tinospora cordifolia* (stem)*, Syzygium cumini* (bark)*, and Mangifera indica* (seed), in equal proportions. The plants were selected on the basis of their traditional use and ethnopharmacological studies. These medicinal plants were collected from Gandhamardhan Hill, Bargarh district, Odisha, and authenticated by a local taxonomist. The plant parts were collected fresh, dried in a tray dryer and pulverized to powder. The APF was subjected to aqueous extraction via Soxhlet extraction and lyophilized to a powder form.

4.2.3. Phytochemical analysis of APF

4.2.3.1. Fourier transform infrared (FT-IR) spectroscopic analysis

The extracts of *T. cordifolia*, *S. cumini* and *M. indica* were subjected to FT-IR spectroscopic analysis to identify the presence of different functional groups via Bruker Alpha-II FT-IR spectroscope (Gorzsás & Sundberg, 2014). The powder samples were placed on the sample holder and shielded with a black lid to minimize background interference. The spectrometer, set to reflectance mode, captured the spectrum for each sample through 32 scans at 4 cm^{-1} intervals. The resulting average spectrum was then saved in Excel format for further analysis.

4.2.3.2.Gas chromatography–mass spectrometry (GC–MS)

Gas chromatography–mass spectrometry (GCMS) analysis was carried out with a Shimadzu Nexis GC-2030 coupled with a Shimadzu GCMS-QP2020 NX mass spectrometer as described earlier (Babu *et al.,* 2024). An SH-I-5Sil MS ultra inert capillary column of size 30 m × 250 µm × 0.25 µm was used for separation. The elution process was fixed with a pretrained programme, i.e., an initial temperature of 60°C with a hold time of 2 minutes, which subsequently increased to 240°C at a rate of 5°C min^{-1} and a hold time of 15 minutes. With a continuous flow rate of 1.0 ml $min^{-1,}$ the carrier gas (helium)

was passed through the column. The sample (1000 µg/ml) was injected in split mode with a split ratio of 20:1. The mass-selective detector facilitates ion formation via electron impact at 200°C for MS detection. The transfer line was maintained at 280°C, and masses were scanned in the quadrupole from m/z 35 to 400u. The obtained mass spectra were searched on the basis of their percentage probability, score and reverse score for the identification of compounds in the NIST 2020 database (MS Search; NIST, MSS Ltd. Manchester, England).

4.2.3.3. Total phenol and flavonoid contents (TPC & TFC)

The assay was carried out for the developed APF as described in the previous sections 3.2.4.1 and 3.2.4.2.

4.2.3.4.DPPH radical scavenging assay

The DPPH radical scavenging assay of APF was performed as described in section 3.2.5.1.

4.2.3.5. Ferric Reducing Antioxidant Power Assay (FRAP)

Ferric reducing/antioxidant power (FRAP) assays were carried out as described in section 3.2.5.3.

4.2.3.6. ABTS radical scavenging assay

The ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assay of APF was performed as described in section 3.2.5.2.

4.2.4. In vitro enzymatic assay of APF

4.2.4.1. α-Amylase assay

The α-amylase inhibitory activity of APF was determined as described in section 3.2.7.1.

4.2.4.2. α-Glucosidase assay

The α-glucosidase inhibitory assay of APF was performed as described in section 3.2.7.2.

4.2.5. In vivo antidiabetic study of APF

4.2.5.1. Animal maintenance

The animal study was approved by the Institutional Animal Ethical Committee (IAEC) of the Department of Biotechnology & Bioinformatics, Sambalpur University, Burla (vide approval No. SU/BTBI/IAEC/2023/02). Twelve Winstar rats (198±5 g) were

selected, and the animals were maintained for two weeks before the experiment was started under standard laboratory conditions, i.e., 25 ± 2°C and 12 hr. light/dark cycle.

4.2.5.2. Acute toxicity study

The oral acute toxicity study was performed according to the Organization for Economic Cooperation and Development (OECD) guidelines as described in section 3.2.8.2. APF extract was given at doses of 400, 800, 1000, 2000, and 2500 mg/kg body weight.

4.2.5.3.Diabetes development and experimental grouping

The chemical induction of diabetes was performed as described in section 3.2.8.4.

The Group I normal control animals were fed 0.9% saline water; the Group II diabetic control (alloxan-induced) animals were fed 0.9% saline water; the Group III positive control animals were treated with metformin at 10 mg/kg b.w.; and the Group IV treatment control animals were treated with the APF extract at 250 mg/kg b.w. All the animals were treated with their respective drugs for four weeks. After the treatment period, the animals were sacrificed via mild anaesthesia (isoflurane), and the pancreata were collected in 10% neutral buffered formalin (NBF). Blood was subsequently collected from each group via cardiac puncture (Kanhar & Sahoo, 2019).

4.2.5.4. Histopathology and immunohistochemistry study

The processing of pancreata and H&E and IHC staining were performed as described in section 3.2.8.7.

4.2.5.5. Quantification:

The pancreatic islet area and the proportion of β-cells in the pancreas in all the treatment groups were quantified via ImageJ software and compared with those in the control group. The myosin Va intensity of the endocrine region and adjacent acini of all the treatment groups was subsequently calculated and compared with that of the control group via ImageJ software.

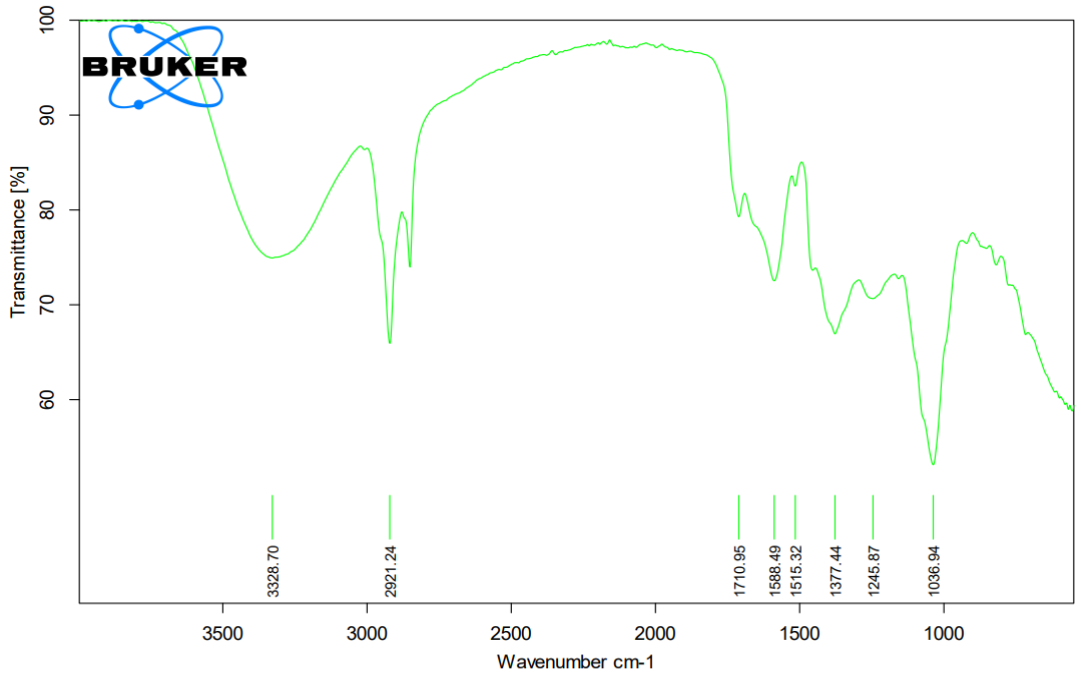
4.3. Results

4.3.1. Phytochemical analysis of APF

4.3.1.1. Fourier transform infrared (FT-IR) analysis

FTIR analysis revealed the presence of ketone, alkane, phenol, and carboxylic acid functional groups in the extracts of the three plants, demonstrating their antidiabetic activity via different mechanisms. In *T. cordifolia*, amides at 3328.70 cm^{-1} (NH stretch) and ketones at 1710.95 cm^{-1} (C=O stretch) contribute to protein synthesis, increase insulin

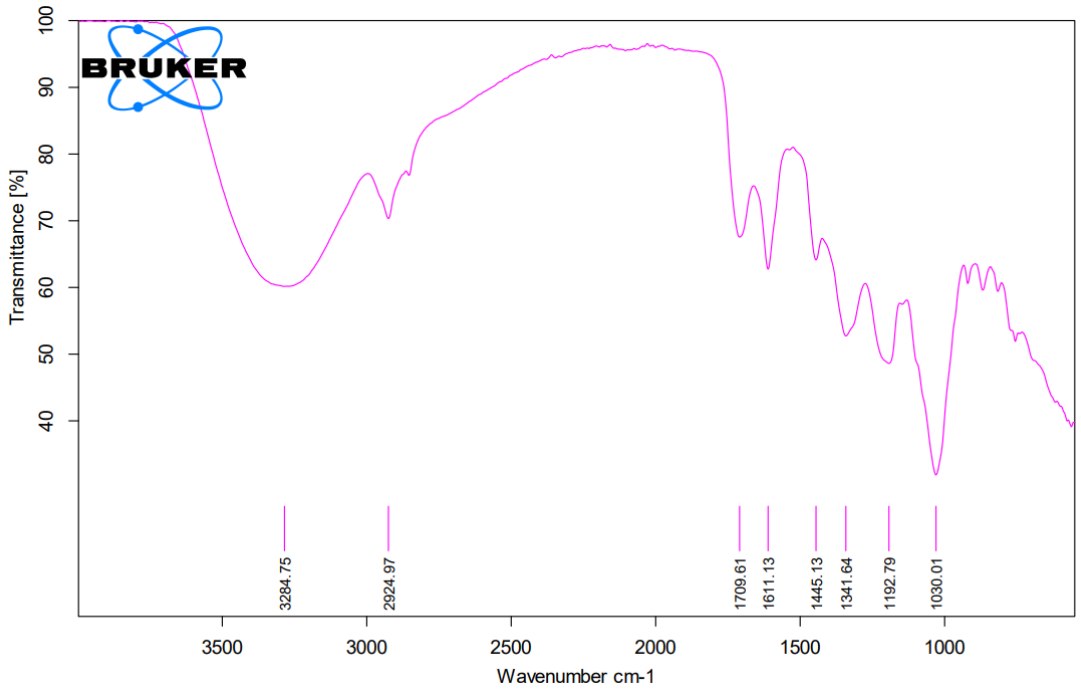
sensitivity, and facilitate glucose metabolism (Figure 4.1. A). For *S. cumini*, phenols at 3284.75 cm^{-1} (ArO-H H bonded), carboxylic acids at 1709.61 cm^{-1} (Dimer C=O), and alkenes at 1611.13 cm^{-1} (C=C stretch) present antioxidant properties, increase insulin secretion, and participate directly in metabolic pathways, respectively (Figure 4.1. B). Additionally, the carboxylic acids at 3257.13 cm^{-1} (Dimer OH) identified in *M. indica* contribute significantly to glucose metabolism (Figure 4.1. C).



A. *T. Cordifolia*

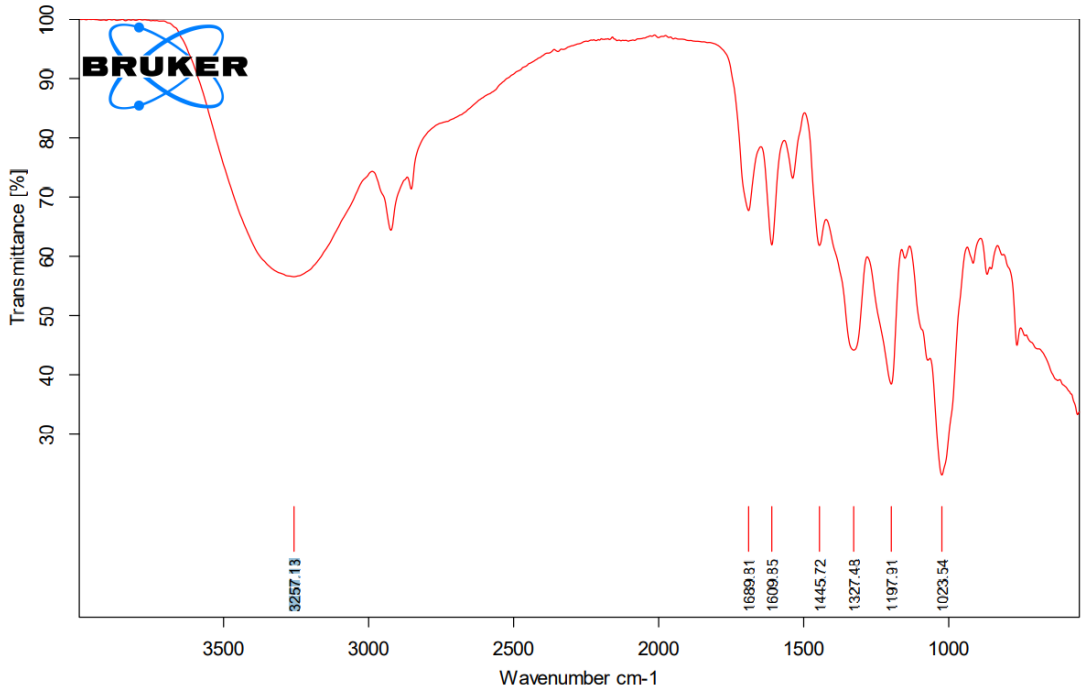
Extract wave no	Class	Functional Assignment
3328.70	Amides	NH stretch (H-bond)
2921.24	Alkanes	CH stretch
1710.95	Ketones	C=O stretch
1588.49	Aromatics	Ar C-C stretch
1515.32	Misc.	Arom. Nitro
1377.44	Misc.	S=O sulphate ester
1245.87	Ethers	C-O stretch

1036.94	Alkyl halides	C-F stretch



B. *S. Cumini*

Extract wave no	**Class**	**Functional Assignment**
3284.75	Phenols	ArO-H H bonded
2924.97	Alkanes	CH stretch
1709.61	Carboxylic acids	Dimer C=O
1611.13	Alkenes	C=C stretch
1445.13	Misc.	S=O sulphate ester
1341.63	Misc.	S=O sulfonic acid
1192.79	Esters	C-O stretch
1030.01	Carboxylic acids	C-O stretch



C. *M. indica*

Extract wave no	Class	Functional Assignment
3257.13	Carboxylic acid	Dimer OH
1689.81	aldehydes	C=O strech
1609.85	amides	NH2 in plane bend
1445.72	Aromatics	Ar C-C stretch
1327.48	Misc.	S=O sulfone
1197.91	Alkyl halides	C-F stretch
1023.54	Misc.	P-OR esters

Figure 4.1: Functional wave of the extract with their respective class and functional assignment.

4.3.1.2.Gas chromatography–mass spectrometry (GC–MS)

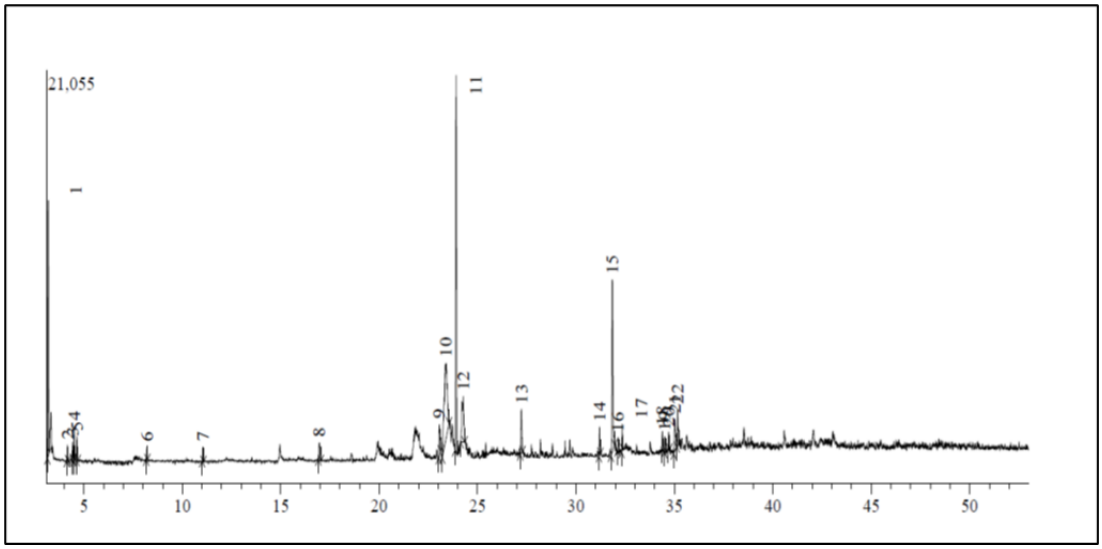
The chromatograms represent the identified compounds from the extracts of the *T. cordifolia*, *S. cumini,* and *M. indica* extracts. The details of the identified phytoconstituents are detailed in the accompanying table below. The chromatograms display the identified

phytochemicals, characterized by their retention times, molecular formulas, and fragmentation patterns (Table 4.1).

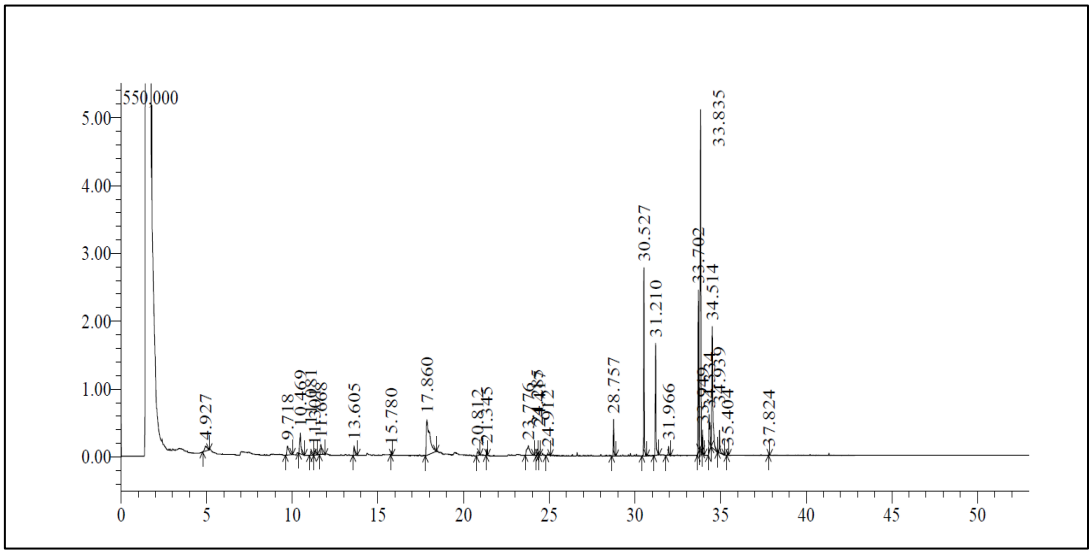
Table 4.1: Identified bioactive phytoconstituents from the plant extract.

Identified compounds from *T cordifolia*				
Compound Name	**Ret Time**	**Area**	**Area %**	**Molecular formula**
Vanillic acid	23.059	5189	2.489	$C_8H_8O_4$
Alpha-l-rhamnopyranose	23.399	41822	20.066	$C_6H_{12}O_5$
Diethyl Phthalate	23.909	48963	23.493	$C_{12}H_{14}O_4$
(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol	27.231	6882	3.302	$C_{10}H_{12}O_3$
n-Hexadecanoic acid	31.846	26232	12.586	$C_{16}H_{32}O_2$
1-Tridecyne	34.386	1820	0.873	$C_{13}H_{24}$
Identified compounds from *S cumini*				
1,2,3-Benzenetriol	17.860	715437	11.142	$C_6H_6O_3$
9,12-Octadecadienoic acid (Z, Z)-, methyl ester (Linoleic acid methyl ester)	33.702	561561	8.745	$C_{19}H_{34}O_2$
Thymol	15.780	13065	0.203	$C_{10}H_{14}O$
9-Octadecenoic acid, methyl ester (E)-(Elaidic acid, methyl ester)	33.949	77571	1.208	$C_{19}H_{36}O_2$
Oleic Acid	34.514	760813	11.848	$C_{18}H_{34}O_2$
n-Hexadecanoic acid	31.210	493112	7.679	$C_{17}H_{34}O_2$
Identified compounds from *M indica*				

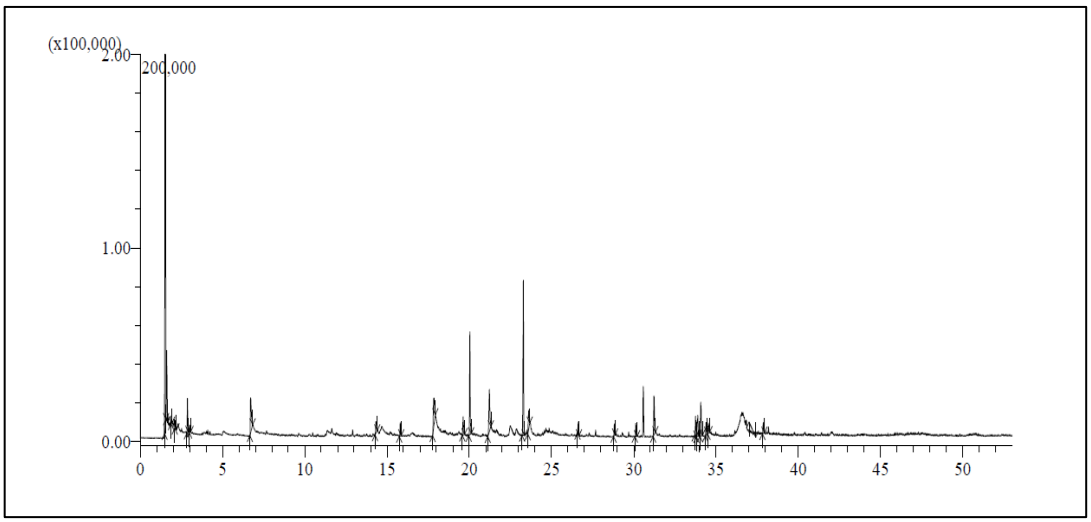
1,2,3-Benzenetriol	15.803	16804	0.520	$C_6H_6O_3$
Quinic acid	23.612	40373	1.250	$C_7H_{12}O_6$
n-Hexadecanoic acid (Palmitic acid)	31.231	60523	1.874	$C_{16}H_{32}O_2$
9,12-Octadecadienoic acid (Z, Z)-, methyl ester (Linoleic acid methyl ester)	33.759	25055	0.776	$C_{19}H_{34}O_2$
Phytol	34.075	50212	1.555	$C_{20}H_{40O}$



T. cordifolia



S. cumini



M. indica

Figure 4.2: GC–MS chromatograms of *T. cordifolia*, *S. cumini,* and *M. indica* plant extracts.

By reviewing both the analytical analysis (FTIR and GC–MS) results, we planned to study the antidiabetic potential of the mixture of three plants. The developed polyherbal formulation leverages the synergistic effects of the phytoconstituents, and enhancing the antidiabetic activity more effectively than individual plant extracts do.

4.3.1.3. Total phenolic and flavonoid contents

The phenolic (TPC) and flavonoid (TFC) contents of the plant increased its biological activity, including anticarcinogenic, antioxidant, antimutagenic, free radical inhibition, oxidative stress, and anti-inflammatory activity. The total phenolic and flavonoid contents of APF were calculated to be 8.957 mg/GAE g and 5.786 mg/Q.E. g D.W., respectively (Figure 4.3).

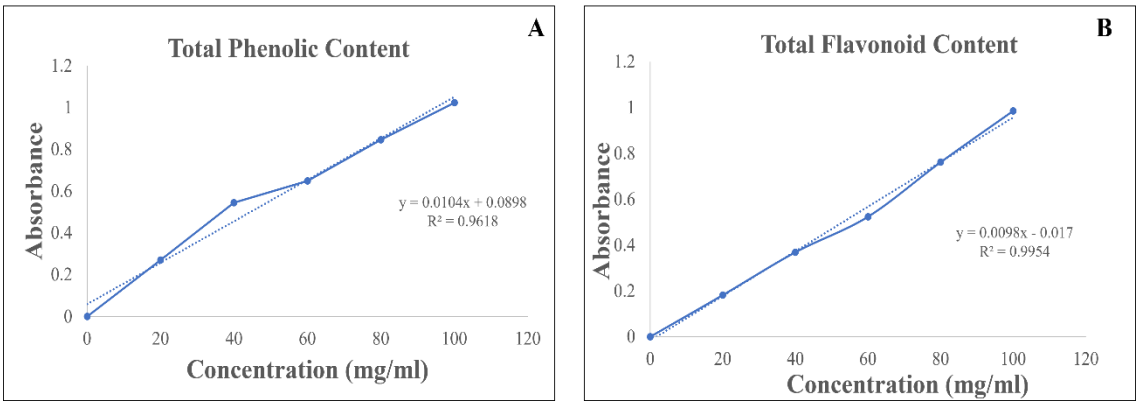


Figure 4.3: Standard curves for TPC and TFC. APE resulted in high TPC and TFC contents, which are responsible for the high free radical scavenging activity.

4.3.1.4. Free radical scavenging activity

Free radical scavenging activity is a crucial measure for evaluating the antioxidant potential of compounds. In the present study, we measured the free radical scavenging activity, including DPPH, FRAP and ABTS, of the APF extract. The calculated IC_{50} values for free radical scavenging activity via DPPH, FRAP and ABTS were 27.07, 41.31, and 25.92 µg/ml, respectively (Figure 4.4).

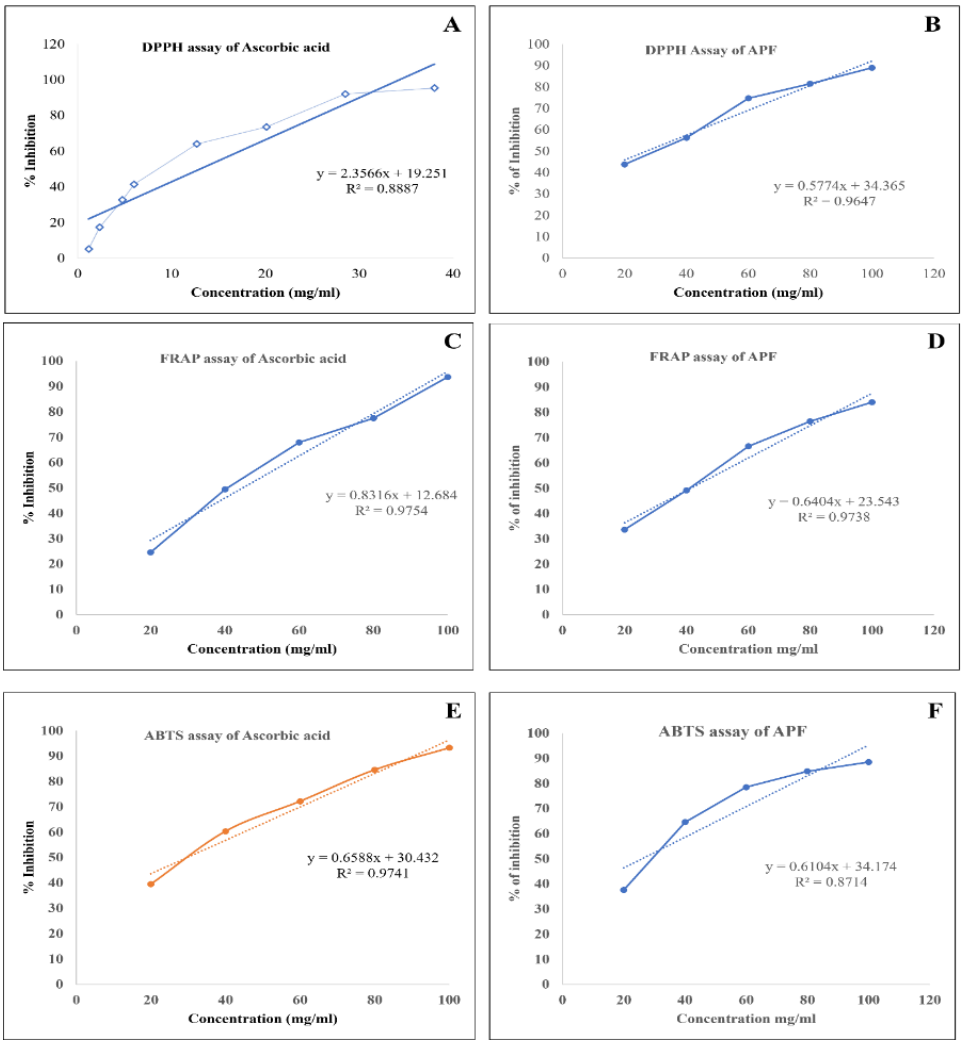


Figure 4.4: Free radical scavenging activity of standard ascorbic acid (A, C, E, G) and APF extracts (B, D, F).

4.3.2. In vitro enzymatic assay of APF

In vitro antidiabetic assays are essential for evaluating the potential of extracts to manage diabetes. These assays typically focus on mechanisms such as the inhibition of carbohydrate-hydrolysing enzymes, the enhancement of insulin secretion, and the protection of pancreatic β-cells. The calculated IC_{50} values of APF for inhibiting α-amylase and α-glucosidase activity were 42.92±0.24 and 30.60±0.17 µg/ml, respectively (Figure 4.5), which were comparable with those of the standard control.

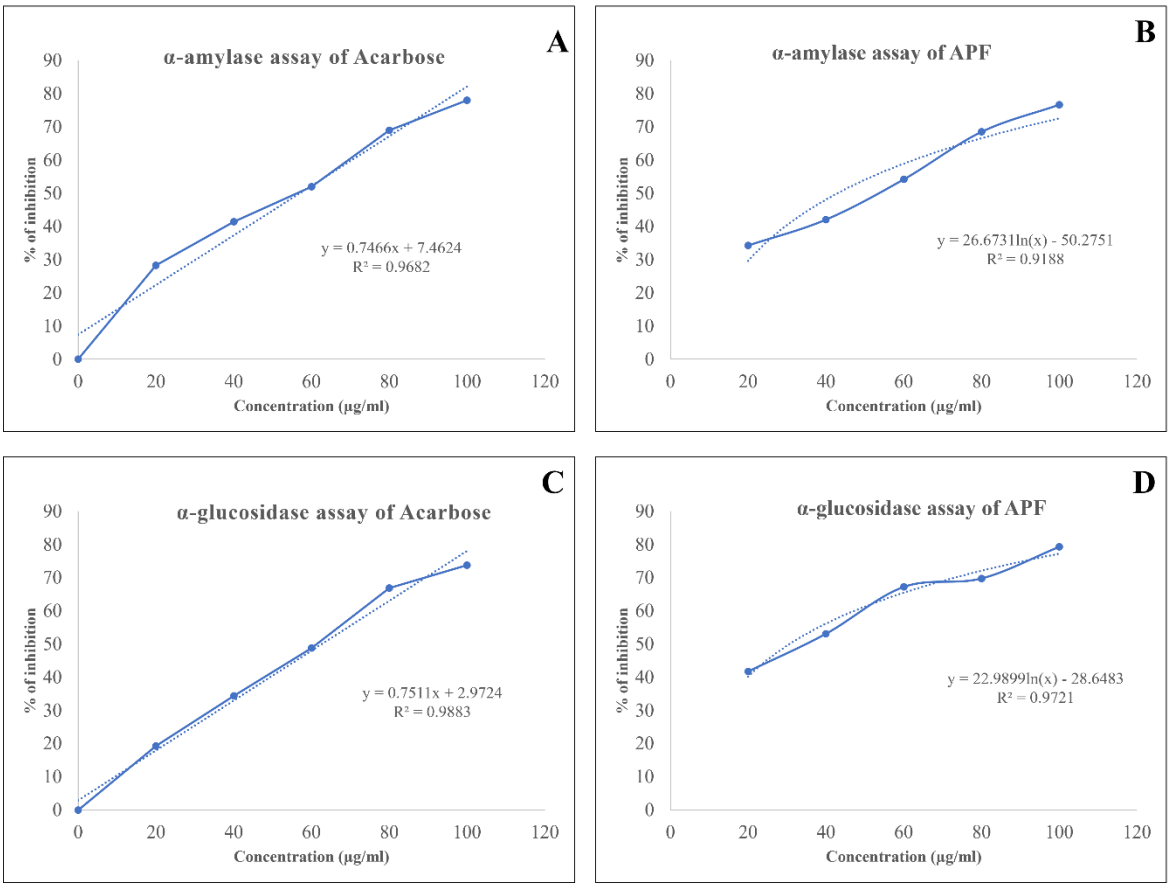


Figure 4.5: α-Amylase and α-glucosidase activities of APF. The results revealed that significant inhibition of both enzymes prevented an increase in the blood sugar level.

4.3.3. In vivo antidiabetic study of APF

4.3.3.1. Acute toxicity study

The acute toxicity study suggested that no mortality was observed in the animals from groups I-III at their respective doses. In contrast, groups IV and V had mortality rates of 2/6 and 3/6, respectively. As a 50% mortality rate was observed at 2500 mg/kg body weight, the lethal dose (LD_{50}) was 2500 mg/kg body weight. The effective dose of APF extract was calculated to be 250 mg/kg b.w. and was subsequently administered to diabetic animals.

4.3.3.2. Effects of APF on blood sugar levels and HbA1c levels

Biochemical parameters such as blood sugar and HbA1c levels were measured in all groups of animals and compared with those in the control group. The blood sugar level of the normal control group was 106±2.3 mg/dl, whereas the diabetic control group had a significantly higher level of blood sugar, i.e., 478±3.57 mg/dl. The blood sugar level of the APF-treated group decreased to 117±3.03 mg/dl, which was comparable to that of the metformin-treated (113 ± 1.56 mg/dl) group (Table 4.2 A).

The HbA1c level was reported to be 4.94±0.31% in the normal control group, whereas a significant increase (17.76±0.42%) was observed in the diabetic control group ($p<0.001$). Upon the administration of APF extract, the HbA1c level significantly decreased to 5.92±0.19% ($p<0.001$), which was comparable to that of the metformin-treated group (5.21±0.1%) (Table 4.2 B).

Table 4.2: Increases in blood glucose and HbA1c levels upon the induction of diabetes and the corresponding treatment groups.

A. Blood glucose levels	
Normal control	106 ± 2.3 mg/dl
Diabetes control	478 ± 3.57 mg/dl
Positive control (Metformin-treated)	113 ± 1.56 mg/dl
Treatment control (APF -treated)	117 ± 3.03 mg/dl
B. HbA1C Levels	
Normal control	4.94 ± 0.31%
Diabetes control	17.76 ± 0.42%
Positive control (Metformin-treated)	5.21 ± 0.1%
Treatment control (APF -treated)	5.92 ± 0.19%

4.3.3.3. Histomorphological studies

The islet size in the different groups varied upon the induction of diabetes and the respective treatments. The islet size in the normal control group ranged from 130–200 µm in diameter; however, the diabetic control group showed a significant decrease in islet size, ranging from 60–80 µm, compared with the normal control group ($p<0.001$). Comparatively larger islets (ranging from 70–95 µm) were observed in the metformin-treated group than in the diabetic control group. This relatively large islets in the metformin-treated group may be due to the proliferation of nonβ cells (such as α- and δ cells). Compared with the control group, the APF-treated groups presented significantly greater islet sizes ranging from 110–185 µm ($p<0.001$) (Table 4.3 A). The significant increase in islet size may be due to the synergistic effects of the APF extract on the regeneration of pancreatic β-cells.

The normal control group presented a mantle core architecture of the cell subtype (total islets and β-cells), whereas the diabetic control group presented destruction of β-cells by the chemical alloxan and its byproduct dialuric acid via reactive oxygen species (ROS). The β-cell proportion of the normal islets was 64%. In contrast, the diabetic control group exhibited complete destruction of β-cells in the islet area. The positive (metformin-treated) control group revealed negative staining for insulin (β-cells) in the islet, suggesting its inability to regenerate lost β-cells. The treatment control (APF-treated) groups showed regeneration of the lost pancreatic β-cells; the calculated proportion of β-cells was 49.07% (Figure 4.6, Table 4.3B).

Table 4.3: Size of islets and proportion of pancreatic β-cells in the experimental group.

A. Islet's size	
Group	**Size of islets**
Normal control	130-200 µm
Diabetes control	60-80 µm
Positive control (Metformin-treated)	70-95 µm
Treatment control (APF-treated)	110-185 µm
B. Regeneration of β-cells	
Group	**Proportion of β-cells**
Normal control	64%
Diabetes control	-
Positive control (Metformin-treated)	-
Treatment control (APF-treated)	49.07%

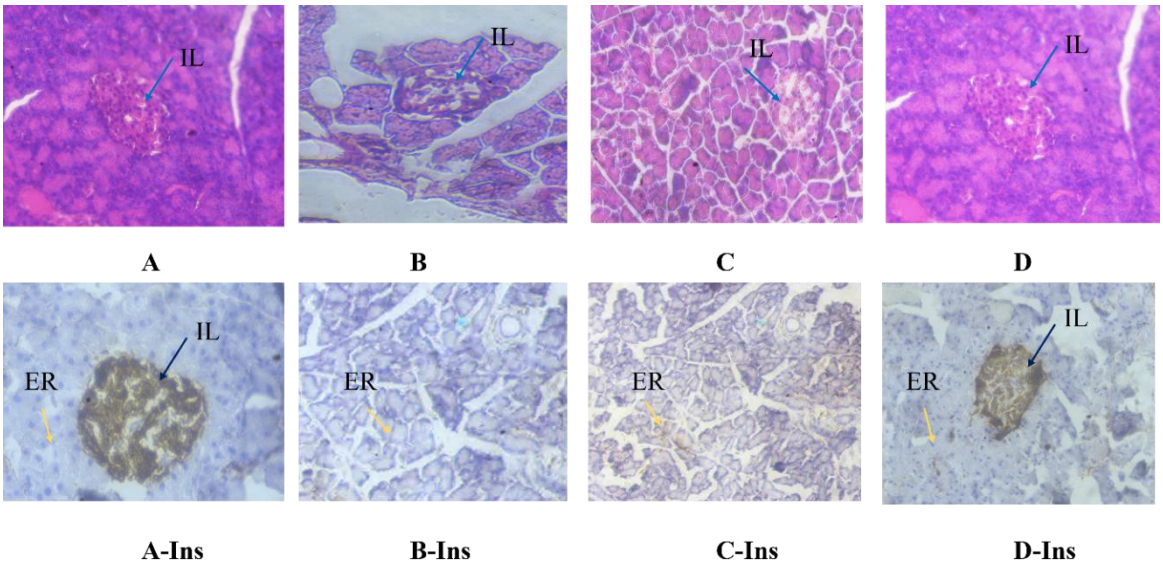


Figure 4.6: Photomicrographs of H&E (A-D) and IHC (anti-insulin) staining of all the experimental groups (A-ins–D-ins). A. Normal, B. diabetic, C. metformin-treated, D. APF-treated group. IL-Islets of Langerhans and ER: Exocrine region of pancreas.

IHC staining with the anti-myosin antibody of the pancreatic islets revealed that the normal group presented the customary intensity of myosin Va, with a higher intensity in the islets (70.285 ± 2.38); in contrast, the diabetic group presented a significant decrease in the intensity of myosin Va in the endocrine region (11.265 ± 2.15, $p<0.001$). This may be due to the destruction of insulin-secreting pancreatic β-cells. Compared with the diabetic control group, the APF-treated group presented a significant increase in myosin intensity (57.082 ± 1.28, $p<0.001$). Conversely, the islets in the metformin-treated group (13.720 ± 1.75) were significantly less intense than those in the normal control group ($p>0.001$); however, the intensity in the metformin-treated group was slightly greater than that in the diabetic control group (Figure 4.7, Table 4.4).

During diabetes, the endocrine region of the pancreas is primarily affected. However, hyperglycemic conditions do not directly impact the exocrine region of the pancreas. Regardless of diabetes status or treatment, the exocrine region had no effect on the intensity of myosin Va, with the calculated mean intensity of the exocrine region being 30.81 (Table 4.4).

Table 4.4: Varying intensities of myosin Va in the endocrine and exocrine regions of the experimental group.

Group	**Myosin intensity in Endocrine region**	**Myosin intensity in Exocrine region**
Normal control	70.285 ± 2.38	29.482 ± 1.07
Diabetes control	11.265 ± 2.15	32.652 ± 1.96
Positive control (Metformin-treated)	13.720 ± 1.75	31.008 ± 1.46
Treatment control (APF-treated)	57.082 ± 1.28	30.840 ± 2.07

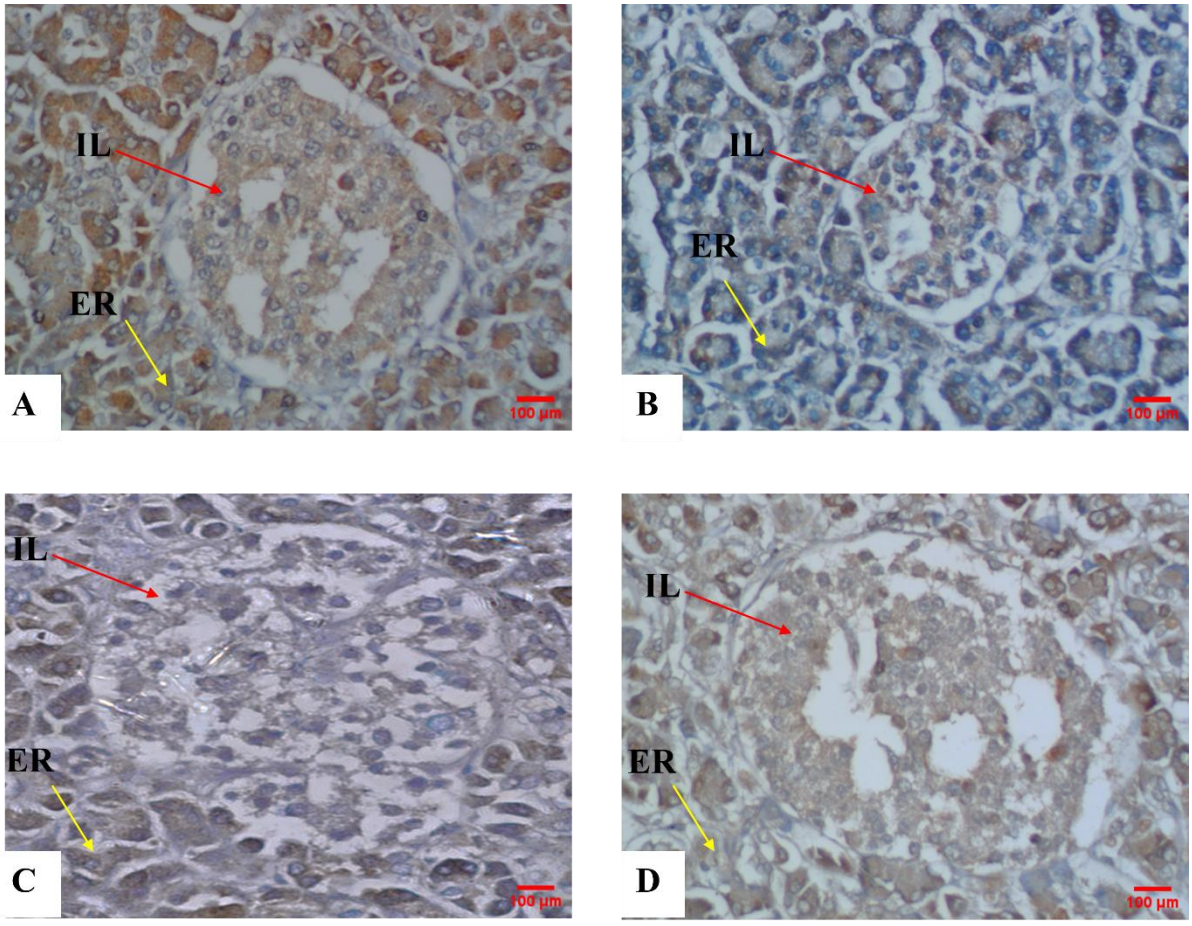


Figure 4.7: Photomicrograph of the IHC staining (anti-myosin) of all the experimental groups (A-D). A. Normal, B. diabetic, C. metformin-treated, D. APF-treated (the herbal-treated group shows a restoration of myosin Va expression after treatment).

NB: IL-Islets of Langerhans and ER: Exocrine region of the pancreas.

4.4. Discussion

Despite significant advancements in diabetes treatment, including islet transplantation, *in situ* regeneration of pancreatic β-cells remains a formidable challenge, highlighting the need for continued research and innovation in this critical area. The insulin-secreting pancreatic cell subtype (β-cell) is crucial for maintaining blood glucose levels. Functional deficits (any alteration or impaired insulin secretion) in these β-cells are key driving factors in the progression of diabetes. A recent *in vivo* antidiabetic study suggested that insulin resistance in muscles is responsible for impaired insulin secretion and destabilizes β-cell compensation via glucose and lipid toxicity. Over time, type II DM becomes type I DM, suggesting severe β-cell dysfunction and insulin dependence, forming a clinical scenario that is similar to type I DM, which requires insulin therapy (Saisho, 2014, 2015; Standl, 2007).

Researchers have identified a number of active compounds that not only decrease hyperglycemic conditions but are also capable of regenerating pancreatic β-cells and restoring their functions. Among the diverse array of antidiabetic medicinal plants, many have the potential to regenerate pancreatic β-cells in diabetes. Plants such as *Madhuca longifolia*, *Marsilea quadrifolia*, *Nigella sativa*, *Sarcopoterium spinosum*, and *Triticum aestivum* have already been tested in cellular and diabetic animal models (Aguayo-Mazzucato & Bonner-Weir, 2018; Fiorina *et al.,* 2008; Rajab, 2010; Shapiro *et al.,* 2016). In this context, the developed APF has the potential to lower blood glucose levels and promote the regeneration of lost pancreatic β-cells. This restoration potential of APF may be due to the synergistic interactions between phytoconstituents such as alkaloids, phenols, and flavonoids. This finding aligns with previously published literature on reducing blood sugar levels via *in vivo* models (Rudolf, 2023). FTIR analysis revealed the presence of functional groups such as ketones, alkanes, phenols, and carboxylic acids, which exhibit antidiabetic properties via different mechanisms (Nie & Cooper, 2021). GC–MS analysis revealed the presence of phytoconstituents such as vanillic acid, n-hexadecanoic acid, tetradecanoic acid, 12-methyl ester, and tridecanoic acid methyl ester in the extract, which influence lipid metabolism by reducing lipid accumulation and insulin sensitivity and improving lipid profiles, subsequently enhancing the function of pancreatic β-cells (Ganesan *et al.,* 2024). In addition, the identified phytochemicals, such as 1,2,3-benzoenetriol (pyrogallol) and 9,12-octadecadienoic acid (oleic acid methyl ester), help restore pancreatic β-cells and enhance their functions (Karakose *et al.,* 2018).

Limited literature has reported the expression of myosin Va in the diabetic pancreas. For the first time, we evaluated the regeneration of pancreatic β-cells and the differential

expression patterns of myosin Va both in together in the diabetic pancreas. Controlled exocytosis of secretory vesicles is a key insulin release mechanism. The myosin Va motor protein plays a crucial role as a regulator in the exocytosis of insulin granules. Previous scientific evidence on the role of myosin in exocytosis revealed that Myo Va facilitates the movement of large dense core vesicles (LDCVs) towards the final stage of exocytosis by disrupting the direct connections between myosin Va and the actin filament. The exocytosis of secretory vesicles involves a complex network of actin filaments facilitating their movement towards the plasma membrane (Bittins *et al.,* 2009; Rudolf *et al.,* 2011; Sun *et al.,* 2014). Additionally, herbal products influence the molecular regulation of pancreatic β-cells, leading to normal insulin secretion. In both the normal, diabetic, and treatment control groups, myosin Va intensity had no effect on the exocrine pancreas. This is because DM is primarily associated with dysfunction of the endocrine pancreas. DM may affect the exocrine pancreas through fibrosis, metaplasia, and other structural alternations rather than changes in myosin Va expression (Wright *et al.,* 2024). These results suggest that the developed APF specifically impacts the endocrine pancreas by enhancing insulin secretion.

Owing to this integral role of Myosin Va in the tracking of secretory vesicles from the above literature, we emphasized the differential expression pattern of Myo Va in the pancreata of the normal, diabetic, and treatment control groups. Long-term oxidative stress leads to the degradation of myosin molecules. The current findings regarding the variable expression pattern of myosin Va aligned with the previous results, i.e., a high intensity of myosin Va was observed in the endocrine region of the normal control group of animals. In contrast, a significant decrease was observed in the diabetic control group of animals. This substantial decrease in the endocrine region was due to hyperglycemic conditions, the ROS produced by alloxan, and its byproduct, dialuric acid, which directly leads to the selective destruction of pancreatic β-cells. A previous study suggested that, compared with that in the normal control group of animals, the expression pattern of myosin in the muscular and nerve termini in diabetic patients was significantly lower or that myosin was not expressed in these areas. Additionally, myosin expression was significantly lower in the brains of diabetic animals than in those of normal control animals. The results revealed that the loss of myosin may be due to increased protein levels or organelle dysfunction because of hyperglycemic conditions (Chaudhury *et al.,* 2014; Da Costa *et al.,* 2011; Kögel *et al.,* 2010).

Compared with the diabetic control group, the metformin-treated group presented a larger islet size, which may be due to the proliferation of non-beta cells in the endocrine

pancreas. A previous study suggested that these non-beta cells proliferate in response to various stimuli, such as injury and metabolic stress (H. Huang *et al.,* 2021). However, a significant increase in the islet size in the APF-treated group was observed, possibly due to the regeneration of pancreatic β-cells (Yin *et al.,* 2006).

Compared with the diabetic control group, the metformin-treated group presented a slightly greater intensity of myosin Va. However, the APF-treated group presented significantly greater myosin Va intensity than did the diabetic control group. This result signifies that metformin is insulin sensitive but does not impact the functions of lost pancreatic β-cells. The regeneration of pancreatic β-cells after treatment with herbal drugs may be due to neogenesis (differentiation of β-cells from ductal or extra pancreatic precursor cells) or trans-differentiation (conversion of alpha cells or other endocrine cells to β-cells). However, the myosin Va expression pattern in the exocrine pancreas was not affected by diabetes or treatment. These findings demonstrate that the drug's action is specific to endocrine cells. Myosin molecules are present in both endocrine (including alpha cells) and exocrine cells of the pancreas. The concentration of myosin does not increase in individual cells; rather, its appearance is observed in newly regenerating cells. Previous studies on the expression of myosin have suggested that it is highly expressed in endocrine tissues such as the pituitary gland, adrenal medulla, pineal gland, and islets of Langerhans, and also been reported that myosin Va helps in the transportation of insulin granules towards the exocytosis process (Espindola *et al.,* 2008). In the APF-treated group, hyperglycemic conditions were reduced, and immunohistochemistry (IHC) (anti-insulin) confirmed the regeneration of lost pancreatic β-cells upon administration. Therefore, the myosin Va motor protein may help in the transportation of β-cells toward the final stage of exocytosis and help normal insulin secretions maintain high blood sugar levels. Previous studies on the role of myosin in exocytosis emphasized that, in association with the cortical actin network, myosin helps the movement of dense core secretory vesicles (chromaffin cells, neuroendocrine cells, and insulin granules) toward the final stage of exocytosis (Espindola *et al.,* 2008; Jacobs *et al.,* 2009).

The differential expression pattern of myosin Va in the experimental group revealed that it was directly associated with the transportation of insulin granules within the endocrine region toward the final stage of exocytosis and controlled hyperglycemic control. Previous studies have shown that the function of myosin Va is the same, i.e., it involves the transport of different secretory granules, such as chromaffin cells (which help in neurotransmitter secretion), the intracellular transport and maintenance of cellular

structure and functions, and the movement of insulin granules within pancreatic β-cells towards the plasma membrane and the pigment granules of melanocytes and the smooth endoplasmic reticulum of neurons (Brozzi *et al.,* 2012; Espindola *et al.,* 2008; Rosé *et al.,* 2003; Waselle *et al.,* 2003). Islet transplantation has attracted considerable interest in the management of diabetes. Scientific evidence suggests that the transplantation of islets leads to insulin independence and improved glucometabolic control, with a high success rate of approximately 60%. In recent decades, the transplantation technique and related outcomes have significantly improved (Rudolf *et al.,* 2011).

The strategy of selection was aimed at narrowing down the most promising candidates for detailed single-plant evaluations, providing a clearer understanding of their therapeutic contributions. Consequently, single plants, *Tinospora cordifolia* and *Mangifera indica,* were chosen for further study on the basis of their ethnopharmacological importance, and the antidiabetic efficacy of these plants has been documented in the literature.

4.5. Conclusion

The preclinical studies conducted on the animal model have provided valuable insight into the potential of the ayurvedic polyherbal formulation as an alternative source of medication. The biochemical parameters revealed a significant reduction in blood glucose levels and restoration of HbA1c levels in the APF-treated group. In the IHC study, anti-insulin staining revealed the ability of APF to restore pancreatic β-cells, and anti-myosin staining revealed a differential expression pattern in the endocrine region of the experimental pancreatic tissue. As we did not directly study the molecular approaches of the restoration of myosin Va molecules for exocytosis of insulin granules, the increased expression of myosin Va in the endocrine region may be a surrogate marker of the secretory potential of the β-cells of the islets of Langerhans. Furthermore, research should focus on the molecular mechanism behind the reported results, specifically the involvement of myosin Va in the exocytosis of pancreatic β-cells. Clinical studies are needed to determine the effectiveness and safety of APF in humans. Investigating the impact of the formulation on diabetic complications, such as neuropathy and retinopathy, may offer a more complete picture of its therapeutic potential.

Chapter 5

Ethnopharmacological importance of *Tinospora cordifolia* in blood sugar regulation via *in vitro* and *in vivo* studies in a rodent model

Abstract

Diabetes mellitus is a chronic metabolic disorder, affecting millions globally and rising at an alarming rate. Ethnopharmacological approaches have emerged as an adjunct therapy due to their complementary approach in managing various chronic health conditions, including diabetes. Giloy, botanically identified as *Tinospora cordifolia*, holds a revered status in traditional medicinal systems in controlling blood sugar levels.

FTIR, GC-MS analysis, and quantitative assessment (TPC, TFC) were utilized to confirm the presence of bioactive compounds in *T. cordifolia* extract. Enzyme inhibitions and lipid accumulation assay were carried out in *in-vitro* study to examine its glucose lowering effect. Diabetic model was developed using alloxan in Wistar rats, followed by treatment with *T. cordifolia* extract. H&E and IHC staining was done to study the microarchitecture of pancreatic islets.

T. cordifolia extract showed the presence of several bioactive compounds as identified from FTIR and GC-MS analysis; subsequently, with quantified TPC and TFC. The extract inhibited digestive enzymes and enhanced the lipid accumulations, with IC_{50} values of 80.55 µg and 81.67 µg/ml, respectively. The loss of the pancreatic β-cells proportion in diabetes, as observed in *in vivo* study, was reversible with *T. cordifolia* treatment.

The inhibitions of digestive enzymes and improved lipid accumulation were attributed to secondary metabolites like phenol and alkaloids in the *T. cordifolia* extract. Significant restoration of pancreatic β-cells was observed in the extract treated group compared with the control group. *T. cordofolia* holds promise as an adjunct therapy option for diabetes.

5.1. Introduction

Diabetes mellitus represents a persistent metabolic irregularity characterized by reduced insulin secretion, actions, or a blend of both (Galicia-Garcia *et al.,*2020). Hyperglycemia, a hallmark feature of diabetes, is the primary instigator of numerous detrimental consequences linked with this condition, including disruptions in glucose and lipid metabolism and alterations in hepatic enzyme activity (Dilworth *et al.,*2021). The global burden of diabetes mellitus has increased, and the adverse side effects of synthetic drugs underscore the urgency of exploring alternative medicines with fewer/no side effects (Sugandh *et al.,*2023). Among its plethora of medicinal benefits, one that has garnered significant attention is its purported antidiabetic potential (Gupta *et al.,*2024).

Tinospora cordifolia, commonly known as Giloy, stands in traditional medicinal systems such as Ayurveda, Siddha, and Unani for its therapeutic properties (Saha & Ghosh, 2012). In conventional medicine, various parts of this plant (stem, roots, and leaves) are applied for adaptogenic, immunomodulatory, antioxidant, anti-inflammatory, and antidiabetic properties (Anjum *et al.*,2023; Sankhala *et al.*,2012). This plant is rich in phytochemicals such as alkaloids, diterpenoids, glycosides, steroids, flavonoids, and polysaccharides (Upadhyay *et al.*,2010), which subsequently constitute valuable resources in natural medicine (Joladarashi *et al.*,2011). Previous studies have explained the antidiabetic potential of this plant; however, further research, including well-designed preclinical and clinical trials, is needed to validate these findings and fully elucidate the underlying mechanisms (Saha & Ghosh, 2012).

The current study primarily examines the antidiabetic potential of *T. cordifolia* by evaluating its ability to increase insulin sensitivity and suppress lipid biosynthesis in target cells, as well as alterations in the cellular microarchitecture of pancreatic islets in a diabetic animal model.

5.2. Materials and methods

5.2.1. Consumables

The chemicals used in this study, such as DMEM, FBS, penicillin/streptomycin, alloxan, metformin, and β-glucosidase, were obtained from Sigma Aldrich, USA. The primary antibodies, such as anti-glucagon from Bioss, USA, the primary antibody anti-insulin, the secondary antibody, and the DAB chromogen, were obtained from Pathnsitu, California. Xylene, propanol, the DPX mounter, and $FeCl_3$ were procured from Merck, India.

5.2.2. Sample Collections

The stem of the plant was collected from Gandhamardhan Hill, Bargarh District, Odisha, in January 2024. The plants were validated and identified by a local taxonomist. The live sample of the plant species was grown in the herbal garden of Sambalpur University, Odisha, India. The freshly collected plant parts were dried in a tray dryer and passed through a pulverizer. The powder sample was subjected to hydroalcoholic (70%) extraction via a microwave digestion system, followed by lyophilization.

5.2.3. Phytochemical analysis

5.2.3.1. Fourier transform infrared (FT-IR) analysis

FTIR analysis of the hydroalcoholic extract of *T. cordifolia* was performed as described in section 4.2.3.1.

5.2.3.2.Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis of the hydroalcoholic extract of *T. cordifolia* was carried out as described in section 4.2.3.2.

5.2.3.3. Total phenolic and flavonoid contents

The total phenolic and flavonoid (TPC & TFC) contents of the extracts were calculated as described in section 4.2.3.3. & 4.2.3.4.

5.2.3.4.DPPH radical scavenging activity

The DPPH free radical scavenging activity of the extract was determined as described in section 4.2.3.4.

5.2.4. In vitro study

5.2.4.1. α-Amylase enzymatic assay

The α-amylase inhibitory assay was performed as described in section 4.2.4.1.

5.2.4.2. β-Glucosidase enzymatic assay

For β-glucosidase activity, 50 µl of 100 mM phosphate buffer, 10 µl of β-glucosidase solution (1 U/ml), and 20 µl of different concentrations of extract (1 mg/ml) were mixed properly, and the reaction mixture was incubated for 15 min at 37°C. After the incubation period, 20 µl of 5 mM P-NPG was added and incubated for another 30 min. Then, 0.1 M Na_2CO_3 was added to terminate the reaction mixture, and the absorbance was measured at 405 nm via a microplate reader (Bio-Rad). Acarbose was used as a standard control (Tamil *et al.,*2010). The result was calculated as a percentage of inhibition via the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control-Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

5.2.4.3. Cell culture and maintenance

The 3T3-L1 adipocyte line was maintained as described in section 3.2.7.3.

5.2.4.4. Cytotoxicity assay

The cytotoxicity assay of *T. cordifolia* extract was performed as described in section 3.2.7.4.

5.2.4.5.Lipid accumulation assay

The lipid accumulation assay was conducted as previously described (Lahrita *et al.,*2015). Briefly, after the preadipocytes were differentiated into adipocytes, the cells were treated with various concentrations of *T. cordifolia* extract for 48 hours. Oil red O staining was performed to detect the accumulated lipid droplets. After incubation, the cells were washed with ice-cold PBS and stained with Oil Red O (ratio) for 20 mins. The cells were subsequently washed with PBS and dissolved in ethanol to remove the excess stain. The optical density (OD) was measured via a microplate reader (Bio-Rad) at 520 nm.

5.2.5. In vivo antidiabetic study

5.2.5.1. Animal maintenance

The animals were maintained prior to the experiment as described in section 4.2.5.1.

5.2.5.2. Acute toxicity

The acute toxicity of the *T. cordifolia* extract was evaluated orally as described in section 4.2.5.2.

5.2.5.3.Oral subacute toxicity

The oral subacute toxicity study was performed as described in section 3.2.8.3.

5.2.5.4. Induction of diabetes

The chemical induction of diabetes in animals was developed as described in section 4.2.5.3.

5.2.5.5. Antidiabetic evaluation of T. cordifolia

Group I consisted of normal control animals fed 0.9% saline water. The diabetic animals were randomly divided into three groups: Group II, the untreated diabetic control (given 0.9% saline water); Group III, the positive control (treated with 10 mg/kg b.w. metformin); and Group IV, the treated control (treated with 250 mg/kg b.w. *T. cordifolia* extract). The animals were orally administered the drug or the plant extracts for four weeks. Before dosing, blood glucose levels were measured every other day. At the end of the experiment, the animals were sacrificed via mild anaesthesia. Blood samples were collected via cardiac puncture. Pancreatic tissues from each group were dissected and harvested in 10% neutral buffered formalin (Dra *et al.,*2019).

5.2.5.6. Histopathological and immunohistopathological studies

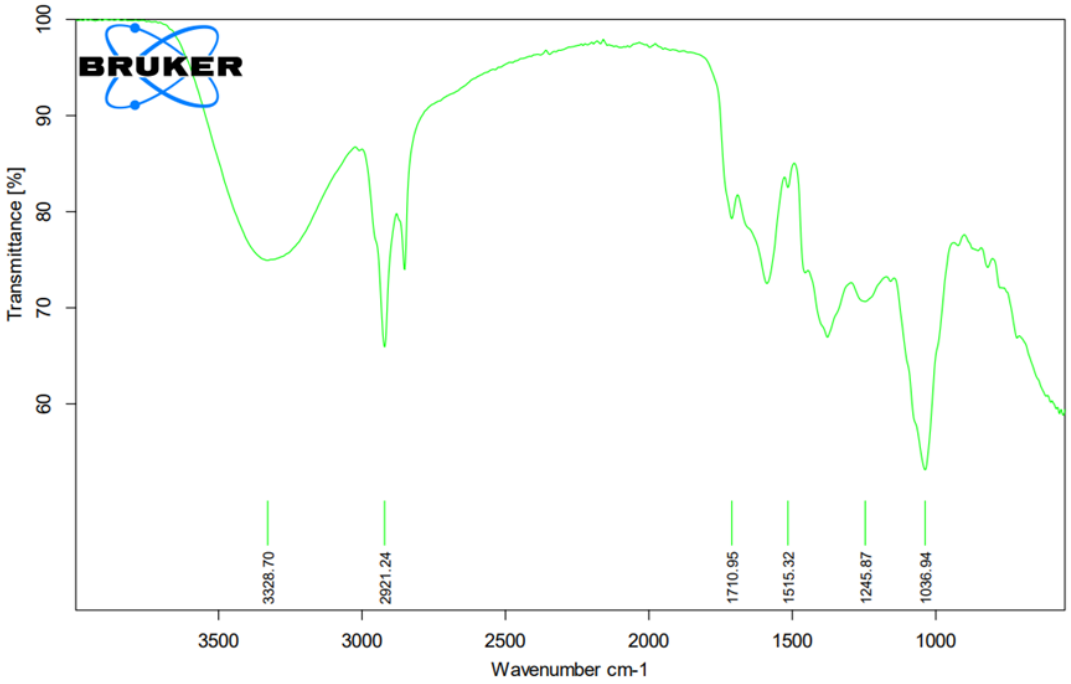
H&E staining of vital organs and H&E and IHC staining of the pancreatic islets were performed as described in section 4.2.5.4.

5.3. Results

5.3.1. Phytochemical analysis

5.3.1.1. FTIR analysis

The FTIR analysis of the *T. cordifolia* extract revealed a number of diverse functional groups with diverse biological activities. The key peaks included amides (NH stretch) and alkynes (C-H stretch) at 3328.70 cm^{-1}, alkanes (CH stretch) at 2921.24 cm^{-1}, ketones (C=O stretch) at 1710.95 cm^{-1}, and various others, such as ethers (C-O stretch) and alkyl halides (C-F stretch).



T. Cordifolia

Extract Wave no	Class	Functional Assignment	Class	Functional Assignment
3328.70	Amides	NH stretch (H-bond)	Alkynes	C-H stretch
2921.24	Alkanes	CH stretch	Alkanes	CH_2
1710.95	Ketones	C=O stretch	Carboxylic acids	Dimer C=O
1515.32	Misc.	Arom. Nitro	Misc	N=O nitroso
1245.87	Ethers	C-O stretch	Esters	C-O stretch
1036.94	Alkyl halides	C-F stretch	Amines	C-n stretch

Figure 5.1: FTIR chromatograms and identified functional groups from the *T. cordifolia* extract.

*5.3.1.2.*Gas chromatography–mass spectrometry (GC–MS)

A representative base chromatogram of the hydroalcoholic extract of the *T. cordifolia* plant is presented in Figure 5.2, and the identified phytoconstituents are listed in Table 5.1. Chromatograms depict the identified phytochemicals on the basis of their characteristic retention time, molecular formula, and fragmentation pattern.

Table 5.1: Compounds identified from the GC–MS analysis of the *T. cordifolia* extract.

Peak No	Compound Name	Ret Time	Area	Area %	Mechanism action of antidiabetic properties
1.	2,2-Dimethoxybutane	3.190	26305	12.621	-

2.	Methyl vinyl ketone	4.417	1651	0.792	-
3.	3,3-Dimethoxy-2-butanone	4.504	3196	1.534	-
4.	2,2,4-Trimethyl-3-pentanone	8.197	1102	0.529	-
5.	Butanoic acid, 1,1-dimethyl ethyl ester	11.037	1546	0.742	-
6.	Ethanone, 1-(2-hydroxy-5-methyl phenyl)-	16.946	3337	1.601	-
7.	Vanillic acid	23.059	5189	2.489	Inhibitions of α-amylase, glucosidase and β-glucosidase activity
8.	Alpha-l-rhamnopyranose	23.399	41822	20.066	Protects the pancreatic β-cells
9.	Diethyl Phthalate	23.909	48963	23.493	Protects the pancreatic β-cells
10.	β-D-Glucopyranose, 4-O-. beta. -D-alactopyranosyl	24.275	14728	7.067	-
11.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol	27.231	6882	3.302	Inhibitions of α-amylase, glucosidase and β-glucosidase activity
12.	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	31.201	2848	1.366	-
13.	n-Hexadecanoic acid	31.846	26232	12.586	Enhance lipid metabolism.
14.	1-Tridecyne	34.386	1820	0.873	Inhibitions of α-amylase, glucosidase & β-glucosidase activity, and trypsin phosphate 1B protein
15.	1-Dodecyn-4-ol	34.519	2258	1.084	-
16.	Tridecanoic acid, methyl ester	35.021	5752	2.760	-
17.	Carbonic acid, isobutyl cyclohexyl methyl ester	35.161	5078	2.437	-

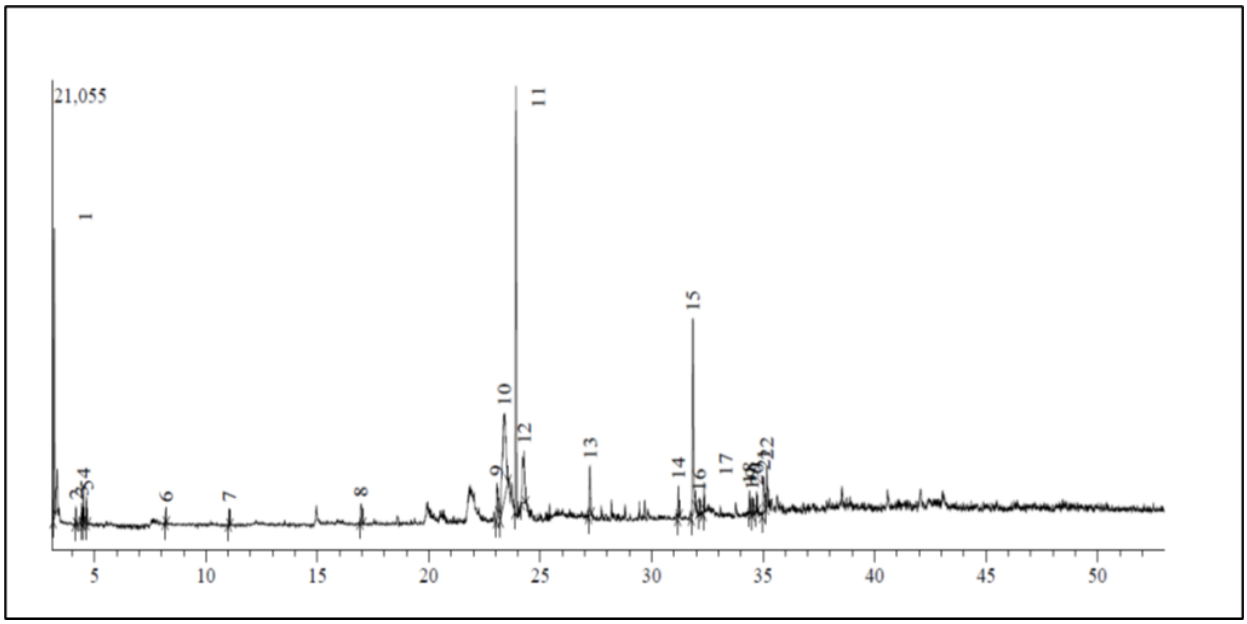


Figure 5.2: Chromatograms obtained from the GC–MS analysis of the *T. cordifolia* plant extract.

5.3.1.3. Phenolic and Flavonoid contents

The phenol and flavonoid contents in the extract are responsible for the antioxidant activity, which is subsequently crucial for different biological activities. The extracts of *T. cordifolia* contained 27.85 mg/g GAE D.W. and 4.138 mg/QE phenol and flavonoid, respectively. The yield was calculated from the standard gallic acid and quercetin calibration curves (Figure 5.3).

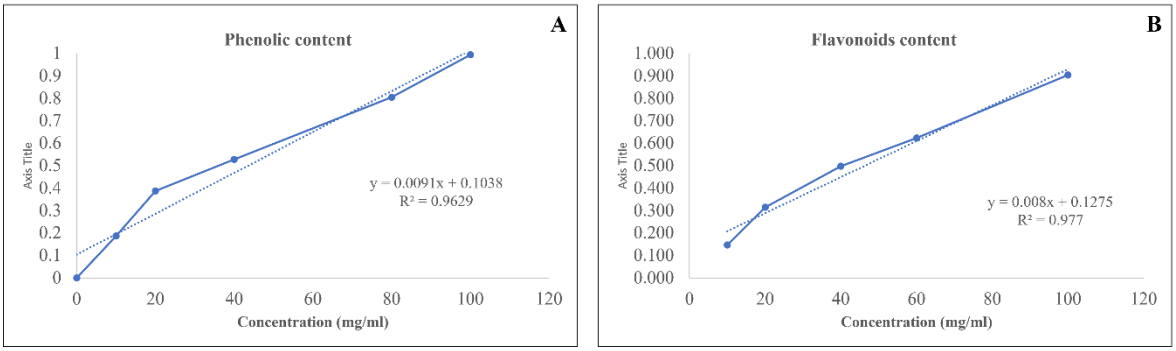


Figure 5.3: Standard calibration curves of gallic acid (A) and quercetin (B).

5.3.1.4. DPPH scavenging activity

The assessment of the antioxidant activity of a plant extract is crucial because of its direct ability to protect pancreatic β-cells from oxidative damage. The *T. cordifolia* extract scavenged free radicals at a minimal concentration, and the calculated IC_{50} was 13.122 µg/ml, which was comparable with the standard ascorbic acid, which has an IC_{50} value of 5.40 µg/ml (Figure 5.4).

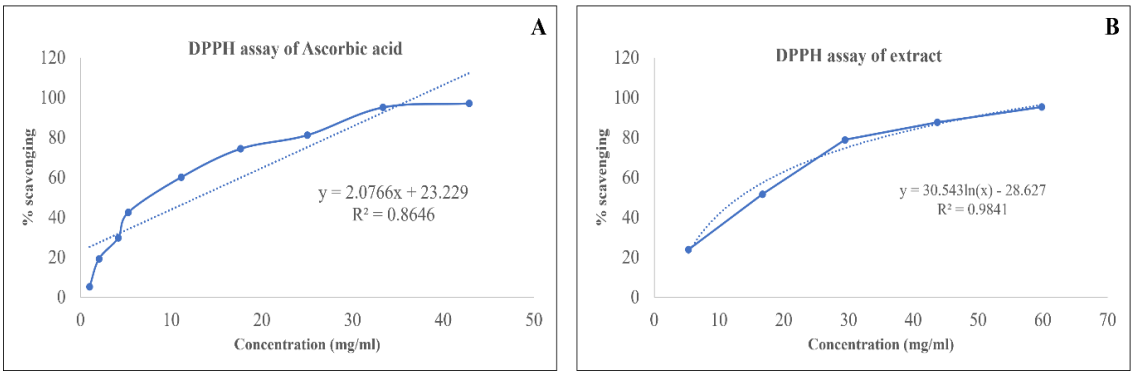


Figure 5.4: DPPH radical scavenging activity of the standard (A) and *T. cordifolia* extracts (B).

5.3.2. *In vitro* study

5.3.2.1. Enzyme inhibition assay

The hydroalcoholic extract of *T. cordifolia* inhibits α-amylase enzymes. The highest inhibition of the extract was calculated to be 78%. The calculated IC_{50} value for the extract was 80.55 µg/ml, and that of the standard was 49.32 µg/ml. Similarly, the extract showed dose-dependent inhibitory activity for the β-glucosidase enzyme at 50% or more. The highest inhibitory activity was calculated to be 96.62%. The calculated IC_{50} value for *T. cordifolia* was 81.67 µg/ml, and that for acarbose was 44.36 µg/ml (Figure 5.5).

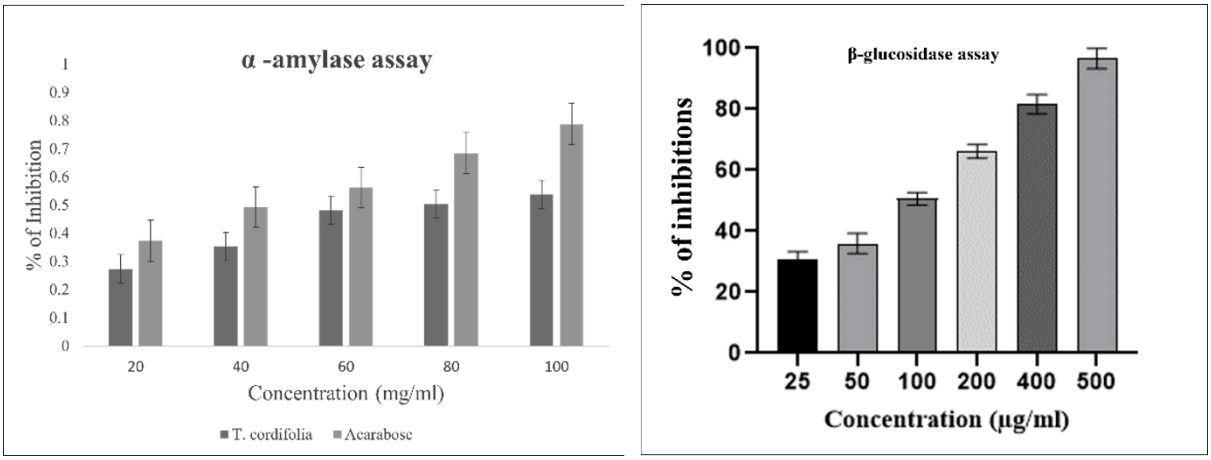


Figure 5.5: Enzyme inhibition assay of the *T. cordifolia* extract. **A.** α-Amylase inhibition assay and **B.** β-glucosidase inhibition assay of the extract.

5.3.2.2. Cytotoxicity assay

The MTT results indicate the noncytotoxic effects of the *T. cordifolia* extract, even at relatively high concentrations. The calculated IC_{50} value was 618.97 µg/ml, indicating the nontoxic effect of the plant extract at a relatively high concentration (Figure 5.6).

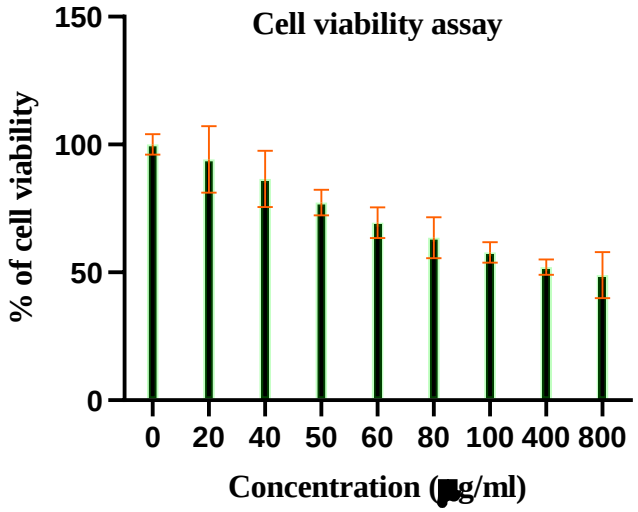


Figure 5.6: Cell viability assay using 3T3-L1 *T. cordifolia* extract.

5.3.2.3. Lipid accumulation

T. cordifolia extract inhibited lipid accumulation in a dose-dependent manner. The calculated IC_{50} value was 48.68 µg/ml, indicating its enhanced efficacy in reducing lipid accumulation and inhibiting adipogenesis (Figure 5.7).

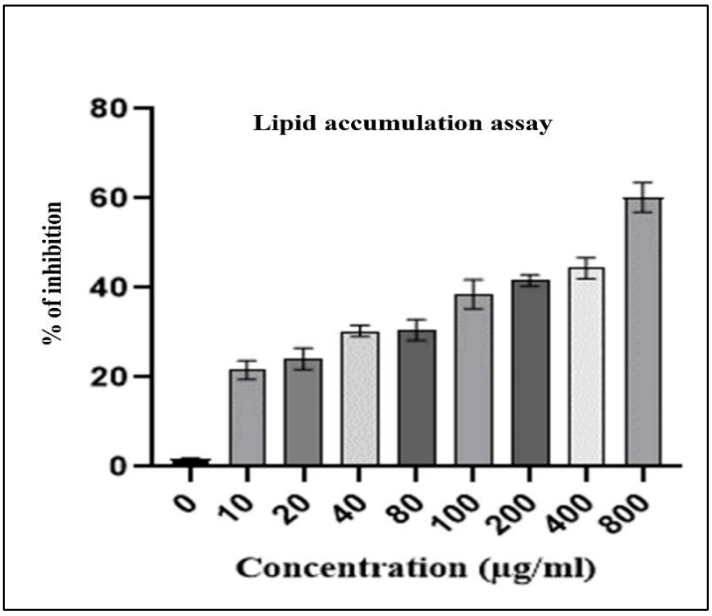


Figure 5.7: Dose-dependent inhibition of lipid accumulation by the extract

5.3.3. In vivo antidiabetic study

5.3.3.1. Acute toxicity assessment

The orally administered *T. cordifolia* extract at a single dose of 5000 mg/kg b.w. did not cause any symptoms of toxicity or mortality in the treated animals. All the treated

animals exhibited normal behaviours and no clinical alternations throughout the experimental period.

5.3.3.2.Oral subacute toxicity assessment

The subacute toxicity of the *T. cordifolia* extract did not induce any death or toxicity symptoms in the animals. Throughout the experiment, no significant clinical changes or mortality were observed. The physiochemical parameters, such as body weight, food and water intake (Table 5.2), haematological parameters (Table 5.3), and biochemical parameters (Table 5.4), did not significantly differ from those of the normal control group. The observed haematological and biochemical parameters were within the reference range.

H&E staining of the examined vital organs revealed no pathological alterations. The brain tissue showed intact neuronal structures without signs of inflammation or necrosis. Similarly, the kidney tissue displayed normal glomerular and tubular architecture, indicating the absence of nephrotoxicity. Liver sections exhibited well-preserved hepatic cells with no evidence of hepatocellular damage or fibrosis. Heart tissue also appeared normal, with no signs of myocardial injury or inflammation. The lung tissue exhibited healthy alveolar structures without inflammation, fibrosis, or cellular necrosis (Figure 5.8).

Table 5.2: Physiochemical parameters of normal and *T. cordifolia* extract-treated animals.

Body Weight (in gm)		
Days	**Control**	**Treated**
1	194.7±6.26	206.2±10.3
7	196.9±6.45	208.5±10.27
14	198.8±7.09	210.4±9.94
21	201.3±6.46	212±11.67
28	202.8±6.4	214.7±11.83
Food intake (in gm)		
1	14.76±1.69	16.93±2.78
7	14.82±1.86	17.09±2.88
14	15.7±2.33	16.09±2.32
21	14.76±1.69	15.32±1.9
28	14.76±1.69	15.48±2.02
Water intake (in ml)		
1	18.42±4.68	22.1± 2.78
7	21.13±2.55	21.76±2.6

14	21.74±2.42	21.92±2.36
21	21.8±2.37	21.33±1.39
28	22.18±2.74	19.11±4.06

Table 5.3: Haematological parameters of normal and *T. cordifolia* extract-treated animals.

Parameters	Normal Control	*T. cordifolia* treated	Ref Range
WBC (10^3/L)	5.25±1.07	5.84±0.77	3.50 – 10.00
Neutrophils (10^3/L)	2.87±1.09	4.73±1.17	1.60 - 7
Lymphocytes (10^3/L)	2.63±0.75	2.15±0.34	1-3
Monocytes (10^3/L)	0.45±0.19	0.51±0.1	0.20 – 0.80
Eosinophils (10^3/L)	0.3±0.11	0.37±0.05	0.00 – 0.50
Basophil (10^3/L)	1.3±3.56	0.12±0.04	0.00 – 0.15
NLR	1.89±0.19	1.98±0.32	1-3
PLR	0.02±0	0.06±0.08	0.017-0.028
RBC (10^12/L)	5.09±0.3	5.16±0.45	4.20-6.0
Haemoglobin (g/dL)	14.84±0.59	14.02±0.9	13-17
HCT	41.74±1.52	43.88±2.55	39-52
MCV (fL)	83.86±2.02	84.12±3.96	76 - 100
MCH (pg)	28.71±1.05	29.58±2.21	26 - 34
MCHC (g/L)	33.23±1.02	33.66±0.82	32-35
RDW-CV	13.26±0.78	14.09±0.95	11-16
RDW-SD (fL)	41.18±3.94	41.56±2.73	37 - 49
PLT (10^3/L)	239.5±38.65	261.4±46.25	150 - 400
PCT (mL/L)	0.28±0.05	0.28±0.05	0.15 - 0.40

Table 5.4: Biochemical parameters of normal and *T. cordifolia* extract-treated animals.

Parameter	Normal Control	*T. cordifolia* treated	Ref range
Glucose (GLU) (mg/dl)	108.33±5.9	95.4±9.36	74 - 143
Albumin (ALB) (g/dl)	3.03±0.12	2.97±0.44	2.3 - 4.0
Urea (UREA) (mg/dl)	27.83±6.32	33.81±3.04	15 - 58
Creatinine (CREA) (mg/dl)	0.88±0.17	1.2±0.42	0.5 - 1.8
Cholesterol (CHOL) (mg/dl)	161.53±13.82	132.5±13.24	109 - 202

Triglycerides (TG) (mg/dl)	95±7.87	103.3±20.68	40 - 165
Alanine Transaminase (ALT) (U/L)	41.03±14.53	90.52±16.83	10 - 125
Aspartate Aminotransferase (AST) (U/L)	23.36±6.74	22.27±15.17	0 - 50
Total Protein (TP) (g/dl)	6.38±0.57	5.74±1.22	5.2 - 8.2
Magnesium (MG) (mg/dl)	1.61±0.08	1.65±0.25	1.5 - 2.1
Phosphorus (PHOS) (mg/dl)	6.38±0.42	4.39±0.79	3.0 - 6.20
Calcium (CA) (mg/dl)	9.32±1.56	9.66±1.08	8.7 -11.8
Direct Bilirubin (DBIL) (mg/dl)	0.2±0.01	0.32±0.07	0 - 0.5
Total Bilirubin (TBIL) (mg/dl)	0.52±0.08	0.41±0.29	0 - 0.9
High-density Lipoprotein (HDL) (mg/dl)	57.8±6.98	56.06±3.72	35 - 88
Gamma-glutamyl Transferase (GGT) (U/L)	5.61±0.34	4.24±1.71	0 – 10
Alkaline Phosphatase (ALP) (U/L)	175.1±31.72	55.65±29.7	0.1 - 212

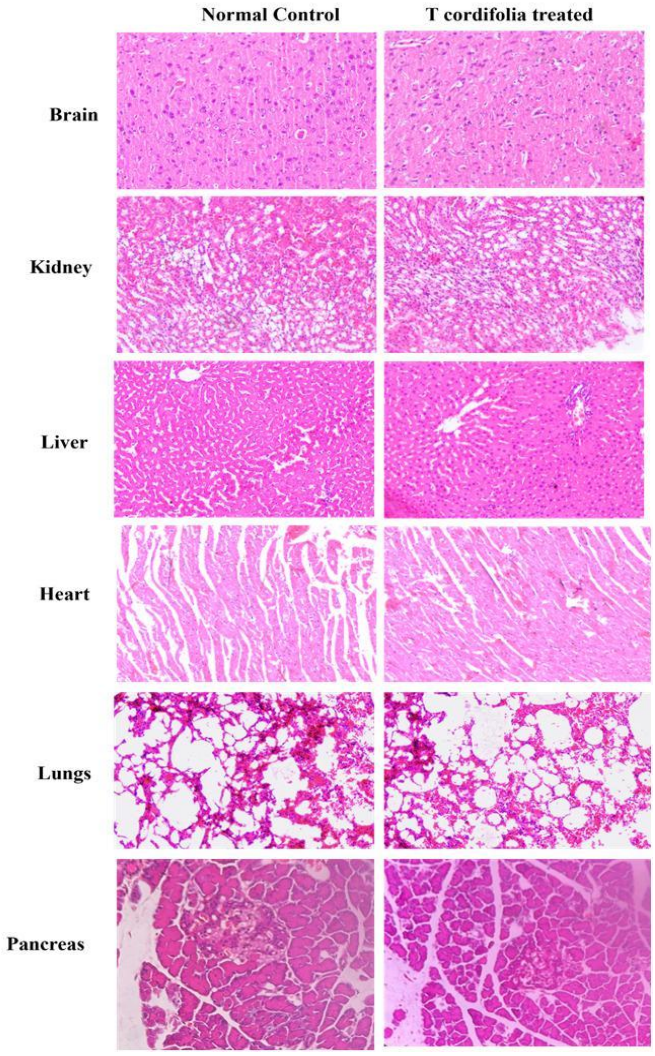


Figure 5.8: Assessment of the toxicity of *T. cordifolia* extract in various organs, including the brain, kidney, liver, heart, and pancreas, in both the normal control and treated groups. H&E staining revealed no significant changes or toxicity symptoms. Additionally, H&E staining of the pancreatic islets in the experimental group revealed no alterations in cell subtype architecture.

5.3.3.3. Effects of extract on physiochemical parameters and HbA1c levels

A comparative study of body weight and blood glucose levels in different experimental groups, included in Table 5.4 below, provides critical insights into the antidiabetic efficacy of *T. cordifolia*. Moreover, the trends in blood glucose levels offer indirect insights into long-term glucose control. A significant decrease in body weight was observed in the untreated diabetic control group (123 ± 1.06 g) compared with the normal control group (207 ± 0.92 g) ($p<0.001$). However, the body weights of the geese in the treatment group markedly improved after four weeks. The positive control (metformin-treated) group and the extract-treated group had mean body weights of 211 ± 1.11 g and 209.3 ±1.24 g ($p<0.001$), respectively (Table 5.5A). This illustrates the positive effects of the treated drugs on physiological parameters.

A significant increase in blood glucose levels ($p<0.0001$) was observed in the untreated control group (492 ± 0.98 mg/dl) compared with the normal control group (111 ± 2.32) ($p<0.0001$). In contrast, the metformin-treated group (114 ± 4.67) and extract-treated group (118 ± 5.35) presented markedly lower blood glucose levels (Table 5.5B). A substantial reduction in blood glucose levels was observed in both treatment groups, exemplifying their antidiabetic efficacy.

Table 5.5. Comparative study of body weight and blood glucose levels in different experimental groups on different days.

A. Body weight (in gm)					
Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	190 ± 2.4	195 ± 1.02	198 ± 3.21	201 ± 1.02	207 ± 0.92
Untreated Diabetic	225 ± 5.65	213 ± 2.12	198 ± 1.02	156 ± 3.12	123 ± 1.06
Metformin treated	231 ± 3.54	218 ± 4.54	202 ± 1.78	206 ± 4.43	211 ± 1.11
Cordifolia treated	191.6 ± 2.47	141.3 ± 2.8	170.3 ± 6.42	205 ± 3.55	209.3 ±1.24
B. Blood glucose levels (in mg/dl)					
Normal control	98 ± 4.09	100 ± 2.34	103 ± 4.01	108 ± 1.23	111 ± 2.32
Untreated Diabetic	107 ± 2.12	223 ± 6.7	322 ± 3.33	468 ± 2.09	492 ± 0.98

Metformin treated	111 ± 3.44	354 ± 1.02	336 ± 1.33	203 ± 5.78	114 ± 4.67
Cordifolia treated	106 ± 3.02	258 ±	183 ± 4.12	142 ± 9.27	118 ± 5.35

5.3.3.4. Effects of extract on biochemical parameters

The biochemical estimation of different serum markers in the experimental groups, as detailed in Table 5.6, provides a comprehensive overview of the physiological impacts of *T. cordifolia* treatment on diabetic conditions. The parameters measured highlight the metabolic and organ-specific effects of *T. cordifolia*, offering insights into its therapeutic efficacy. The albumin levels were markedly higher in the untreated diabetic control group (5.81 ± 0.487 g/dl) than in the normal control (2.41 ± 0.39 g/dl), positive control (2.59 ± 0.51 g/dl) and extract-treated groups (2.1 ± 0.54 g/dl). Elevated albumin levels in untreated diabetic controls indicate potential renal dysfunction, which is a common complication of diabetes. *T. cordifolia* treatment helps maintain albumin levels, suggesting its protective role against diabetic nephropathy. The urea and creatinine levels were significantly lower in the untreated diabetic control group than in the normal control group (9.16±2.16 and 26.42±1.74 mg/dl and 44.27±2.67 and 1.346±0.08 mg/dl, respectively), but the treatment groups (positive and extract-treated) recovered (49.89 ±3.49 & 1.38±0.15 mg/dl and 34.17 ± 2.38 & 0.56±0.20 mg/dl, respectively). Cholesterol levels were substantially lower in the untreated diabetic control group (7.316 ± 4.09 mg/dl) than in the normal control group (119.93±3.65 mg/dl). However, upon treatment, it was reversed to near that of the normal treatment control group, i.e., for the positive control (118.41±3.24 mg/dl) and treatment (112.48±2.43 mg/dl) groups. Triglyceride levels were significantly greater in the untreated diabetic control group (184.88 ± 3.00 mg/dl) than in the normal control group (134.16±12.89 mg/dl). The treated group significantly recovered (positive control, 115.41±10.94 mg/dl; treated control, 45.185 ± 3.72 mg/dl) and was comparable with the normal control. These results indicate the lipid-lowering effects of *T. cordifolia*, which can help mitigate cardiovascular risks associated with diabetes. The total protein level in the treated group (6.03 ± 1.44 g/dl) was within the normal range (5.2 - 8.2 g/dl), indicating overall nutritional balance.

HbA1c levels provide a long-term view of glucose control. The treated group showed a significant reduction of 6.5% from the untreated diabetic control group at 18.7%, closely matching the normal control group (5.56%) and the positive control group (6.16%). These findings indicate that *T. cordifolia* reduces immediate blood glucose levels and helps control long-term glycemic conditions. Hence, the biochemical estimations indicated that

T. cordifolia has a broad spectrum of beneficial effects on various metabolic and organ-specific parameters under diabetic conditions. Its ability to normalize glucose levels, improve liver and kidney function, and regulate lipid profiles underscores its potential as a comprehensive antidiabetic therapy.

Table 5.6. Biochemical estimation of different serum markers in the experimental groups

Parameter	Normal control	Untreated Diabetes	Positive control	Treated	Range	Unit
Glucose (GLU)	128.9 ± 3.86	243.8 ± 8.43	98.74 ±7.48	78.51 ± 4.46	74.0 - 143.0	mg/dl
Albumin (ALB)	2.41 ± 0.39	5.81 ± 0.487	2.59 ±0.51	2.1 ± 0.54	2.3 - 4.0	g/dl
Urea (UREA)	44.27 ± 2.67	9.16 ± 2.16	49.89 ±3.49	34.17 ± 2.38	15.0 - 58.0	mg/dl
Creatinine (CREA)	1.346 ±0.08	26.42 ± 1.74	1.38 ±0.15	0.56 ±0.20	0.5 - 1.8	mg/dl
Cholesterol (CHOL)	119.93 ±3.65	7.316 ± 4.09	118.41 ±3.24	112.48 ±2.43	109.0 - 202.0	mg/dl
Triglycerides (TG)	134.16 ±12.89	184.88 ± 3.0	115.41 ±10.94	45.185 ±3.72	40 - 165	mg/dl
Alanine Transaminase (ALT)	80.56 ±3.74	154.95 ± 2.9	107.81 ±6.51	105 ±2.94	10 - 125	U/L
Aspartate Aminotransferase (AST)	21.45 ±3.12	324.3 ± 4.57	34.01 ±2.27	25.36 ±2.08	0 - 50.0	U/L
Total Protein (TP)	6.1 ±0.95	2.81 ± 0.55	5.68 ±0.69	6.03 ±1.44	5.2 - 8.2	g/dl
Magnesium (MG)	2.28 ±0.36	0.566 ± 0.23	1.63 ±0.18	1.08 ±0.54	1.50 - 2.10	mg/dl
Phosphorus (PHOS)	4.26 ±0.45	48.08 ± 4.60	4.16 ±0.32	4.91 ±4.65	3.00- 6.20	mg/dl
Calcium (CA)	8.83 ±0.22	71.96 ± 4.00	9.154 ±0.45	9.253 ±0.25	8.70- 11.80	mg/dl
Direct Bilirubin (DBIL)	0.891 ±1.74	4.08 ± 0.52	1.975 ±0.29	0.413 ±0.012	0 - 0.50	mg/dl
Total Bilirubin (TBIL)	0.085 ±0.09	10.7 ± 0.32	0.028 ±0.01	0.318 ±0.13	0 - 0.90	mg/dl
High-density Lipoprotein (HDL)	61.66 ±2.1	384.4 ± 55.4	68.26 ±1.40	64.18 ±28.43	35.0 - 88.0	mg/dl
Gamma-glutamyl Transferase (GGT)	1.88 ±0.27	12.65 ± 0.68	1.185 ±0.48	2.3 ±0.77	0 - 10.0	U/L
Alkaline Phosphatase (ALP)	17.82 ±0.88	254.4 ± 10.9	35.55 ±4.70	7.83 ±0.58	0.1 - 212.0	U/L

5.3.3.5. Histopathological Study

Immunohistochemical (IHC) analysis of pancreatic islets from different experimental groups revealed that the islets were stained with anti-synaptophysin and anti-insulin antibodies (Figure 5.9). Panels NC-TC present H&E staining of the normal control (NC), untreated diabetic control (UC), positive control treated with metformin (PC), and *T. cordifolia* treated (TC) groups. In the normal control group, the pancreatic islets exhibited strong staining for anti-synaptophysin (NCS) and anti-insulin (NCI), indicating intact synaptic function and vigorous secretion of insulin. These findings serve as a baseline reference for evaluating the effects of diabetes and subsequent treatments on pancreatic islet health. The untreated diabetic control group presented significantly reduced synaptophysin (UCS) staining and a complete reduction in insulin (UCI) levels. The diminished presence of these markers underscores the extent of β-cell damage and the consequent loss of insulin-producing capability in diabetic conditions. In the positive control group treated with metformin, there was an evident improvement in the staining intensity for synaptophysin (PCS), but the insulin (PCI) staining was negative. This improvement suggests that metformin therapy helps partially restore synaptic function but does not impact pancreatic β-cells. Notably, the *T. cordifolia*-treated group presented staining patterns for synaptophysin (TCS) and insulin (TCI) that were comparable to those of the normal control group. The robust staining in this group indicated that *T. cordifolia* prevents further damage and promotes significant recovery of both synaptic integrity and insulin production in the pancreatic islets (Figure 5.9). These findings suggest that *T. cordifolia* has a profound therapeutic effect, potentially restoring pancreatic islet function to near-normal levels.

The normal proportion of pancreatic β-cells in the endocrine region was calculated to be 65%. However, no positive staining was observed in the untreated diabetic or positive control groups. In contrast, the β-cells in the treated group regenerated 34% of the total β-cells, suggesting that the plants could restore β-cell function.

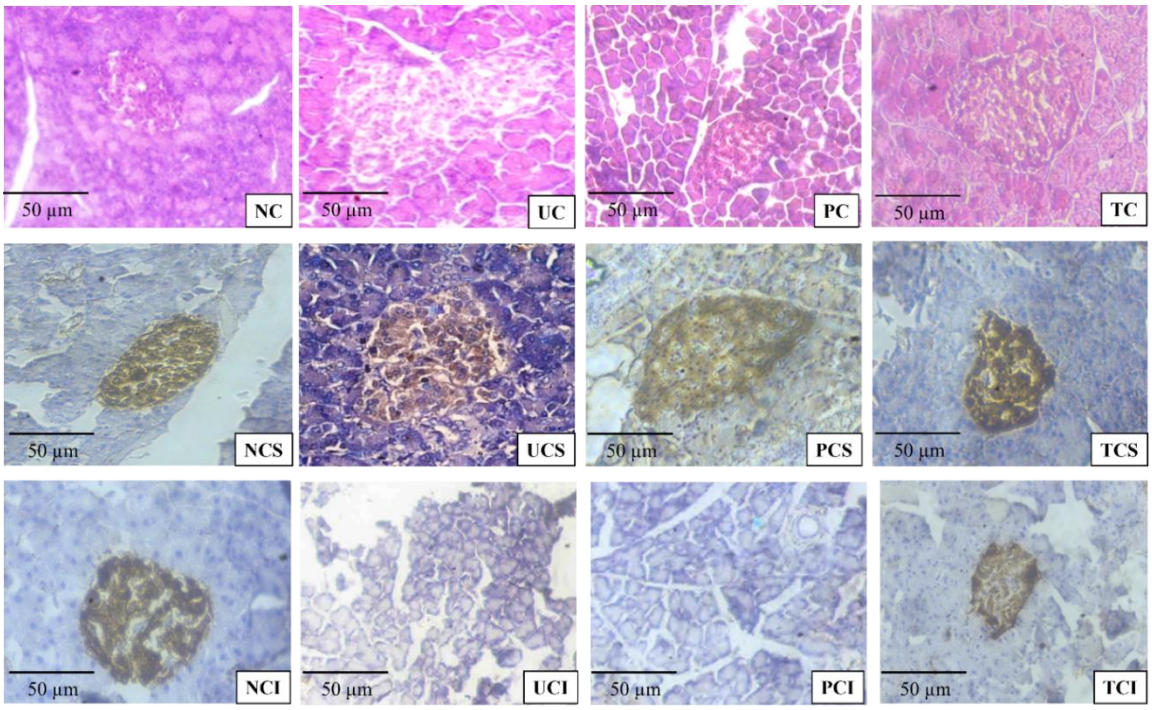


Figure 5.9: Haematoxylin and eosin staining of pancreatic islets from the normal control (NC), untreated diabetic control (UC), metformin-treated (PC), and *T. cordifolia* extract-treated (TC) groups. ; IHC staining with anti-synaptophysin antibody of pancreatic islets from the normal control (NCS), untreated diabetic control (UCS), metformin-treated (PCS), and *T. cordifolia* extract-treated (TCS) groups; IHC staining with anti-insulin antibody of pancreatic islets from the normal control (NCI), untreated diabetic control (UCI), metformin-treated (PCI), and *T. cordifolia* extract-treated (TCI) groups.

5.4. Discussion

Ethnopharmacology provides a compelling framework for investigating the therapeutic potential of medicinal plants *(Chaudhary et al.,2024)*. *T. cordifolia*, an important medicinal plant, possesses antidiabetic activity due to the presence of secondary metabolites such as alkaloids, tannins, flavonoids, and saponins (Gupta *et al.,*2024). Studies on crude extracts obtained from plants such as *Withania coagulans, Mangifera indica, Syzygium cumini, Curcuma longa,* and *Azadirachta indica* have shown the inhibition of carbohydrate digestive enzymes such as α-amylase and glucosidase (Ganorkar *et al.,*2023, Chouhan *et al.,*2017). The same result was obtained in the present study. The *T. cordifolia* extract significantly inhibited carbohydrate digestive enzymes such as α-amylase and β-glucosidase at a minimal concentration. This inhibition can help reduce the breakdown of carbohydrates into simple sugars in the digestive tract, thereby managing postprandial blood glucose levels. The analytical analysis revealed the presence of compounds such as vanillic acid, (E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxy-phenol,

and 1-tridehene in the extract, which inhibits carbohydrate digestive enzymes and tyrosine phosphate 1B (PTP-1B) protein, which helps in regulating insulin signalling by promoting glucose uptake by cells and improving insulin sensitivity, which subsequently helps in the regulation of blood glucose levels (Della Pina *et al.,*2011; Numonov *et al.,*2019; Oke *et al.,*2021).

The isoquinoline alkaloid-rich fraction from the stem of *T. cordifolia*, comprising palmatine, jatrorrhizine, and magnoflorine bioactive compounds, exhibited insulin-mimicking and insulin-releasing effects both *in vitro* and *in vivo*. The controlled blood glucose levels in the treated group (*T. cordifolia* extract) may be due to the presence of these compounds (Saha & Ghosh, 2012; Sharma *et al.,*2015). Additionally, research has shown that many plants, such as *Trigonella foenum-graecum, Momordica charantia, Aloe-barbadensis miller, Cyamposis tertragonoloba,* and *Cyamposis tertragonoloba,* exhibit antidiabetic activity, as evidenced by a reduction in glycosylated hemoglobin (HbA1C), plasma oxidative stress indicators, and alterations in antioxidant enzyme levels (Tripathi & Chandra, 2009). The results of the present study revealed a substantial reduction in the HbA1c level in the treated group (*T. cordifolia* extract), which was comparable with that in the normal control group and aligned with the findings of previous studies. GC–MS analysis revealed the presence of compounds such as n-hexadecanoic acid in the extract, which have the potential for lipid metabolism, subsequently leading to a reduction in postprandial glucose and HbA1c levels (Della Pina *et al.,*2011).

The preclinical investigation of *the* antidiabetic potential of *T. cordifolia* in Wistar rats represents a pivotal step in elucidating its pharmacological profile and therapeutic efficacy by shedding light on its therapeutic mechanisms and translational implications. Alkaloids derived from *T. cordifolia* demonstrate insulin-mediated activities due to their interaction with the insulin hormone (Behl *et al.,*2022; Tran *et al.,*2020). *T. cordifolia* also lowered brain lipid levels and blood glucose in a diabetic rat model, indicating its potential lipid-lowering and antidiabetic properties (Saha and Ghosh, 2012). A previous antidiabetic study suggested that many plants, including *Prangos ferulacea*, *Myrcia bella*, *Cichorium intybus*, *Azadirachta indica*, *Averrhova bilimbi*, *Andrographis paniculata*, *Allium sativum*, and *Acacia arabica* (Kooti *et al.,*2016), substantially reduce lipid metabolism by reducing biochemical parameters such as cholesterol, triglyceride, and high-density lipoprotein levels. Our findings revealed that the treated group (*T. cordifolia*) presented lipid-lowering ability, which is crucial for managing diabetes. Additionally, the antidiabetic effect of *T. cordifolia* is evident, as it reduces systemic glucose levels and enhances insulin efficiency

by increasing its concentration in the bloodstream (Joladarashi *et al.,*2011; Saha & Ghosh, 2012; Tiwari *et al.,*2018).

In addition, the regeneration of pancreatic β-cells is attributed mostly to phytoconstituents such as flavonoids and tannins found in the plant extract. Scientific evidence suggests that plants such as *Syzygium cumini, Melia azadirachta, Momordica charantia, Gymnena sylvestre, Aegle marmelos, Panax ginseng, and Pterocarpus marsupium* can regenerate pancreatic β-cells through the activation of different signalling pathways. The current study illustrates the regeneration of pancreatic β-cells by *T. cordifolia* extract, which may be due to the synergistic effects of flavonoids and other phytochemicals present in the plant extract. Although we have not directly examined the mechanism of regeneration capacity, GC–MS analysis revealed the presence of compounds such as diethyl phthalate (DEP) and alpha-L-rhamnopyranose (Elmaidomy *et al.,*2020; Huang *et al.,*2021), which have been reported to protect pancreatic β-cells from oxidative stress and increase insulin secretion to maintain glucose homeostasis. In addition, the synergistic effects of these phytoconstituents present in the extract are capable of regenerating lost pancreatic β-cells in a rodent model.

5.5. Conclusion

The preclinical investigation of *the* antidiabetic properties of *T. cordifolia* in Wistar rats underscores its potential as a natural therapeutic agent for managing diabetes mellitus. This study revealed significant improvements in physiochemical parameters (such as body weight, blood glucose levels, and glycated hemoglobin), indicating its efficacy in restoring metabolic balance and improving pancreatic function. An immunohistochemical study revealed the restoration of both the synaptophysin- and insulin-positive cell subtypes after extract treatment, demonstrating the regenerative capacity of the plant. These findings align with traditional uses and highlight the importance of integrating ethnopharmacological knowledge with modern scientific research to develop effective antidiabetic therapies. On the basis of the results observed in preclinical studies, it is advisable to explore its full therapeutic potential and establish its role in modern health care.

Chapter-6

Antidiabetic Potential of *Mangifera Indica*: Insights from *In Vitro* and *In Vivo* Studies

Abstract

Background: Diabetes mellitus is a chronic endocrine disorder characterized by elevated blood glucose levels. Owing to the adverse side effects of synthetic drugs, natural products attract research interest because of their fewer side effects and prominent efficacy. In the present study, we examined the antidiabetic effects of the seeds of *Mangifera indica*.

Materials & Methods: Phytochemical analysis (quantitative and free radical scavenging activity), *in vitro* enzymatic assays, and *in vivo* antidiabetic studies were performed on the hydroalcoholic extract of *M. indica*. Additionally, an immunohistochemistry approach was utilized to illustrate the alteration of the cellular architecture in the pancreatic islets of extract-treated diabetic rats.

Results: The hydroalcoholic extract exhibited good phenolic and flavonoid contents, prominent radical scavenging ability, and antidiabetic activity in an *in vitro* enzymatic assay. At a dose of 200 mg/kg body weight, the hydroalcoholic extract significantly decreased the blood glucose and HbA1c levels and substantially improved the serum marker levels (GLU, ALB, UREA, CREA, CHOL, TG, SGPT, SGOT, TP, HDL, and ALP). The immunohistological study revealed 40% regeneration of pancreatic β-cells upon extract treatment.

Conclusion: The hydroalcoholic extract has prominent antidiabetic activity, as evidenced by both *in vitro* and *in vivo* studies, by modulating the cellular composition and regenerating the β-cells of the pancreatic islet.

6.1. Introduction

Diabetes is a persistent health condition affecting millions of people worldwide. The recent focus on drug discovery has intensified the unravelling of the mechanisms of action of bioactive molecules with antidiabetic properties and the exploration of phytochemistry (Unuofin & Lebelo, 2020). There is a growing interest in medicinal plant therapy for managing diabetes mellitus because of its perceived advantages and minimal side effects. DM affects an estimated 451 million individuals worldwide, making it a significant health challenge and a leading cause of mortality (Kooti *et al.,* 2016b; Unuofin & Lebelo, 2020). Conventional antidiabetic medications often have numerous adverse effects, prompting researchers to delve into alternative therapeutic sources, such as medicinal herbs and natural products(Salehi, Ata, *et al.,* 2019). These natural reservoirs harbor bioactive compounds capable of effectively reducing blood glucose levels and modulating various mechanisms associated with DM, including β-cell function, insulin resistance, glucose absorption, and glucagon-like peptide-1 homeostasis (Tran *et al.,* 2020b).

Mangifera indica, commonly referred to as the mango tree, is recognized as a tropical fruit-bearing tree renowned for its potential medicinal properties, particularly in the management of diabetes. (Ahmad *et al.,* 2023) Different parts of mango trees, including leaves, bark, flowers, and seeds, have been utilized in traditional medicine. Traditional medicinal practices, such as Ayurveda and traditional Chinese medicine, have employed mango-derived remedies to address a spectrum of ailments, from gastrointestinal issues to inflammatory conditions (Ambu *et al.,* 2020). Recent scientific studies have revealed the potential health benefits of mangoes, elucidating their rich nutritional profile, which is rich in vitamins, minerals, antioxidants, and dietary fibre (Lebaka *et al.,* 2021).

Furthermore, investigations into mango bioactive compounds, such as polyphenols, carotenoids, and terpenoids, have revealed their diverse therapeutic properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects(Kumar *et al.,* 2021). In particular, emerging evidence suggests the potential antidiabetic effects of certain bioactive compounds found in mangoes. However, further research, including well-designed clinical trials, must validate these findings and fully elucidate the underlying mechanisms (Chaudhury *et al.,* 2017). *Mangifera indica* has a rich history of traditional use, with indigenous populations across Asia, South America, India, and East Africa utilizing its fruit for treating diabetes and associated ailments. Exploring its therapeutic potential in diabetes management represents a promising avenue in modern medicine.

This study aims to provide an overview of preclinical evidence regarding the antidiabetic properties of *M. indica*, with a focus on *in vitro* and *in vivo* studies. By synthesizing findings from preclinical investigations, this study sought to elucidate the therapeutic potential of *M. indica* in diabetes management.

6.2. Materials and methods

6.2.1. Chemicals

The chemicals and antibodies used in the current study, such as α-amylase and glucosidase, p-NPG, TPTZ, DPPH, ABTS, alloxan, and metformin, were manufactured from Sigma Aldrich, USA. The primary anti-insulin antibody, secondary antibody, and DAB chromogen were obtained from Pathnsitu, Livermore, California. The primary antibody against glucagon was purchased from Bioss, USA. Xylene, propanol, and DPX mounter were obtained from Merck, India.

6.2.2. Herbal formulation preparation

Seeds of *M. indica* were collected from Gandhamardan hill, Bargarh district, Odisha, India, in November 2023. A taxonomist from the Regional Plant Research Centre (RPRC), Bhubaneswar, identified and authenticated the sample. The freshly collected plant parts were shade-dried and pulverized to powder. The powder was subjected to hydroalcoholic extraction (methanol: water) via a microwave extraction system and lyophilized.

6.2.3. Phytochemical analysis

6.2.3.1. Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the *M. indica* plants were determined as described in section 5.2.3.3.

6.2.3.2. DPPH assay

The DPPH assay of the plant extract was performed as described in section 5.2.3.4.

6.2.4. In vitro enzymatic assay

6.2.4.1. β-Glucosidase inhibition assay

The β-glucosidase inhibitory activity of the plant extract was determined as described in section 5.2.4.2.

6.2.5. *In vivo antidiabetic study*

6.2.5.1. *Animal maintenance & ethical clearance*

The animal ethical clearance was obtained from the Department of Biotechnology and Bioinformatics, Sambalpur University, Burla, Odisha vide: SU/BTBI/IAEC/2023/02. The animals were maintained and acclimatized as described in section 5.2.5.1.

6.2.5.2. *Acute toxicity study*

The acute toxicity of the plant extract was determined as described in section 5.2.5.2.

6.2.5.3. Subacute toxicity study

Oral subacute toxicity of the extract was performed as described in section 5.2.5.3.

6.2.5.4. *Chemical Induction of Diabetes*

The chemical induction of diabetes and grouping for the respective drug treatments were performed as described in section 5.2.5.4.

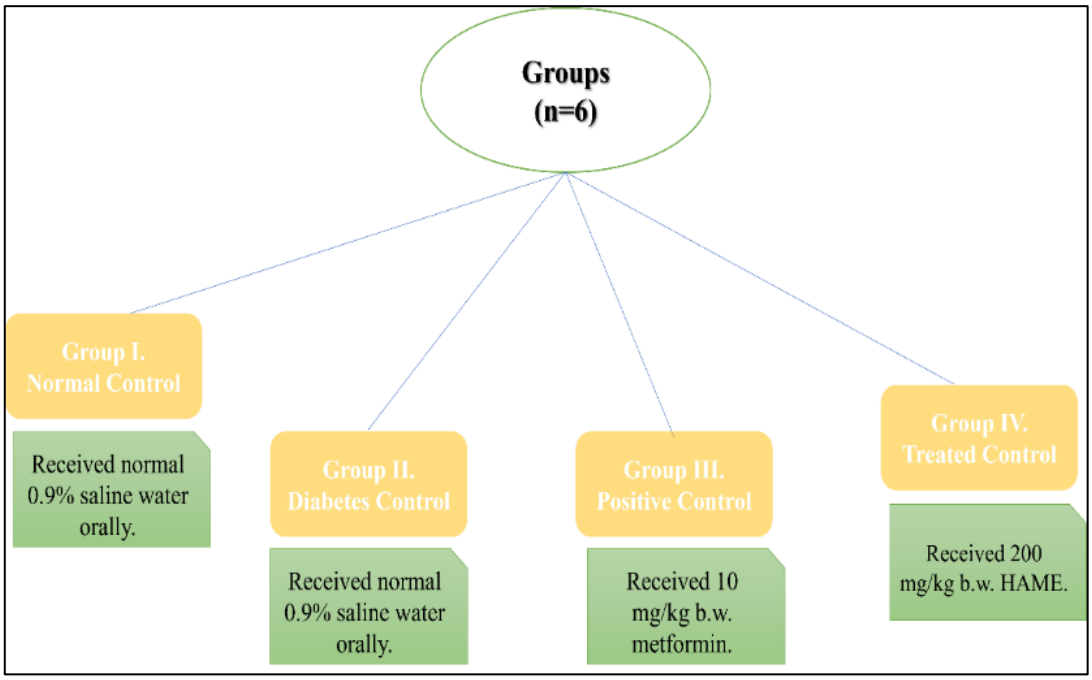


Figure 6.1: Experimental design and animal grouping. The animals were grouped into 4 groups, with 6 animals in each group. All the groups were treated differently, as shown in the figure.

6.2.5.5. *Biochemical estimation*

The biochemical serum marker study was performed as described in section 5.2.5.5.

6.2.5.6.Histopathological study

Histopathological and immunohistochemistry studies of the pancreatic islets were performed as described in section 5.2.5.6.

6.3.Results

6.3.1. Phytochemical analysis

6.3.1.1. Total phenolic and flavonoid contents

Estimating the total phenolic and flavonoid contents in medicinal plants is crucial in the field of drug discovery. Hence, in the present study, the TPC and TFC of the plant extracts were calculated. The plant extract presented the highest percentage of phenolic compounds. The calculated total phenolic content in the extract was 17.02 mg GAE/g, and the flavonoid content was 2.35 mg QE/g dry weight (Figure 6.2).

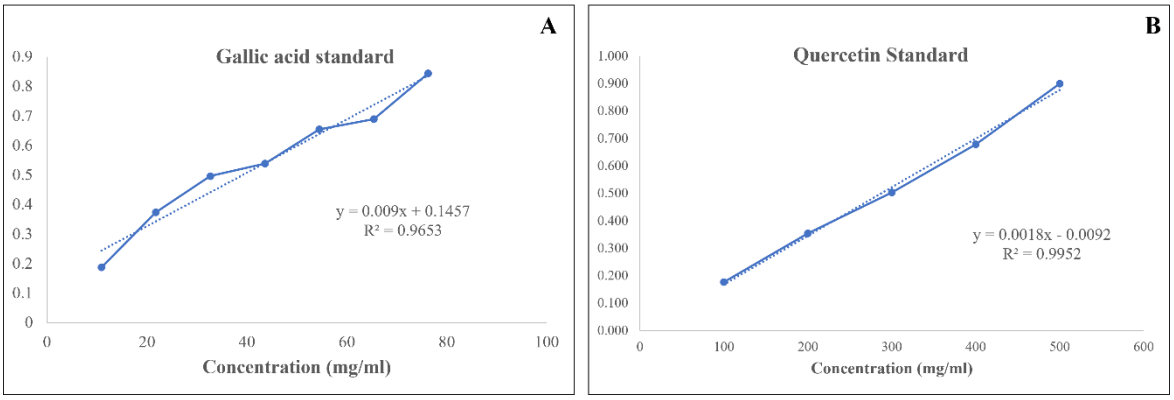


Figure 6.2: Standard plots of gallic acid (A) and quercetin (B).

6.3.1.2.DPPH activity

These results indicate that the plant extract has better antioxidant properties than the standard. The scavenging activity was evaluated at different concentrations, and the highest percentage of inhibition was calculated to be 97.09%. The calculated IC_{50} of the plant extract was 50.54 µg/ml (Figure 6.3).

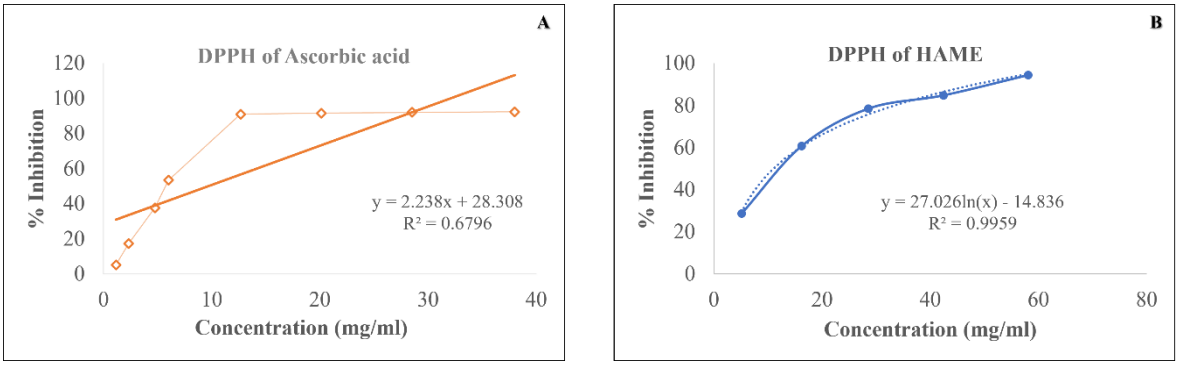


Figure 6.3: Free radical scavenging activity of ascorbic acid (A) and the plant extract (B).

6.3.2. *In vitro* enzymatic assay

The plant extract showed better inhibition activity in an *in vitro* β-glucosidase inhibition assay. The calculated IC_{50} value of the plant extract for β-glucosidase was 76.77 µg/ml, which was comparable with that of the standard (43.65 µg/ml) (Figure 6.4).

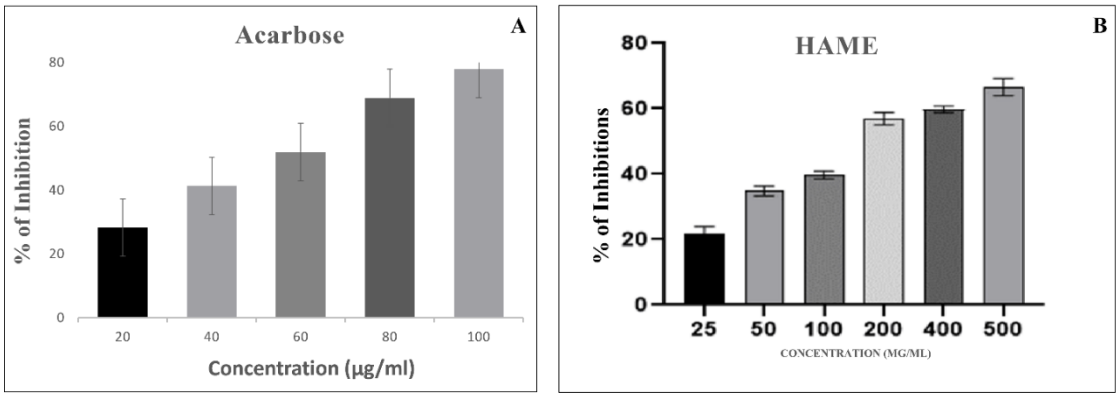


Figure 6.4: *In vitro* β-glucosidase enzymatic assay of the standard control (**A**) and the plant extract (**B**).

6.3.3. In vivo antidiabetic study

6.3.3.1. Acute oral toxicity

Orally administered plant extract at a dose of 2000 mg/kg b.w. did not cause any symptoms of mortality or toxicity to the animals. All the treated animals showed normal behaviour with no clinical symptoms of mortality or other symptoms of toxicity throughout the experiment.

6.3.3.2. Subacute toxicity study

Subacute toxicity studies of the plant extract revealed no deaths or toxicity-related clinical symptoms in the treated animals. H&E staining of vital organs revealed no pathological alterations compared with the normal control (Figure 6.5).

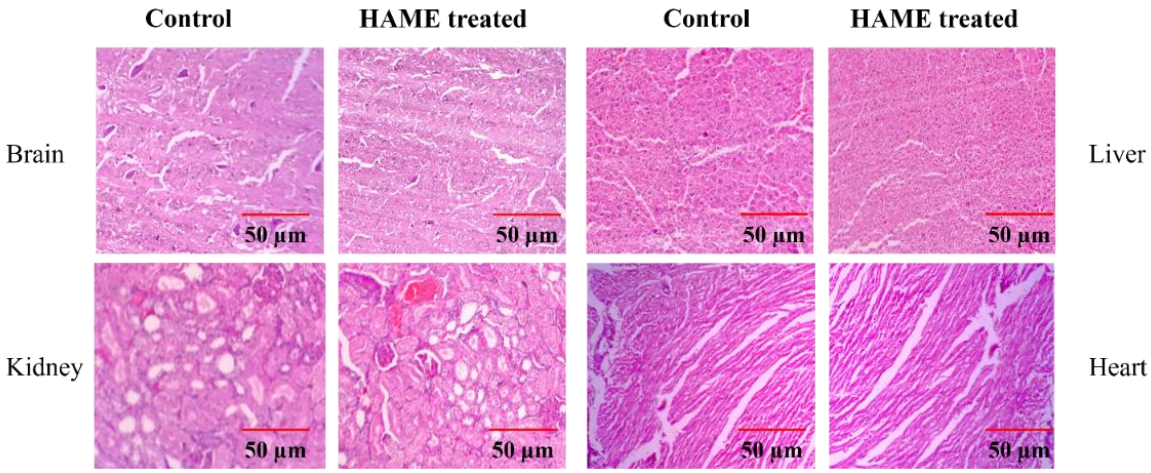


Figure 6.5: H&E staining toxicity of different organs treated with the plant extract. **A)** Brain, **B)** kidney, **C)** liver & **D)** heart.

6.3.3.3. Effects of extract on blood glucose levels, body weights, and HbA1c levels

Parameters such as body weight and blood glucose levels were measured at different day intervals and compared with those of the control group. The normal control group of animals presented a b.w. of 236±5.78 g, whereas the diabetic control group presented a significant decrease in b.w. to 191.25±9.93 g. However, the positive control (metformin-treated) and plant extract-treated groups of animals presented improvements in body weight of 186±3.04 g and 194.5±1.6 g, respectively (Table 6.1. A). The blood glucose level was greater in the diabetic control group (471±3.2 mg/dl) than in the normal control group (111.5±2.8 mg/dl). However, compared with the positive control group (110±1.45 mg/dl), the plant extract-treated group presented a decrease in the blood glucose level to 121±9.4 mg/dl (Table 6.1. B). The glucose hemoglobin (HbA1c) level in the normal control group was 5.56%, but it increased to 18.7% in the diabetic control group. In contrast, the plant extract significantly decreased the HbA1c level to 5.8%, which was comparable to that of the metformin-treated group (5.76%) (Figure 6.6).

Table 6.1: Comparison of blood glucose levels and body weights of experimental animals on different days of treatment.

A. Bodyweight				
	Day 0	**Day 7**	**Day 21**	**Day 28**
NC	215 ± 1	228 ± 4	227.6 ± 0.4	236 ± 5.78
DC	282 ± 2.2	277.5 ± 4.5	247 ± 6.7	191.5 ± 9.93
PC	160 ± 3.01	140 ± 2.45	152 ± 1.67	186 ± 3.04
TC	166.2 ± 5	176.5 ± 1.78	182.3 ± 3.2	194.5 ± 1.65
B. Blood Glucose level				
NC	109.5 ± 2.1	112.5 ± 3.4	119.6 ± 1.88	111.5 ± 2.8
DC	103 ± 6.3	294 ± 6.2	366.3 ± 4	471.5 ± 3.1
PC	97 ± 2.59	330 ± 2.76	144 ± 1.12	110 ± 1.45
TC	95.5 ± 1.5	265 ± 3	152 ± 2.8	121.7 ± 9.4

NC: Normal control, DC: Untreated Diabetic, PC: Metformin treated & TC: extract treated.

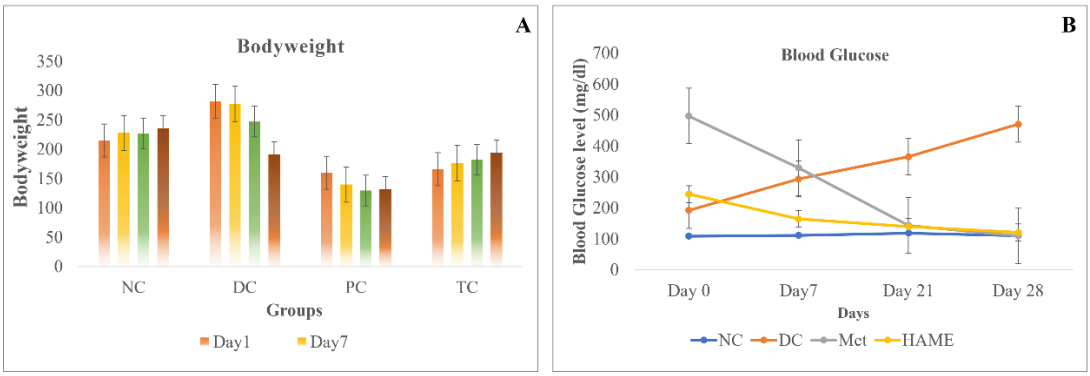


Figure 6.6: Graphical representation of the comparison of body weight **(A)** and blood glucose level **(B)** with those of the normal control, diabetes control, positive control, and plant extract treatment control groups.

6.3.3.4. Effects of extract on biochemical serum markers

In the present study, parameters such as GLU, ALB, ALP, UREA, CREA, CHOL, TG, SGPT, SGOT, TP, and HDL were included. Compared with normal controls, diabetic controls have elevated levels of this serum biomarker. The plants in the extract-treated and metformin-treated groups returned to the normal control group, as shown in Table 6.2 below. Elevated protein levels destroy pancreatic β-cells, leading to insufficient insulin secretion. The results revealed that the total protein level (2.43±0.40 g/dl) was lower in the negative control group than in the normal control group (6.4±0.85 g/dl). However, the values in the plant extract-treated and metformin-treated groups were nearly normal, i.e., 5.99±0.07 and 5.5± 1.62 g/dl, respectively.

Table 6.2: Effects of plant extracts on diabetic animal blood serum levels.

Parameter	Normal	Negative	Positive	Treated	Range	Unit
GLU	123.1 ± 3.4	318.3 ± 5.15	97.14 ± 7.88	96.32 ± 4.34	74.0 - 143.0	mg/dl
ALB	2.833 ± 03	8.32 ± 0.15	12.45 ±0.15	3.18 ± 0.82	2.3 - 4.0	g/dl
UREA	44.27 ±2.67	32.56 ± 0.31	49.89 ±3.49	33.00 ± 2.25	15.0 - 58.0	mg/dl
CREA	1.34 ±0.085	12.85 ± 1.23	1.38 ±0.15	0.595 ± 0.04	0.5 - 1.8	mg/dl
CHOL	112.4 ± 2.9	62.23 ± 1.82	118.4 ±3.24	123.01 ± 2.77	109.0 - 202	mg/dl
TG	134.16 ±12.8	202.5 ± 1.48	105.41 ±5.9	108.16 ± 7.57	40.0 - 165.0	mg/dl
ALT	80.56 ±3.74	451.32 ± 6.1	107.81 ±6.51	65.65 ± 2.70	10.00 - 125	U/L
AST	31.45 ±2.12	145.48 ± 2.3	34.01 ±2.27	21.26 ±1.40	0 - 50.0	U/L

TP	6.4 ±0.85	2.43 ±0.40	5.99 ±0.07	5.5 ±1.62	5.2 - 8.2	g/dl
DBIL	4.42 ±0.35	4.3516 ± 0.29	1.97 ±0.293	0.39 ±0.06	0 - 0.50	mg/dl
TBIL	0.62 ±0.27	11.08 ± 0.50	0.08 ±0.02	0.2 ±0.10	0 - 0.90	mg/dl
HDL	61.66 ±2.12	513.36 ±6.37	78.68 ±5.74	53.47 ±158	35.0 - 88.0	mg/dl

6.3.3.5. Histopathological study

H&E staining confirmed the normal architecture of the pancreatic tissue (Figure 6.7). Significant structural alterations were revealed in the diabetic control group via H&E staining; however, those in the plant extract-treated group were nearly normal. Consequently, these tissues were further used in immunohistochemistry (IHC) studies.

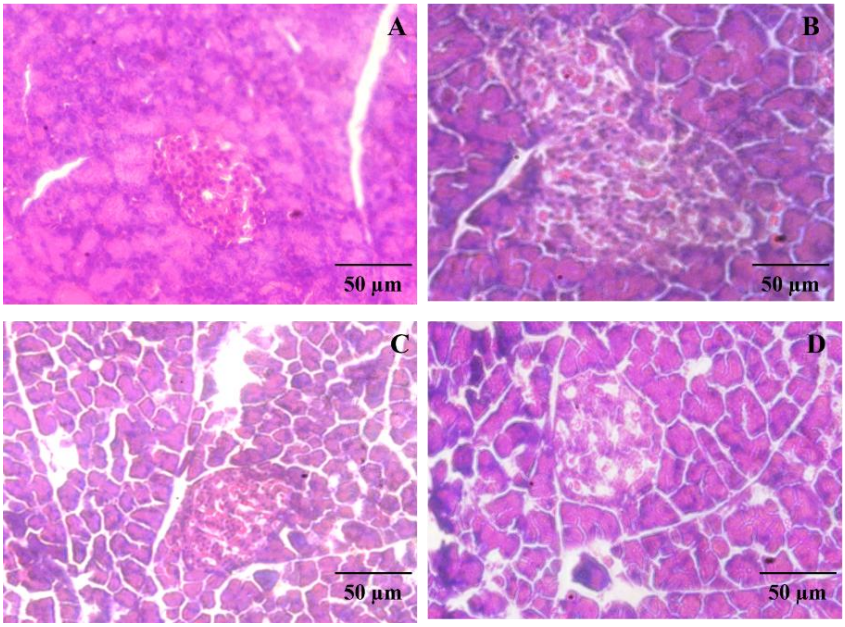


Figure 6.7: H&E staining of pancreatic islets. **A)** Pancreatic islets of the normal control group. **B)** The diabetic control group presented disrupted architecture, C) the positive control group, and D) the plant extract-treated group.

6.3.3.6. Immunohistochemistry study

IHC staining with an anti-insulin antibody revealed a normal distribution of β-cells located at the core of the islet in the normal pancreas (Fig. 6.8A). In contrast, the diabetic control pancreas did not stain for insulin (Fig. 6.8B) because of selective destruction of the pancreatic β-cells with alloxan, leading to elevated blood glucose levels. The positive control group also resembled the negative control group (Fig. 6.8C), which presented negative staining. Although the positive control group displayed a decrease in blood

glucose and HbA1c levels, it did not impact the β-cells of the islet. However, the damaged β-cells regenerated in the animals treated with the plant extract (Fig. 6.8D). ImageJ analysis revealed 40% regeneration of pancreatic β-cells in the plant extract treatment group compared with the normal group. IHC staining with an anti-glucagon antibody revealed that α-cells were predominantly located at the periphery, accounting for 17% of the total islet area. In contrast, the total islet area of the diabetic control group increased to 42%. In the positive control group, it decreased to 36%, whereas in the plant extract-treated group, it was 22%, which was located at the periphery of the islet (Fig. 6.9).

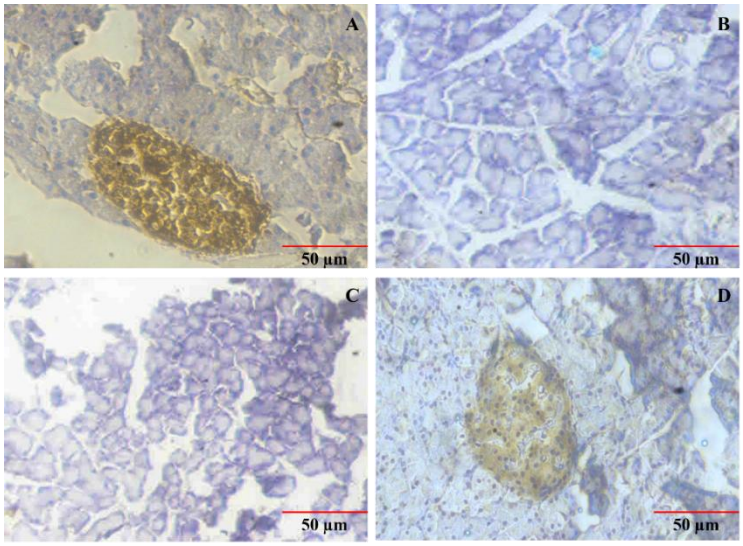


Figure 6.8: IHC staining with an anti-insulin antibody. **A)** Pancreatic β-cell area of the normal control group, **B**) diabetic control group, **C**) positive control group, and **D**) treated group.

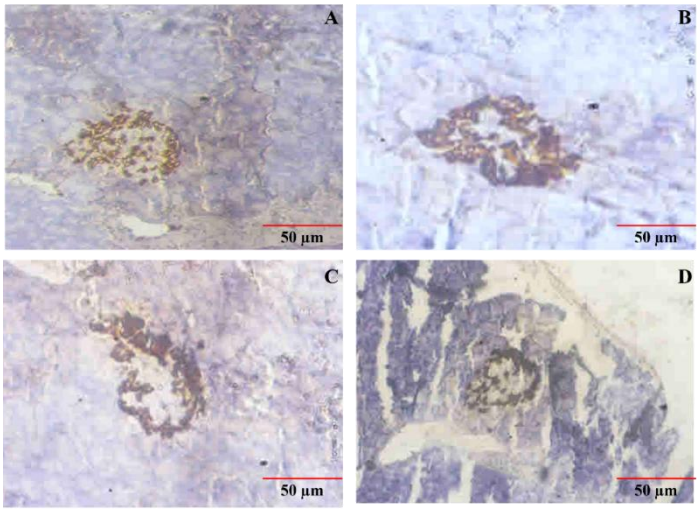


Figure 6.9: IHC staining with an anti-glucagon antibody. **A)** Normal control group, **B**) diabetic control group, **C**) positive control group, and **D**) the plant extract-treated group.

6.4. Discussion

Diabetes has become a major headache in many developing countries worldwide. Nevertheless, an effective medication for DM has not yet been developed. The currently used synthetic antidiabetic drugs decrease blood glucose levels but have many adverse side effects, such as microvascular and macrovascular complications (Zofou *et al.,* 2023). The current study aimed to validate the antidiabetic efficacy of hydromethanolic extracts of mango seeds in *in vitro* and *in vivo* antidiabetic models and to study microarchitectural changes in the pancreatic islets of diabetic and treatment groups. For many decades, medicinal plants have been traditionally used to manage and treat many life-threatening diseases, but many of them have not yet been scientifically proven (Yikna & Yehualashet, 2021). Many studies have revealed that compounds isolated from plant products are more effective for the long-term treatment of DM. Additionally, it has been demonstrated that natural products are resources for discovering new potent candidates for targeting many diseases (Nabi *et al.,* 2013; Salehi, Ata, *et al.,* 2019). It has been observed that bioactive molecules derived from various medicinal plant sources have notable antidiabetic activity via multiple pathways. The effect may work alone or in concert with other effects (Yikna & Yehualashet, 2021). DPPH is a commonly used method to determine the free radical scavenging activity of natural products. It determines the ability of a natural compound to scavenge free radicals and damage macromolecules such as nucleic acids, polysaccharides, and lipids (Konaté *et al.,* 2014). In the present study, the plant extract showed better free radical scavenging activity at minimal concentrations. Free radical scavenging activity may be due to the presence of many antioxidant compounds, such as phenols, flavonoids, and carotenoids. These antioxidant compounds donate electrons to free radicals to stabilize them and prevent them from causing damage (Peshev *et al.,* 2013).

The secondary metabolites present in medicinal plants have improved pharmacological and biological activity, and these metabolites directly interact with receptors, the cell membrane, and nucleic acids (Zofou *et al.,* 2023). The present study revealed the presence of secondary metabolites in the hydroalcoholic extract, which resulted in better scavenging activity. Additionally, these phytoconstituents have the potential to lower blood glucose levels via the reduction of oxidative stress (ROS) (LAMİCHHANE *et al.,* 2022). Previous studies revealed that many such medicinal plants are capable of decreasing blood glucose levels in diabetic animals, i.e., the hydroalcoholic extract of *T. schimperi* at a dose of 500 mg/kg b.w. for 14 days in alloxan-induced animals, which is capable of decreasing blood glucose levels (Taye *et al.,* 2020), and the ethanolic and aqueous crude extracts of *M.*

stenopetala leaves at a dose of 500 mg/kg significantly reduce blood glucose levels after a treatment period of 14 days in STZ-induced animals (Toma *et al.,* 2015). Similarly, in the present study, the HAME treatment group presented a significantly lower blood glucose level (121.7 ± 9.4 mg/dl) than did the diabetic control group (471.5 ± 3.1 mg/dl). The decline in blood glucose levels may be due to the extrapancreatic effect of HAME, such as glucose utilization and glycolytic or glycogenic enzyme activity in peripheral tissues (Sukalingam *et al.,* 2015a).

The current study revealed a significant decrease in body weight after the induction of alloxan in the animals, which was due to the diabetic condition and initial adjustment to the treatment regimen. Previous reports have shown that phytoconstituents in plants harm the body weight of diabetic-induced animals. After the treatment period, animals treated with the plant extract presented a substantial increase in body weight (16%), indicating a potential beneficial effect on weight maintenance, which was comparable with that of the standard control. In addition to the blood glucose level and body weight in DM, the glycolate hemoglobin level plays a crucial role; this level was 3–4 times greater in the diabetic control group than in the normal group of animals. Reports on diabetes suggest that several plants, i.e., *Cinnamomum cassia, Catharanthus roseus,* and *Gymnema Sylvestre*, have decreased HbA1c levels to normal levels after a few days of treatment (Semwal *et al.,* 2021b; X. G. Yao *et al.,* 2013). The results of the HbA1c level in the present study align with those of a previous report; plant extract can decrease the HbA1C level to 5.56% after a treatment period of 28 days. The above findings support the potential of plant extracts as complementary or alternative therapies for managing diabetes-related complications and improving overall metabolic health.

Two common mechanisms have been utilized in the regeneration of destroyed pancreatic β-cells in diabetes, i.e., regeneration of β-cells from nonbeta cells or stem/progenitor cells and increased cellular proliferation of preexisting β-cells (Kimani *et al.,* 2023). Previous reports on DM revealed that the ethanolic extract of *Spondias mombin* regenerates pancreatic β cells after a treatment period of 12 weeks (Eluehike & Onoagbe, 2020). The bioactive compound kinsenoside, which is isolated from the *Anoectochilus roxburghii* plant, can potentially regenerate pancreatic β-cells. Another compound, momordicin, which is extracted from *Momordica charantia* plants, regenerates pancreatic β-cells in alloxan-induced diabetic animals (Cicero *et al.,* 2004; Oh, 2015; Y. Zhang *et al.,* 2007). In addition, the most commonly found compounds, quercetin, resveratrol, and silymarin (from *Silybum marianum*), inhibit the apoptosis of β cells (Jose *et al.,* 2011; Oh,

2015). In our study, the plant extract regenerated the destroyed β-cells after four weeks of treatment. The results of the IHC study of pancreatic tissues shed light on microarchitectural changes.

The normal control group shows the mantle-core architecture of the α- and β-cells within the islets. This arrangement is crucial for the normal secretion of insulin and the maintenance of glucose homeostasis. On the other hand, the diabetic control group displayed a disturbed architecture of the islet, characterized by an absence of positive staining for insulin. In contrast, the proportion of glucagon-positive cells increased to 42%, which led to an increase in the blood glucose level in the body. This finding is consistent with the impaired insulin production and function typically observed in diabetic conditions. Interestingly, the metformin-treated group had negative staining for the insulin-positive cell subtype. These findings suggest that metformin helps manage blood glucose levels via a reduction in hepatic glucose production and may not have a direct effect on pancreatic β cells; however, when the cells are stained with an anti-glucagon antibody, the proportion of the total islet area decreases to near normal. The plant extract-treated group exhibited a well-preserved architecture of the islet and regenerated pancreatic β cells, indicating a mantle-core architecture of α and β cells. This observation is significant, as it indicates the potential of the extract to not only reduce blood glucose levels but also contribute to the regeneration of pancreatic β cells. Overall, these findings from the IHC study provide valuable insights into the cellular mechanisms underlying the therapeutic effects of plant extracts in managing diabetes and restoring pancreatic β-cell function. Further research into the specific molecular pathways involved in this regeneration process could uncover new avenues for developing effective treatments for diabetes and related complications.

6.5. Conclusion

Natural products have gained significant interest in managing and treating major health concerns, including diabetes, on a global scale. This study demonstrated that *Mangifera indica* has promising efficacy in various assays, including radical scavenging, *in vitro* enzyme inhibition, and *in vivo* antidiabetic studies. Additionally, it has potential by lowering hyperglycemia through gluconeogenesis inhibition and regenerating pancreatic β-cells. Comprehensive studies on antidiabetic activity, such as blood glucose level evaluation, body weight, HbA1C levels, biochemical parameters, histopathology, and immunohistochemistry analyses, affirm the extract's effectiveness. These findings underscore the promising role of *Mangifera indica* in diabetes management.

Conclusion

The aqueous polyherbal formulation (APE) prepared from the sixteen medicinal plants traditionally known for its antidiabetic properties demonstrated significant efficacy in our comprehensive study. UPLC–Q-TOF-MS/MS analysis revealed a total of 60 bioactive compounds in APE, 39 of which have antidiabetic properties. The in silico analysis of APE highlighted a strong interaction between verbascoside B and the target proteins (insulin-like growth factor-I and GLUT4), with impressive docking scores of -12.433 and -17.825 kcal/mol, respectively, compared with those of the standard drug (-2.605 and -3.332 kcal/mol). This strong binding affinity suggests that verbascoside B may increase insulin secretion and promote GLUT4 translocation, thereby facilitating glucose uptake in cells. The *in vitro* carbohydrate digestive enzyme assay demonstrated significant inhibition of the enzyme at a minimal concentration. The extract is nontoxic to cells even at relatively high concentrations. Notably, the glucose uptake assay in MIN6 cells revealed a dose-dependent improvement in glucose uptake, surpassing the efficacy of the standard drug metformin. An *in vivo* antidiabetic study further demonstrated that APE significantly decreased elevated blood glucose and HbA1c levels and normalized biochemical parameters related to glucose metabolism, lipid profiles, liver enzymes, and kidney function markers in diabetic animal models. Immunostaining of the pancreata of all the experimental groups revealed significant restoration of pancreatic β-cells (47%) in the APE-treated group, which was lost in the diabetic group, underscoring its potential in diabetes management. Given the undesirable side effects of current modern therapies (such as sulfonylurea, biguanides, and GLP-I agonists), aqueous polyherbal formulations could be preferred for the treatment of diabetes. The aqueous polyherbal formulation offers a promising alternative with fewer side effects and potent antidiabetic properties.

Following a rigorous evaluation process encompassing bioactive compound profiles, pharmacological efficacy, and traditional use of the above plants, three plants (*Tinospora cordifolia*, *Syzygium cumini*, and *Mangifera indica*) have emerged with exceptional promise. Consequently, these compounds were chosen for further exploration to investigate their synergistic effects on diabetes management.

The ayurvedic polyherbal formulation (APF) was prepared from the above selected plants. FTIR analysis revealed 23 diverse functional groups and 17 active bioactive phytoconstituents. Owing to the presence of these diverse constituents, it notably inhibits carbohydrate digestive enzymes. The acute and subacute toxicity studies confirmed the

nontoxic nature of the extract in animal models. *In vivo,* the antidiabetic study demonstrated a significant restoration of blood glucose, HbA1c, and biochemical serum markers related to liver enzymes, as well as kidney function markers, following treatment in diabetic models. Immunostaining revealed significant restoration of pancreatic β-cells and myosin Va molecules in response to APF treatment, which were lost in diabetic patients. This restoration underscores the role of myosin Va in the exocytosis of insulin granules, which is essential for normalizing insulin secretion from pancreatic islets. Furthermore, islet size, crucial for glucose homeostasis, was significantly reduced in the diabetic model group but notably increased in the APF-treated group. The increase in islet size is likely due to the synergistic effects of APF on the regeneration of pancreatic β-cells.

The selection process focused on identifying the most promising plants for detailed analysis, aiming to uncover their specific therapeutic benefits. As a result, *Tinospora cordifolia* and *Mangifera indica* were chosen on the basis of their ethnopharmacological significance and well-documented antidiabetic potential in the scientific literature, and further investigations are needed to better understand their roles and contributions to diabetes treatment.

Seventeen bioactive compounds, ten of which have antidiabetic activity, were identified from *Tinospora cordifolia* via GC–MS analysis. Both T. *cordifolia* and *M. indica* significantly inhibited carbohydrate digestive enzymes, and improved lipid accumulation was observed in the treated cells. The *in vivo* study revealed a significant decrease in blood glucose, HbA1c, and biochemical serum marker levels near those of normal controls after treatment with these herbal drugs. Immunostaining revealed a restoration of the lost pancreatic β-cells in the diabetic animal models.

In conclusion, both the developed polyherbal formulations and individual medicinal plants demonstrated superior efficacy compared with traditional systems, offering efficient therapeutic outcomes with fewer side effects. These findings underscore the potential of these formulations as viable alternatives for diabetes management, paving the way for further clinical exploration and application.

Future Perspectives

Compared with traditional systems, the developed polyherbal formulations and individual medicinal plants exhibit superior efficacy. These formulations not only offer more efficient therapeutic outcomes but also present fewer side effects than modern therapies do. Their eco-friendly nature ensures that they do not harm any living organisms, aligning with sustainable and ethical practices.

Future research should focus on extensive clinical trials to validate these findings and explore the molecular mechanisms underlying their therapeutic actions. Additionally, efforts should be made to optimize the extraction and formulation processes to increase their potency and stability. Collaborations with pharmaceutical industries could pave the way for commercial production and wider accessibility. The potential of these natural products in managing other chronic conditions should also be explored, broadening their applicability in the realm of holistic and integrative medicine.

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LIST OF PUBLICATION

PATENT

1. A Novel Polyherbal Formulation for the treatment and management of Type-II Diabetes Mellitus. DBT BUILDER, Govt. of India, Department of Biotechnology and Bioinformatics, Sambalpur University, Inventors: Pradeep Kumar Naik, **Abhijit Sahu**, Pratyush Pragyandipta, Srichandan Rath, Dibya Ranjan Sahoo, Deepali Naik, Ashirbad Nanda, Satish Kanhar, Debanand Sahu, Pravash Ranjan Mishra. Application No. 202431000868 A, Publication Date: 26/01/2024, Indian Patent Office Journal No. 04/2024 Dated 26/01/2024

RESEARCH ARTICLES

1. **Sahu A,** Rath S, Naik D, Mishra P R and Naik P K. Antidiabetic Potential of *Mangifera Indica*: Insights from In Vitro and In Vivo Studies, *International Journal for Modern Trends in Science and Technology*, 2024, 10(10), pages. 17-27. https://doi.org/10.46501/IJMTST1010003 (Published).
2. Mishra P, **Sahu A**, Naik P K, Ravi P K. Islet Dimensions and Its Impact on the Cellular Composition and Insulin-Secreting Capacity: Insights into the Role of Non-beta Cells. *Cureus,* 2024, 16(1): e52428. DOI 10.7759/cureus.52428 (Published).
3. **Sahu A**, Mishra P R, Pragyandipta P, Rath S, Nanda A, Kanhar S, Sahoo D R, Naik E, Naik D and Naik P K. Unvealing the therapeutic potential of polyherbal formulations for diabetes management via endogenous pancreatic β-cell regeneration. (Under Review).
4. **Sahu A**, Mohanty B, Rath S, Sahoo D R, Naryan R K, Sahoo P K, Naik P K and Mishra P R. Evaluation of an Ayurvedic Polyherbal formulation for antidiabetic activity based on β-cell restoration and recovery of Myosin Va in Diabetic Pancreas. (Under review).
5. **Sahu A**, Nayak S, Naik P K, and Mishra P R. Deep Learning Based Detection and Quantification of Pancreatic Islet Cell Subtypes in Immunohistochemistry Images: From Microscope to Machine. (Under review).
6. **Sahu A,** Mishra P R, Rath S, Sahoo D R, Naik D, Pragyandipta P, Babu S K, and Naik P K. Ethnopharmacological Importance of *Tinospora cordifolia* in blood sugar regulation based on in-vitro & in-vivo study in rodent model. (In peer review).

7. **Sahu A**, Chaudhury M, Pragyandipta P, Sahoo D R, Ravi P K, Swain S, Naik P K, Chaudhury A, and Mishra P R. Efficacy of *Swertia chirayita* in Blood Glucose Regulation: Insights from *In Vitro* and *In Vivo* Models. (Communicated).
8. Swain S, **Sahu A**, Singh P, Rout S, Parida G K, Mishra P R and Agrawal K. Potential of Liver Serum Enzymes and SUVmax in Primary Tumors as Predictive Biomarkers with Correlational Evidence. *Cureus*, 2024, 16(4): e58532. DOI 10.7759/cureus.58532 (Published).
9. Priyanka K, Sahoo R N, Nanda A, Kanhar S, Das C, **Sahu A**, Naik P K and Nayak A K. Wound Healing Activity of Topical Herbal Gels Containing Barringtonia acutangula Fruit Extract: In silico and In vivo Studies. *Chemistry & Biodiversity*, 2024 p.e202400147. DOI doi.org/10.1002/cbdv.202400147 (Published).

CONFERENCE PRESENTATION

1. **Abhijit Sahu,** Pravash R Mishra, Srichandan Rath, Dibya Ranjan Sahu, & Pradeep Kumar Naik. "Ethnopharmacological Role of *Tinospora cordifolia* in Diabetes Management: *In Vitro* & *In Vivo* Studies". Poster presentation at 2nd Annual Research Day 2024 and 1st Regional Research Conclave organized by All India Institute of Medical Sciences, Bhubaneswar, Odisha.
2. **Abhijit Sahu,** Mehaghni Chaudhury, Anita Bhushan, Akanksha Das, Praveen Kumar Ravi, Sashikanta Swain, Pradeep Kumar Naik, Pravash Ranjan Mishra, and Arun Chaudhury. "Chirayita Restores Insulin and Myosin Va in Diabetic Rat Pancreas". Poster presentation at Annual Scientific Meeting & Postgraduate CourseACG-2024. Organized by American College of Gastroenterology, Philadelphia, Pennsylvania, USA.
3. **Abhijit Sahu,** Pravash Ranjan Mishra and Pradeep K. Naik. "A Step Closer to a Natural Solution: Impact of the Polyherbal Formulation on Diabetes in Animal Model". Oral presentation at National Conference on Emerging Trends in Biotechnology and Bioinformatics (ETBB 2024) organized by the Department of Biotechnology and Bioinformatics, Sambalpur University, Odisha
4. **Abhijit Sahu,** Pravash Ranjan Mishra and Pradeep K. Naik. "Effects of the polyherbal formulation on the microarchitecture of Pancreatic Islet & its modulation in Diabetic Animal". Poster presentation at Odisha Research Conclave-2022. Organized by Odisha State Higher Education Council and Ravenshaw University, Odisha.

5. **Abhijit Sahu,** Pravash Ranjan Mishra and Pradeep K. Naik. **"**Method Development for the Architectural study of Human pancreatic islets to assess the Antidiabetic potential of Herbal formulation". Poster presentation at Odisha Research Conclave 2022. Jointly organized by Odisha State Higher Education Council and Ravenshaw University.

Attendance in Seminar/Symposia

1. International Webinar on "Biotechnology Bioinformatics and Natural products and Therapeutics" Department of Biotechnology and Bioinformatics, Sambalpur University, Odisha. 29th June to 5th July 2020.
2. National conference on "Current Research Trend in Biotechnology, Bioinformatics and Intellectual Property Management" held on 3rd to 4th march, 2020 organized by Department of Biotechnology and Bioinformatics, Sambalpur University, Sambalpur, Odisha.
3. International webinar on "Life Science Research in Pre and Post Covid Era" held on 16th August 2020 organized by Department of Botany, School of Applied Science, Centurion University of Technology and Management, Odisha, India.
4. National Webinar on "Introduction to preclinical trials" held on 15th June 2020 organized by Department of Biotechnology, Rajah Serfoji Govt. College, Thanjavur, Tamil Nadu.
5. International webinar on "Porosome: The secretory Nanomachine in cells" held on 7th November 2020, organized by Department of Zoology Utkal University, Bhubaneswar and Zoological society of Orissa.

Certifications/Training/Workshop

1. Workshop on "Hands-on-Training on Flow Cytometry" organized by the Institute of Chemical Technology, Mumbai under the scheme of DST STUTI, with the Department of Pharmaceutical Engineering & Technology IIT BHU from 29th November to 5th December 2022.
2. Workshop On "Animal cell culture techniques and Screening of Drug molecules (ACTSDM-2022) at Sambalpur university by Department of Science and Technology (DST) under STUTI Programme.

ACADEMIC HONORS AND AWARDS

1. Best poster paper presentation award, 2023 at ORC-2023. Jointly organized by Odisha State Higher Education Council and Sambalpur University, Odisha. Poster Title: “Effects of the polyherbal formulation on the microarchitecture of Pancreatic Islet & its modulation in Diabetic Animal”.
2. Outstanding poster presentation award, 2024, at Annual Scientific Meeting & Postgraduate CourseACG-2024. Organized by American College of Gastroenterology (ACG), Philadelphia, Pennsylvania, USA. Poster title: “Chirayita Restores Insulin and Myosin Va in Diabetic Rat Pancreas”.