DETERMINATION OF MECHANISM OF ACTION (INSULIN SIGNALLING GENES) OF EXTRACTS OF *Spondias mombin* LEAVES IN TYPE 2 DIABETIC RAT MODEL

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COLLEGE OF POSTGRADUATE STUDIES

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SUPERVISED BY

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DECLARATION

I, **Balogun Christopher Segun** (PG/BMS1605035), of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Benin, Benin City, hereby declare that the information contained in this dissertation work is the outcome of authentic academic research that I conducted. I confirm that the dissertation has not been submitted to obtain a degree in any other institution, either in it's entirely or in part. All sources of data and scholarly dissertation information utilized in this dissertation are appropriately acknowledged

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DEDICATION

This work is dedicated to God Almighty and my family for their unwavering support and encouragement throughout this journey.

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ABSTRACT

Diabetes mellitus (DM), particularly type 2 diabetes (T2DM), represents a global health crisis characterized by persistent hyperglycemia due to insulin resistance and β-cell dysfunction. With projections estimating 783 million affected adults by 2045, current pharmacotherapies like metformin and sulfonylureas face limitations including side effects, high costs, and therapeutic inertia. This has spurred interest in medicinal plants as complementary therapies. *Spondias mombin L.* (hog plum), traditionally used in Nigeria for diabetes management, exhibits antioxidant, anti-inflammatory, and hypoglycemic properties, yet its molecular mechanisms remain underexplored. This study investigates the effects of ethanol extract of *S. mombin* on insulin signaling pathway genes (IR-Insulin Receptor, AKT-AKT serine/theorine kinase, PPAR-γ- Peroxisome Proliferator activated receptor Gamma, DPP-4- Dipeptidyl peptidases 4) in high-fat diet (HFD) and streptozotocin (STZ)-induced T2DM rats, aiming to validate its ethnomedicinal use and elucidate gene-modulating potential. Male Wistar rats (150-200 g) were acclimatized, then divided into five groups (n=6): Group 1: normal control , Group 2: positive control, Group 3: diabetic control , Group 4: 200mg/kg of extract and Group 5: 400mg/kg of extract. T2DM was induced via 2-week HFD followed by low-dose STZ (40 mg/kg i.p.). Treatments were administered orally for 2 weeks. Body weights and organ weights (pancreas, liver) were measured. Liver RNA was isolated for semi-quantitative RT-PCR analysis of IR, AKT, GLUT-4, PPAR-γ, DPP-4. Results revealed diabetes-induced body weight loss (147 ± 9.56 g vs. 184.17 ± 11.92 g in controls), reduction in the weight of the pancreas (0.19 ± 0.04 g vs. 0.59 ± 0.14 g), and increase in liver weight (8.56 ± 0.25 g vs. 7.33 ± 0.53 g). Extract treatments restored body weights dose-dependently (189.11 ± 15.99 g at 200 mg/kg; 209.28 ± 17.74 g at 400 mg/kg), surpassing glipizide (170.79 ± 31.55 g). Pancreas weights increased to 0.29 ± 0.04 g and 0.32 ± 0.05 g, while liver weights normalized, indicating protective effects. Gene expression analysis showed downregulation in diabetic controls for IR, AKT, GLUT-4, PPAR-γ, and DPP-4. Extract upregulated these genes dose-dependently, often exceeding glipizide, suggesting enhanced insulin sensitivity, glucose uptake, and metabolic regulation. These findings corroborate *S. mombin*'s antioxidant and anti-inflammatory mechanisms, likely driven by flavonoids and saponins, addressing T2DM's multifactorial nature. The extract's superiority over glipizide highlights its potential as a safe, affordable adjunct therapy, overcoming conventional limitations. This study provides mechanistic evidence supporting phytotherapy in T2DM management, contributing to natural product-based drug discovery. The research aligns with T2DM pathophysiology, involving disruptions in insulin signaling (e.g., IR, AKT, GLUT-4, PPAR-γ, DPP-4) exacerbated by oxidative stress and inflammation. Literature highlights plants' multi-target actions, such as enhancing glucose uptake via PI3K/AKT activation and enzyme inhibition, positioning *S. mombin* as a candidate with validated antihyperglycemic effects in prior model

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes mellitus (DM) is a chronic, heterogeneous metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or a synergistic combination of both (Tegegne *et al*., 2024; Padhi *et al*., 2020). It has evolved into one of the most critical global health emergencies of the 21st century, posing a severe threat to individual health and socioeconomic development (Wang *et al*., 2025; Gkrinia and Belančić, 2025). The epidemiological burden of diabetes is escalating at an alarming rate; recent data indicates that approximately 537 million adults were living with diabetes in 2021, with projections estimating a rise to 783 million by 2045 (Moke *et al*., 2023). This growing prevalence is driven by aging populations, urbanization, and lifestyle transitions leading to obesity and physical inactivity, particularly in low- and middle-income countries (Huang *et al*., 2025; Lin *et al*., 2020).

Clinically, diabetes is categorized into four distinct types: Type 1 diabetes (T1DM), resulting from autoimmune β-cell destruction and absolute insulin deficiency; Type 2 diabetes (T2DM), characterized by a progressive loss of adequate β-cell insulin secretion frequently on the background of insulin resistance; gestational diabetes mellitus (GDM); and specific types due to other causes such as monogenic diabetes syndromes or diseases of the exocrine pancreas (ElSayed *et al*., 2023; Tegegne *et al*., 2024). T2DM is the most prevalent form, accounting for more than 90% of all diabetes cases globally (Młynarska *et al*., 2025; Ye *et al*., 2023).

The pathophysiology of T2DM is multifactorial and complex, primarily driven by insulin resistance in peripheral tissues such as the skeletal muscle, liver, and adipose tissue; and

pancreatic β-cell dysfunction (Galicia-Garcia *et al*., 2020; Albajy and Mihailescu, 2023). At the molecular level, insulin resistance is characterized by disruptions in the insulin signaling pathway. Under physiological conditions, insulin binds to the insulin receptor (IR), triggering a cascade of phosphorylation events involving insulin receptor substrates (IRS), phosphoinositide 3-kinase (PI3K), and protein kinase B (AKT) (Zhao *et al*., 2023). This pathway is crucial for the translocation of glucose transporter-4 (GLUT-4) to the plasma membrane to facilitate glucose uptake (Młynarska *et al*., 2025; Martiniakova *et al*., 2025). In T2DM, defects in this signaling cascade, such as impaired AKT phosphorylation and reduced GLUT-4 expression, lead to hyperglycemia (Zhao *et al*., 2023). Furthermore, the peroxisome proliferator-activated receptor-gamma (PPAR-γ) plays a vital role in regulating lipid metabolism and insulin sensitivity, while dipeptidyl peptidase-4 (DPP-4) degrades incretin hormones, thereby reducing insulin secretion (Padhi *et al*., 2020; Wang *et al*., 2025). Oxidative stress and chronic inflammation further exacerbate these metabolic defects, linking obesity to insulin resistance and β-cell failure (Moke *et al*., 2023; Thomaz *et al*., 2024).

The management of T2DM remains a significant therapeutic challenge. Standard pharmacotherapy includes biguanides (metformin), sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists (Shah *et al*., 2025; Al-Madhagi, 2025). While effective, these synthetic agents are often associated with limitations such as adverse side effects (e.g., hypoglycemia, weight gain, gastrointestinal issues), high costs, and limited accessibility in resource-poor settings (Padhi *et al*., 2020; Tegegne *et al*., 2024). Consequently, there is a growing interest in exploring medicinal plants and natural products as alternative or complementary therapeutic agents. Bioactive phytochemicals from plants, including flavonoids, tannins, and saponins, offer a multi-target approach to managing diabetes by modulating gene

expression, enhancing insulin sensitivity, and reducing oxidative stress with fewer side effects (Rahman *et al*., 2022; Shaik Mohamed Sayed *et al*., 2023).

Spondias mombin L. , commonly known as "Hog plum" or "Iyeye" in Nigeria, is a deciduous tree belonging to the Anacardiaceae family with significant ethnomedicinal value (Akwu *et al*., 2024; Gobinath *et al*., 2022). Traditional healers utilize various parts of the plant, particularly the leaves, to treat digestive disorders, inflammation, and diabetes (Rafiu *et al*., 2025). Recent pharmacological studies have validated the plant's therapeutic potential, demonstrating significant antioxidant, antimicrobial, and anti-inflammatory activities (Gobinath *et al*., 2022). Specifically, methanolic extracts of *Spondias mombin* leaves have shown potent antihyperglycemic and antihyperlipidemic effects in streptozotocin-induced diabetic rat models, comparable to standard drugs like glibenclamide (Gobinath *et al*., 2022). Furthermore, combinatorial treatments involving *Spondias mombin* and metformin have been reported to mitigate hepatorenal injury in diabetic models, suggesting a synergistic potential (Akwu *et al*., 2024).

Despite the established hypoglycemic efficacy of *Spondias mombin*, the precise molecular mechanisms underlying its antidiabetic effects remain to be fully elucidated. While its antioxidant capacity is well-documented (Moke *et al*., 2023), there is a paucity of data regarding its specific influence on the expression of key genes involved in the insulin signaling pathway. This study aims to investigate the effect of *Spondias mombin* leaf extract on the expression of insulin signaling genes, including *Insulin Receptor (IR)*, *Protein Kinase B or AKT Serine/Theorine kinase (AKT)*, *Dipeptidyl peptidases 4 (DPP4)*, *Glucose Trasporter-4 (GLUT-4)*, and *Peroxisome proliferator activated receptor(PPAR-γ)*, in type 2 diabetic rats. By determining whether the extract modulates these molecular targets—potentially by upregulating insulin-

sensitizing genes and down-regulating insulin-degrading enzymes; this research seeks to provide scientific validation for its ethno-medicinal use and identify novel natural leads for diabetes management.

1.2 Statement of the Problem

The growing number of people with type 2 diabetes mellitus (T2DM) is a major public health issue, impacting millions and leading to serious complications like heart disease, nerve damage, and vision problems. This rise in cases is worsened by lifestyle changes, urban living, and an aging population, which put a strain on healthcare systems everywhere. While existing diabetes medications can work well for some, they are often too expensive for many people, especially in lower-income countries. Additionally, these medications might come with unwanted side effects, such as stomach issues, weight gain, and the risk of low blood sugar.

There's an immediate need for accessible, safe, and effective alternatives sourced from nature, particularly plants that can help improve how our bodies manage sugar and insulin levels. Although there are encouraging stories and some early research on traditional remedies, we still lack solid scientific evidence about how *Spondias mombin* leaf extract affects the genes involved in insulin signaling. This missing information makes it difficult to include it in diabetes treatment plans. By conducting thorough research in this area, we could discover new, plant-based options for managing diabetes more effectively.

1.3 Aim of Study

To investigate the effect of *Spondias mombin* leaf extract on the expression of insulin signaling pathway genes (Insulin Receptor, AKT, DPP4, GLUT-4, and PPAR-γ) in experimental type-2 diabetic rats.

1.4 Specific Objectives

i. To induce type 2 diabetes in experimental rats using standard induction methods (e.g., high-fat diet + streptozotocin).

ii. To administer graded doses of *Spondias mombin* leaf extract to diabetic rats.

iii. To evaluate changes in blood glucose levels and insulin sensitivity as preliminary indicators.

iv. To assess the expression levels of insulin signaling genes (IR, AKT, DPP4, GLUT-4, PPAR-γ) using molecular techniques (e.g., qRT-PCR, Western blotting).

v. To compare the gene-modulating effects of the extract with a standard antidiabetic drug (eg glypizide).

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Diabetes Mellitus

2.1.1 Definition and Classification of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Sapra and Bhandari, 2023). The disease encompasses a group of metabolic diseases that lead to inappropriately elevated blood glucose levels, which, if uncontrolled, can cause damage to various organ systems, particularly the eyes, kidneys, nerves, and cardiovascular system (Goyal *et al*., 2023).

DM is broadly classified into four main categories based on etiology and clinical presentation: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and specific types due to other causes such as monogenic diabetes syndromes (e.g., maturity-onset diabetes of the young [MODY] and neonatal diabetes) (Sapra and Bhandari, 2023). T1DM, accounting for 5% to 10% of cases, is characterized by the autoimmune destruction of pancreatic β-cells, leading to absolute insulin deficiency (Goyal *et al*., 2023). Conversely, T2DM accounts for over 90% of all diabetes cases and is defined by a combination of insulin resistance and an inadequate compensatory insulin secretory response (Hossain *et al*., 2024). GDM is hyperglycemia first detected during pregnancy, while other specific types may result from genetic defects, exocrine pancreas diseases, or drug-induced conditions (Sapra and Bhandari, 2023).

2.1.2 Global and Regional Epidemiology

Diabetes has emerged as a critical global health epidemic. In 2021, the International Diabetes Federation (IDF) estimated that approximately 537 million adults aged 20–79 years were living

with diabetes, representing 10.5% of the global population (Hossain *et al*., 2024). This prevalence is projected to rise to 643 million by 2030 and 783 million by 2045 (Hossain *et al*., 2024). The economic burden is substantial, with global healthcare expenditures related to diabetes reaching approximately $966 billion in 2021 (Hossain *et al*., 2024).

Regionally, the burden of T2DM is disproportionately high in low- and middle-income countries (LMICs), where nearly 80% of the diabetic population resides (Hossain *et al*., 2024). Asia faces a particularly rapid increase in T2DM prevalence, with young adults aged 15–39 years experiencing significant incidence rates, driven largely by urbanization and lifestyle transitions toward high-calorie diets and sedentary behaviors (Wang *et al*., 2025). In 2021, countries such as China, India, and the United States recorded the highest numbers of T2DM-related deaths caused by high body mass index (BMI) (Huang *et al*., 2025).

In the United States, T2DM-related mortality has increased substantially over the past two decades. Age-adjusted mortality rates more than doubled from 1999 to 2023, with significant disparities observed across demographic groups; mortality rates are higher among males, Black or African American populations, and residents of rural areas (Ahmed *et al*., 2025). Furthermore, the global prevalence of T2DM correlates strongly with economic development and urbanization, yet recent trends indicate a rising prevalence in lower-income nations, where undiagnosed cases remain a critical concern (Khan *et al*., 2020).

2.1.3 Pathophysiological Mechanisms of Type 2 Diabetes

Insulin Resistance and β-Cell Dysfunction

The pathophysiology of T2DM is fundamentally characterized by two core defects: insulin resistance in peripheral tissues and pancreatic β-cell dysfunction (Goyal *et al*., 2023). Insulin

resistance is defined as a diminished metabolic response to insulin in key target tissues, including skeletal muscle, the liver, and adipose tissue (Daryabor *et al*., 2020). In the early stages of the disease, pancreatic β-cells compensate for insulin resistance by increasing insulin secretion (hyperinsulinemia) to maintain euglycemia (Sapra and Bhandari, 2023). However, as the disease progresses, β-cells become exhausted and undergo functional decline, leading to relative insulin deficiency and overt hyperglycemia (Galicia-Garcia *et al*., 2020).

Recent evidence suggests that β-cell dysfunction may be the primary driver of T2DM pathogenesis, characterized by the loss of β-cell identity and dedifferentiation rather than merely cell death (Yau *et al*., 2025). Transcriptional analysis has revealed that β-cells in T2DM lose the expression of key identity markers such as *MafA* and *PDX1* while upregulating stress response genes (Yau *et al*., 2025). Furthermore, glucolipotoxicity; the combined deleterious effect of elevated glucose and fatty acids which impairs β-cell function by inducing endoplasmic reticulum (ER) stress and mitochondrial dysfunction (Galicia-Garcia *et al*., 2020).

Role of Oxidative Stress and Inflammation

Oxidative stress and chronic low-grade inflammation are pivotal in the progression of T2DM and its complications. Hyperglycemia induces oxidative stress through multiple pathways, including the increased flux of glucose through the polyol pathway, the formation of advanced glycation end products (AGEs), and the activation of protein kinase C (PKC) isoforms (Caturano *et al*., 2025). These processes lead to the overproduction of reactive oxygen species (ROS), which overwhelm endogenous antioxidant defenses (e.g., superoxide dismutase, catalase, and glutathione) (Banaszak *et al*., 2024). Mitochondrial dysfunction further exacerbates ROS production, leading to cellular damage and insulin signaling impairment (Zhao *et al*., 2023).

Additionally, prolonged hyperglycemia can lead to reductive stress (accumulation of NADH), which paradoxically fuels further oxidative stress (Tegegne *et al*., 2024).

Inflammation is intricately linked to insulin resistance, a state often described as "metaflammation" (Galicia-Garcia *et al*., 2020). In obesity and T2DM, adipose tissue macrophages polarize from an anti-inflammatory M2 phenotype to a pro-inflammatory M1 phenotype, secreting cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) (Berbudi *et al*., 2025). These inflammatory mediators activate stress kinases, such as c-Jun N-terminal kinase (JNK) and IκB kinase-β (IKKβ), which inhibit insulin signaling by phosphorylating insulin receptor substrates (IRS) at serine residues (Zhao *et al*., 2023).

Dysregulation of Insulin Signaling Genes

At the molecular level, T2DM is driven by the dysregulation of key genes and proteins within the insulin signaling cascade.

- **Insulin Receptor (IR) and IRS:** Inflammation and oxidative stress impair the function of the insulin receptor and insulin receptor substrate-1 (IRS-1). Specifically, ROS and cytokines promote the serine phosphorylation of IRS-1, preventing its tyrosine phosphorylation and subsequent activation of phosphatidylinositol 3-kinase (PI3K) (Zhao *et al*., 2023).
- **PI3K/AKT Pathway:** The impairment of PI3K activation leads to reduced phosphorylation of AKT (Protein Kinase B). AKT is a critical node that regulates glucose metabolism; its inhibition compromises downstream effectors, including glycogen

synthase kinase 3 (GSK3) and FOXO1, thereby promoting hepatic gluconeogenesis and reducing glycogen synthesis (Galicia-Garcia *et al*., 2020).

- **GLUT-4:** In insulin-sensitive tissues like skeletal muscle and adipose tissue, the translocation of Glucose Transporter-4 (GLUT-4) to the plasma membrane is essential for glucose uptake. In T2DM, oxidative stress and inflammation downregulate GLUT-4 expression and translocation, directly contributing to hyperglycemia (Wang *et al*., 2025; Zhao *et al*., 2023).
- **PPAR-γ:** Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a nuclear transcription factor essential for adipocyte differentiation and lipid metabolism. Its downregulation or impaired activity in T2DM exacerbates insulin resistance and lipotoxicity (Tegegne *et al*., 2024).
- **DPP-4:** Dipeptidyl peptidase-4 (DPP-4) is an enzyme that degrades incretin hormones like glucagon-like peptide-1 (GLP-1). In T2DM, the activity of DPP-4 is effectively unopposed or upregulated relative to the diminished incretin effect, leading to reduced insulin secretion and increased glucagon release; consequently, DPP-4 inhibitors are utilized as therapeutic agents to restore incretin levels (Tegegne *et al*., 2024).

2.2 Complications Associated with Type 2 Diabetes

Type 2 diabetes mellitus (T2DM) constitutes a chronic metabolic disorder characterized by persistent hyperglycemia arising from insulin resistance and pancreatic β-cell dysfunction (Sami *et al*., 2025). The chronic hyperglycemic state, in conjunction with associated metabolic abnormalities such as dyslipidemia and hypertension, precipitates profound vascular damage. These complications are broadly categorized into microvascular pathologies, affecting the retina, kidneys, and nerves, and macrovascular pathologies, manifesting as cardiovascular,

cerebrovascular, and peripheral arterial diseases (Islam *et al*., 2025). The etiology of these complications is multifactorial, involving complex interactions between metabolic disturbances, oxidative stress, and genetic predisposition (Zhou *et al*., 2025).

2.2.1 Metabolic Disturbances and Oxidative Imbalance

The pathophysiology underlying diabetic vascular complications is fundamentally linked to endothelial dysfunction driven by metabolic toxicity (Yang *et al*., 2024). Chronic hyperglycemia induces the overproduction of reactive oxygen species (ROS), primarily through the mitochondrial electron transport chain, which overwhelms endogenous antioxidant defense mechanisms (Singh *et al*., 2022). This oxidative stress activates several deleterious biochemical pathways that compromise vascular integrity.

First, excess intracellular glucose is shunted into the polyol pathway, where aldose reductase reduces glucose to sorbitol, consuming NADPH in the process. The depletion of NADPH impairs the regeneration of glutathione, a critical antioxidant, thereby exacerbating oxidative stress and cellular susceptibility to damage (Goldney *et al*., 2023; Singh *et al*., 2022). Second, hyperglycemia promotes the formation of advanced glycation end products (AGEs). The interaction of AGEs with their receptor (RAGE) induces a proinflammatory signaling cascade involving nuclear factor-kappa B (NF-κB), leading to the upregulation of adhesion molecules such as VCAM-1 and ICAM-1, and cytokines like TNF-α and IL-6 (Yang *et al*., 2024; Singh *et al*., 2022).

Third, hyperglycemia activates protein kinase C (PKC) isoforms via the synthesis of diacylglycerol (DAG). PKC activation results in basement membrane thickening, vascular occlusion, and increased permeability by upregulating vascular endothelial growth factor (VEGF)

and transforming growth factor-beta (TGF-β) (Singh *et al*., 2022; Goldney *et al*., 2023). Finally, the hexosamine pathway is upregulated, leading to increased production of UDP-N-acetylglucosamine, which modifies proteins and alters gene expression related to PAI-1 and TGF-β, further contributing to fibrosis and vascular dysfunction (Singh *et al*., 2022). Collectively, these metabolic disturbances result in endothelial-to-mesenchymal transition (EndMT), apoptosis, and impaired vasodilation due to reduced nitric oxide bioavailability (Yang *et al*., 2024).

2.2.2 Microvascular Complications

Microvascular complications are characterized by damage to small vessels and include nephropathy, retinopathy, and neuropathy.

Diabetic Nephropathy (DKD)

Diabetic kidney disease (DKD) affects approximately 30-40% of individuals with diabetes and is a leading cause of end-stage renal disease (Sami *et al*., 2025). Pathologically, DKD involves glomerular hypertrophy, mesangial expansion, and podocyte injury, driven by hemodynamic changes and inflammation (Tariq *et al*., 2024). Hyperglycemia-induced oxidative stress and inflammation lead to fibrosis and glomerulosclerosis. The genetic basis of DKD is significant, with heritability estimates ranging from 34% to 59% (Zhou *et al*., 2025). Genome-wide association studies (GWAS) have identified susceptibility loci such as *ELMO1*, which promotes extracellular matrix accumulation, and *COL4A3*, variants of which are associated with glomerular basement membrane thickness (Tariq *et al*., 2024; Cole *et al*., 2020). Furthermore, variants in the *ACE* gene (insertion/deletion polymorphisms) and *ACACB* gene have been credibly linked to DKD susceptibility and macroalbuminuria (Zhou *et al*., 2025; Sami *et al*.,

2025). In the Emirati population, specific associations with the *PPP1R3A*, *ZNF136*, and *HSPA12B* genes have been identified, linking glycogen synthesis and oxidative stress responses to renal deterioration (Mansour *et al*., 2023).

Diabetic Retinopathy (DR)

Diabetic retinopathy is the most common microvascular complication and a leading cause of blindness (Dal Canto *et al*., 2019). It is classified into non-proliferative (NPDR) and proliferative (PDR) stages, the latter characterized by neovascularization driven by ischemia and VEGF overexpression (Yang *et al*., 2024). The pathogenesis involves pericyte loss, breakdown of the blood-retinal barrier, and neurodegeneration (Goldney *et al*., 2023). Genetic factors account for 25-50% of DR risk (Tariq *et al*., 2024). Highly credible genetic associations include SNPs in the *VEGF* gene (e.g., rs3025039) and the *MCP-1* gene, which are linked to inflammation and angiogenesis (Zhou *et al*., 2025). Exome-wide analyses have also implicated the *SHANK3* and *ZSCAN5A* genes, which may influence fibrinogen levels and inflammatory responses within the retina (Mansour *et al*., 2023). Additionally, polymorphisms in the *GRB2* gene have been associated with sight-threatening retinopathy in multi-ethnic cohorts (Tariq *et al*., 2024).

Diabetic Neuropathy (DPN)

Diabetic neuropathy affects both the peripheral and autonomic nervous systems, leading to pain, ulceration, and amputation (Islam *et al*., 2025). Oxidative stress targets nerve fibers, causing demyelination and axonal atrophy (Goldney *et al*., 2023). Although less studied than other complications, genetic susceptibility is evident. The *SOD2* gene, which encodes the antioxidant

enzyme superoxide dismutase 2, has shown a credible association with peripheral neuropathy, highlighting the critical role of oxidative defense mechanisms (Zhou *et al*., 2025). Other implicated genes include *ADH4* and *SLC11A1*, which are associated with lipid metabolism and iron homeostasis, respectively, influencing nerve health (Mansour *et al*., 2023). Polymorphisms in *ACE* and *MTHFR* are also considered significant risk factors, affecting vascular supply to nerves and homocysteine metabolism (Sami *et al*., 2025).

2.2.3 Macrovascular Complications

Macrovascular complications, encompassing coronary artery disease (CAD), cerebrovascular disease, and peripheral artery disease (PAD), are the primary cause of mortality in T2DM patients (Dal Canto *et al*., 2019). The central pathological process is accelerated atherosclerosis, characterized by endothelial injury, lipid deposition, and plaque formation (Yang *et al*., 2024). Insulin resistance and hyperglycemia promote a pro-thrombotic and pro-inflammatory state, exacerbating arterial stiffness and narrowing (Islam *et al*., 2025).

Genetic predisposition to cardiovascular disease in diabetes involves loci distinct from those governing hyperglycemia alone. The *TCF7L2* gene, a major risk factor for T2DM, has variants strongly associated with CAD in diabetic patients (Tariq *et al*., 2024). The 9p21 chromosomal region, specifically the *ANRIL* locus, is a major susceptibility marker for macrovascular disease, influencing vascular smooth muscle cell proliferation (Tariq *et al*., 2024; Sami *et al*., 2025). Furthermore, variants in *GLUL* and *MGMT* have been specifically associated with cardiovascular mortality in individuals with T2DM (Cole *et al*., 2020). In specific populations, genes such as *PCNT* (associated with cardiac structure) and *SEPT14* (involved in ROS repression) have been linked to cardiovascular complications (Mansour *et al*., 2023).

2.2.4 Link to Gene Dysregulation Causing Vascular Issues

The transition from metabolic disturbance to vascular pathology is mediated by gene dysregulation. Hyperglycemia induces epigenetic changes and alters the expression of genes controlling inflammation, oxidative stress, and vascular homeostasis (Tariq *et al*., 2024). For instance, the dysregulation of microRNAs (miRNAs) plays a crucial role; miR-21 is upregulated in DKD, promoting fibrosis via the PTEN/Akt pathway, while miR-126, which is vasculoprotective, is downregulated in T2DM, impairing vascular integrity (Tariq *et al*., 2024).

Gene dysregulation also manifests through the alteration of specific signaling pathways. The PI3K/AKT pathway, central to insulin signaling, is heavily implicated. Its impairment leads to reduced nitric oxide production and defective glucose uptake (Feng *et al*., 2024). Conversely, the aberrant activation of the TGF-β signaling pathway drives endothelial-to-mesenchymal transition (EndMT), a process where endothelial cells lose their phenotype and acquire fibrotic characteristics, contributing to atherosclerosis and renal fibrosis (Yang *et al*., 2024).

Furthermore, individual genetic variants modulate the susceptibility to these dysregulatory processes. For example, the *EPO* gene variant rs1617640 shows a highly credible association with combined microvascular endpoints, suggesting a shared genetic architecture involving erythropoietin regulation (Zhou *et al*., 2025). The *ALR2* gene (aldose reductase) variants influence the flux through the polyol pathway, directly linking genetic variation to the severity of osmotic stress and tissue damage (Tariq *et al*., 2024). Thus, the vascular complications of T2DM are not merely consequences of hyperglycemia but result from a complex interplay where genetic variants determine the susceptibility of vascular tissues to metabolic insults through specific dysregulated molecular pathways.

2.3 Conventional Management of Type 2 Diabetes

The management of Type 2 Diabetes Mellitus (T2DM) has undergone a paradigm shift from a purely glucocentric model to a comprehensive strategy that addresses cardiorenal risk factors and weight management alongside glycemic control. Current consensus reports emphasize a patient-centered approach that integrates lifestyle modifications with a sequential or combination pharmacological strategy (Davies *et al*., 2022).

2.3.1 Lifestyle Modification and Dietary Management

Lifestyle interventions constitute the foundational pillar of T2DM management. The primary objectives are the achievement of glycemic targets, weight reduction, and the mitigation of cardiovascular risk factors. Medical nutrition therapy is essential, with current guidelines recommending an individualized approach rather than a universal diet (Petroni *et al*., 2021). The Mediterranean diet is strongly supported by evidence, demonstrating superior efficacy in reducing glycated hemoglobin (HbA1c) by 0.3–0.47% compared to low-fat diets, while simultaneously improving lipid profiles and reducing cardiovascular events (Milenkovic *et al*., 2021; Yin *et al*., 2022). Furthermore, very-low-calorie diets (VLCDs) and meal replacements (MRs) have emerged as effective strategies for inducing significant weight loss and potentially achieving diabetes remission (Edwards-Hampton and Ard, 2024; Petroni *et al*., 2021). Total diet replacement using MRs has shown greater weight loss efficacy (approximately 10 kg) compared to food-based diets, which is critical given that substantial weight loss (≥10–15 kg) is often required to modify the disease trajectory (Edwards-Hampton and Ard, 2024).

Physical activity complements dietary changes by enhancing insulin sensitivity and glucose uptake. A consensus statement from the American College of Sports Medicine indicates that

regular aerobic exercise can reduce HbA1c by 0.5–0.7% (Kanaley *et al*., 2022). Resistance training is equally vital, particularly for older adults, as it improves skeletal muscle mass and insulin sensitivity, potentially reducing HbA1c by threefold more than calorie restriction alone in sarcopenic populations (Kanaley *et al*., 2022). High-intensity interval exercise (HIIE) has also demonstrated efficacy in improving beta-cell function and reducing postprandial hyperglycemia (Kanaley *et al*., 2022). Furthermore, interrupting prolonged sedentary time with brief bouts of activity can attenuate postprandial glucose and insulin levels, particularly in individuals with higher body mass index (BMI) (Kanaley *et al*., 2022).

2.3.2 Oral Hypoglycemic Agents

Pharmacotherapy is initiated when lifestyle modifications fail to achieve glycemic targets. Metformin remains the preferred first-line oral agent worldwide due to its proven efficacy, safety profile, low cost, and potential cardioprotective benefits (Drzewoski and Hanefeld, 2021; Davies *et al*., 2022). Metformin effectively lowers fasting plasma glucose by inhibiting hepatic gluconeogenesis and enhancing peripheral insulin sensitivity (Cheng *et al*., 2024; Drzewoski and Hanefeld, 2021). It is also associated with pleiotropic effects, including potential anti-aging and anti-cancer properties, and has demonstrated safety in patients with stable chronic kidney disease (CKD) (Drzewoski and Hanefeld, 2021; Petrie, 2024).

Sulfonylureas (SUs) are widely utilized as second-line agents, particularly in resource-constrained settings, due to their high efficacy and affordability (Mohajan and Mohajan, 2024; Trailokya *et al*., 2025). SUs stimulate insulin secretion by binding to the sulfonylurea receptor 1 (SUR1) subunit of ATP-sensitive potassium (K_{ATP}) channels on pancreatic beta-cells, leading to membrane depolarization and calcium influx (Sahin *et al*., 2024). While older generation SUs

like glibenclamide are associated with higher risks of hypoglycemia and cardiovascular issues, newer agents such as gliclazide modified release (MR) exhibit high selectivity for pancreatic SUR1 over cardiac SUR2A receptors, resulting in a lower risk of hypoglycemia and cardiovascular neutrality (Sahin *et al*., 2024; Wang *et al*., 2023).

Alternative oral agents include dipeptidyl peptidase-4 (DPP-4) inhibitors, which enhance incretin levels to stimulate glucose-dependent insulin secretion (Florentin *et al*., 2022). DPP-4 inhibitors are weight-neutral and have a low risk of hypoglycemia, making them suitable for elderly patients and those with renal impairment (Yin *et al*., 2022). Sodium-glucose cotransporter-2 (SGLT2) inhibitors, such as empagliflozin and dapagliflozin, induce glycosuria and have demonstrated significant benefits in reducing heart failure hospitalizations and progression of renal disease (Nelinson *et al*., 2021; Keller *et al*., 2022). Additionally, oral formulations of glucagon-like peptide-1 receptor agonists (GLP-1 RAs), specifically oral semaglutide, utilize absorption enhancers like sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC) to overcome gastrointestinal degradation, offering comparable efficacy to injectable formulations in reducing HbA1c and body weight (Andersen *et al*., 2021; Marassi and Fadini, 2025).

2.3.3 Limitations of Conventional Therapy

Despite the availability of multiple therapeutic classes, achieving and maintaining glycemic goals remains challenging. Several limitations hinder the effectiveness of conventional therapies:

1. **Adherence:** Medication non-adherence is a pervasive issue, with only 54% of patients maintaining good adherence to oral antidiabetic drugs (Piragine *et al*., 2023). Adherence is negatively impacted by complex dosing regimens, polypharmacy, and the

asymptomatic nature of the disease in its early stages (Piragine *et al*., 2023; Chantzaras and Yfantopoulos, 2025).

2. **Side Effects:** Metformin is associated with gastrointestinal adverse events (GI AEs) such as diarrhea, bloating, and abdominal pain in approximately 20–30% of patients, which can lead to treatment discontinuation (Nabrdalik *et al*., 2024; Cheng *et al*., 2024). SUs carry a risk of hypoglycemia and weight gain (1–4 kg), which can deter adherence and complicate weight management efforts (Mohajan and Mohajan, 2024).
3. **Therapeutic Inertia:** A critical barrier to optimal care is therapeutic inertia, defined as the failure to intensify or de-intensify treatment when appropriate (Rodriguez *et al*., 2024). Delays in treatment intensification can persist for years, exposing patients to prolonged hyperglycemia (Almigbal *et al*., 2023). Factors contributing to inertia include clinician time constraints, lack of resources, and patient reluctance to escalate therapy (Rodriguez *et al*., 2024).
4. **Cost and Access:** The high cost of newer agents like SGLT2 inhibitors and GLP-1 RAs creates disparities in access, particularly in low-income populations and developing countries (Galindo *et al*., 2023). Conversely, older drugs like SUs and metformin remain essential in these settings due to their affordability (Trailokya *et al*., 2025).
5. **Resistance and Secondary Failure:** T2DM is a progressive disease characterized by the gradual decline of beta-cell function. Consequently, monotherapies, particularly SUs, often fail to maintain glycemic control over time, a phenomenon known as secondary failure (Sahin *et al*., 2024; Mohajan and Mohajan, 2024).

2.3.4 Mechanism of Action: AKT and PPAR-γ Pathways

To understand the molecular impact of oral hypoglycemic agents beyond simple glucose lowering, it is necessary to examine their interaction with intracellular signaling pathways, specifically the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway and the peroxisome proliferator-activated receptor-gamma (PPAR-γ).

Insulin resistance in T2DM is often characterized by defects in the PI3K/AKT signaling cascade, which is responsible for the translocation of glucose transporter 4 (GLUT4) to the cell membrane in muscle and adipose tissue (Yan *et al*., 2020). Metformin has been shown to restore this pathway. While classically known to activate AMP-activated protein kinase (AMPK), metformin also promotes GLUT4 translocation via the restoration of PI3K/AKT signaling (Yan *et al*., 2020). Furthermore, metformin downregulates thioredoxin-interacting protein (TXNIP), a negative regulator of glucose uptake, thereby enhancing AKT phosphorylation and glucose influx (Yan *et al*., 2020).

Thiazolidinediones (TZDs), such as pioglitazone, function as insulin sensitizers by activating the nuclear receptor PPAR-γ. Upon activation, PPAR-γ heterodimerizes with the retinoid X receptor (RXR) and binds to specific DNA response elements, regulating the transcription of genes involved in glucose and lipid metabolism (Tegegne *et al*., 2024). This activation leads to increased expression of GLUT4 and adiponectin, reduced inflammatory cytokines, and improved insulin sensitivity in peripheral tissues (Alnuaimi *et al*., 2024).

Recent research suggests a convergence of these pathways. Metformin has been observed to activate the PPAR-γ/PI3K/AKT signaling pathway in certain contexts, further elucidating its multifaceted role in combating insulin resistance (Yan *et al*., 2020). Combination therapies

leveraging these mechanisms, such as metformin plus PPAR agonists (e.g., tesaglitazar, a dual PPAR α/γ agonist), have shown superior efficacy in reducing HbA1c, fasting insulin, and HOMA-IR compared to metformin alone (Alnuaimi *et al*., 2024). This synergistic approach targets multiple pathophysiological defects, potentially overcoming the resistance observed with monotherapy (Alnuaimi *et al*., 2024).

2.4 Role of Medicinal Plants in Diabetes Management

2.4.1 Overview of Phytotherapy and Ethnomedicine in Diabetes Treatment

Diabetes mellitus is a chronic, multifactorial metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or a combination of both (Ansari *et al*., 2024). The global burden of diabetes is escalating, necessitating effective therapeutic strategies. While conventional pharmacotherapies such as biguanides (metformin), sulfonylureas, and thiazolidinediones are the standard of care, their long-term use is frequently associated with adverse side effects, including hypoglycemia, lactic acidosis, gastrointestinal distress, weight gain, and hepatotoxicity (Asaad *et al*., 2025; Ansari *et al*., 2023). Consequently, there has been a paradigm shift towards investigating phytotherapy and ethnomedicine as complementary or alternative approaches. Medicinal plants have historically served as primary healthcare resources in developing nations due to their cultural acceptability, accessibility, and affordability (Ajayi *et al*., 2020). Ethnobotanical surveys reveal that traditional practitioners utilize a diverse array of plant species, often prepared as decoctions or infusions, to manage diabetes and its associated complications (Ajayi *et al*., 2020).

Modern pharmacological research validates these traditional practices, demonstrating that medicinal plants are rich reservoirs of bioactive phytochemicals, including flavonoids, alkaloids,

glycosides, terpenoids, saponins, and tannins (Alam *et al*., 2022). These plant-derived secondary metabolites do not merely provide symptomatic relief but target specific molecular pathways involved in glucose homeostasis, mimicking or synergizing with synthetic antidiabetic agents (Ansari *et al*., 2023). For instance, the transition from traditional knowledge to modern drug discovery is exemplified by metformin, which was derived from *Galega officinalis* (Alhajje *et al*., 2024). Current research focuses on elucidating the molecular mechanisms of these phytochemicals to integrate them into evidence-based medical biochemistry.

2.4.2 Mechanisms of Antidiabetic Effects

The antidiabetic efficacy of medicinal plants is mediated through pleiotropic mechanisms that address the complex pathophysiology of diabetes. These mechanisms include the stimulation of insulin secretion, enhancement of peripheral glucose uptake, inhibition of carbohydrate-digesting enzymes, mitigation of oxidative stress and inflammation, and the modulation of gene expression within insulin signaling pathways.

Stimulation of Insulin Secretion

A primary mechanism by which plant extracts exert hypoglycemic effects is the stimulation of insulin secretion from pancreatic β-cells. Certain phytochemicals function as insulin secretagogues, operating through mechanisms similar to sulfonylureas. They induce the closure of ATP-sensitive potassium channels (K_{ATP}) on the β-cell membrane, leading to membrane depolarization, calcium influx, and the subsequent exocytosis of insulin granules (Ansari *et al*., 2024). For example, extracts from *Moringa oleifera* have been shown to enhance insulin levels in diabetic models, potentially by protecting β-cells from glucotoxicity-induced apoptosis or by directly stimulating secretion (Hegazy *et al*., 2025). Additionally, alkaloids such as vindoline and

vindolicine isolated from *Catharanthus roseus* have demonstrated the ability to enhance insulin secretion in pancreatic β-TC6 cells (Alhajje *et al*., 2024). Unlike synthetic secretagogues that may cause β-cell exhaustion, certain plant-derived compounds, such as those found in *Vernonia amygdalina*, have been reported to promote the regeneration of pancreatic β-cells and restore islet architecture, thereby addressing the underlying insulin deficiency (Akpan and Okugbo, 2025).

Enhancement of Glucose Uptake

Improving insulin sensitivity and glucose uptake in peripheral tissues, particularly skeletal muscle and adipose tissue, is critical for managing postprandial hyperglycemia. Medicinal plants enhance glucose transport primarily by promoting the translocation of Glucose Transporter 4 (GLUT4) from intracellular vesicles to the plasma membrane (Clemente-Suárez *et al*., 2025). Flavonoids such as quercetin and kaempferol are potent activators of the AMP-activated protein kinase (AMPK) pathway, a master regulator of cellular energy homeostasis (Liu *et al*., 2025). Activation of AMPK mimics the effects of exercise and metformin, facilitating glucose uptake independently of insulin. Furthermore, plant extracts can sensitize the insulin signaling pathway by enhancing the tyrosine phosphorylation of the insulin receptor substrate (IRS) and activating the Phosphoinositide 3-kinase (PI3K)/Protein Kinase B (Akt) pathway (Pattanaik *et al*., 2024). This cascade results in increased glycogen synthesis and reduced hepatic gluconeogenesis. For instance, polysaccharides from *Polygonatum* species have been shown to upregulate GLUT4 expression and promote glucose consumption in insulin-resistant cells via the PI3K/Akt pathway (Shi *et al*., 2023).

Inhibition of Carbohydrate-Digesting Enzymes

Control of postprandial hyperglycemia is achieved by delaying the digestion of dietary carbohydrates. Medicinal plants contain inhibitors of α-amylase and α-glucosidase, enzymes responsible for breaking down complex polysaccharides into absorbable monosaccharides (Sadeghi *et al*., 2024). By inhibiting these enzymes, plant extracts effectively flatten the postprandial glucose spike, functioning similarly to acarbose but often with fewer gastrointestinal side effects such as bloating and diarrhea (Lam *et al*., 2024). Flavonoids, including luteolin, quercetin, and apigenin, have demonstrated significant inhibitory activity against these enzymes. The structural features of flavonoids, such as the C2-C3 double bond and hydroxyl groups on the B-ring, are critical for their binding affinity to the active sites of these digestive enzymes (Sadeghi *et al*., 2024). *Moringa oleifera* extracts also exhibit strong α-glucosidase inhibitory activity, attributed to their high content of fatty acids like palmitic and α-linolenic acids, providing a dual mechanism of antioxidant protection and enzyme inhibition (Al Shammari *et al*., 2025).

Antioxidant and Anti-inflammatory Effects

Chronic hyperglycemia induces the production of reactive oxygen species (ROS), leading to oxidative stress and low-grade inflammation, which are central drivers of insulin resistance and β-cell failure (Alhajje *et al*., 2024). Medicinal plants are rich sources of antioxidants, particularly polyphenols, which scavenge free radicals and bolster endogenous antioxidant defenses. *Moringa oleifera* leaf extracts, for instance, activate the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, leading to the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Hegazy *et al*., 2025). This amelioration of oxidative stress protects tissues from diabetic complications. Concurrently, plant

bioactives inhibit the nuclear factor kappa B (NF-κB) signaling pathway, suppressing the production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β (Scarpa *et al*., 2024). By reducing systemic inflammation, these compounds improve insulin signaling in peripheral tissues and prevent the progression of diabetic nephropathy and hepatotoxicity (Tahir and Farooq, 2025).

Modulation of Gene Expression in Insulin Signaling

Advanced phytotherapy involves the modulation of gene expression related to glucose metabolism. Polyphenols have been documented to upregulate the expression of genes critical for insulin sensitivity, including *GLUT4*, *IRS1*, *PI3K*, and *Akt* (Hegazy *et al*., 2025). Certain dietary flavonoids can activate Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ), a nuclear receptor that regulates fatty acid storage and glucose metabolism, thereby functioning similarly to thiazolidinediones to improve insulin sensitivity (Pattanaik *et al*., 2024). Additionally, plant secondary metabolites such as alkaloids and stilbenes have been shown to inhibit Protein Tyrosine Phosphatase 1B (PTP1B), a negative regulator of insulin signaling, thereby prolonging the insulin signal (Alhajje *et al*., 2024). Furthermore, some plant extracts inhibit the enzyme Dipeptidyl Peptidase-4 (DPP-4), extending the half-life of glucagon-like peptide-1 (GLP-1) and enhancing glucose-dependent insulin secretion (Tang *et al*., 2025).

2.4.3 Examples of Plants with Documented Antidiabetic Activity

***Vernonia amygdalina* (Bitter Leaf)**

Vernonia amygdalina is a staple in African ethnomedicine, renowned for its hypoglycemic properties. Aqueous extracts of *V. amygdalina* significantly reduce blood glucose levels in

streptozotocin-induced diabetic rats, with efficacy comparable to the sulfonylurea drug glimepiride (Adekemi *et al*., 2024). The plant's antidiabetic action is linked to the regeneration of pancreatic β-cells and the restoration of islet architecture, offering potential for reversing insulin deficiency (Akpan and Okugbo, 2025). Furthermore, *V. amygdalina* ameliorates diabetic hepatotoxicity by normalizing serum liver markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reducing liver inflammation (Adekemi *et al*., 2024). Its phytochemical profile includes sesquiterpene lactones, vernodalol, and luteolin, which contribute to its antioxidant capacity and ability to inhibit α-glucosidase (Degu *et al*., 2024). Synergistic effects have also been observed when *V. amygdalina* is combined with metformin or other plant extracts like *Dacryodes edulis*, leading to enhanced β-cell regeneration (Akpan and Okugbo, 2025).

***Azadirachta indica* (Neem)**

Azadirachta indica, commonly known as Neem, is extensively used in traditional medicine for its broad therapeutic spectrum. Methanolic extracts of *A. indica* seeds have demonstrated dose-dependent hypoglycemic effects in diabetic Wistar rats (Abduallah *et al*., 2023). High doses (350 mg/kg) of the extract were found to normalize blood glucose levels and improve lipid profiles, comparable to standard medication. The antidiabetic efficacy of Neem is attributed to its ability to improve insulin sensitivity, reduce lipid peroxidation, and protect against oxidative stress-induced organ damage (Abduallah *et al*., 2023). Bioactive constituents such as nimbidin and quercetin in Neem leaves contribute to the upregulation of GLUT4 and the inhibition of intestinal glucose absorption (Ansari *et al*., 2024).

***Moringa oleifera* (Drumstick Tree)**

Moringa oleifera is characterized as a "miracle plant" due to its dense nutritional and phytochemical profile. Leaf extracts of *M. oleifera* have shown profound antidiabetic effects by reducing insulin resistance and liver damage (Hegazy *et al*., 2025). Mechanistically, *M. oleifera* upregulates the expression of insulin signaling genes (*IRS1*, *PI3K*, *Akt*, *GLUT4*) while suppressing inflammatory markers (*NF-κB*, *TNF-α*) and oxidative stress indicators (*MDA*) (Hegazy *et al*., 2025). Additionally, the plant exhibits strong α-glucosidase inhibitory activity, aiding in the control of postprandial hyperglycemia (Al Shammari *et al*., 2025). Its ability to activate the Nrf2 antioxidant pathway further supports cellular resilience against diabetic complications, including nephropathy and cardiomyopathy (Mthiyane *et al*., 2022).

Spondias mombin **(Hog Plum)**

Spondias mombin is utilized in traditional medicine for various ailments, including diabetes-related reproductive dysfunction. Aqueous extracts of *S. mombin* leaves have been found to ameliorate hyperglycemia and improve sperm characteristics (motility, count, and viability) in diabetic male rats (Oloye *et al*., 2024). This protective effect on the reproductive system is linked to the plant's antioxidant properties, which mitigate oxidative stress in testicular tissue. Furthermore, solvent extracts of *S. mombin* demonstrate significant inhibitory effects against acetylcholinesterase and oxidative stress, which are relevant for managing diabetes-associated neuropathies (Evuen and Kpomah, 2023).

Polygonatum **Species (Solomon’s Seal)**

Plants of the genus *Polygonatum* are widely used in traditional Chinese medicine for treating diabetes. *Polygonatum* polysaccharides are the primary bioactive components responsible for

their antidiabetic effects. These polysaccharides have been shown to improve insulin resistance, promote insulin secretion, and protect pancreatic β-cells from apoptosis (Shi *et al*., 2023). Mechanistically, they regulate the gut microbiota, inhibit advanced glycation end-product (AGE) formation, and modulate gluconeogenesis. Specifically, saponins and homoisoflavonoids from *Polygonatum* activate the AMPK pathway and enhance cellular glucose uptake, offering a multi-targeted approach to diabetes management (Shi *et al*., 2023).

2.4.4 Gene-Specific Modulation by Plant Metabolites

A converging theme in phytotherapy is the ability of distinct plants to target similar genetic pathways via conserved bioactive metabolites. For instance, quercetin, a flavonoid found ubiquitously in plants like *Moringa oleifera*, *Vernonia amygdalina*, and *Allium cepa*, consistently upregulates *GLUT4* translocation and expression in skeletal muscle via the AMPK pathway (Liu *et al*., 2025; Pattanaik *et al*., 2024). Similarly, compounds such as kaempferol and naringenin share the capacity to inhibit *TNF-α* and *IL-6* expression, thereby reducing systemic inflammation associated with insulin resistance (Pattanaik *et al*., 2024). The modulation of the *PI3K/Akt* pathway is another common target; phytochemicals from *M. oleifera*, *Polygonatum* spp., and traditional Chinese medicines have all been observed to activate this pathway to enhance glucose uptake and glycogen synthesis (Hegazy *et al*., 2025; Shi *et al*., 2023; Tang *et al*., 2025). Furthermore, stilbenes like resveratrol and alkaloids like berberine specifically target PTP1B to enhance insulin signaling sensitivity (Alhajje *et al*., 2024). This genetic redundancy among different medicinal plants suggests that phytotherapy functions through conserved biological mechanisms, offering a robust and scientifically validated basis for their integration into diabetes management protocols.

2.5 *Spondias mombin* (Hog Plum)

2.5.1 Botanical Classification

Spondias mombin Linn. belongs to the kingdom Plantae and the order Sapindales. It is a member of the Anacardiaceae family, a flowering plant family that comprises approximately 73 genera and 850 species (Ogunro *et al*., 2023). The plant falls under the genus *Spondias* and the species *mombin* (Nwidu *et al*., 2018).

2.5.2 Description

Spondias mombin is a deciduous, erect tree that typically grows to a height of 15 to 20 meters, with a trunk diameter ranging between 60 and 75 cm (Nwidu *et al*., 2018; Ogunro *et al*., 2023). The tree is characterized by a wide canopy that can sometimes be densely closed, with branches appearing from approximately 4 meters above ground height (Maria *et al*., 2022). The bark is thick, rough, and grayish to light reddish-brown, often possessing deep fissures and producing a brown resinous substance (Maria *et al*., 2022; Ogunro *et al*., 2023).

Morphologically, the leaves are compound and imparipinnate, measuring 20 to 45 cm in length (Ogunro *et al*., 2023). They are arranged in a spiral, alternate distribution and are clustered toward the tips of the branches (Maria *et al*., 2022). Each leaf consists of 5 to 11 pairs of leaflets that are petiolate, with a glabrous upper surface and a hairy underside (Ogunro *et al*., 2023). A distinctive feature of the leaves is the emission of a green mango-like aroma when pressed (Maria *et al*., 2022).

The fruits of *S. mombin* are drupaceous berries or pseudo-drupes, which are typically elliptical or ovoid in shape and measure 3 to 4 cm in length (Ogunro *et al*., 2023; Maria *et al*., 2022). Initially

green, the fruits turn golden-yellow upon ripening, a color change attributed to their carotenoid composition (Ogunro *et al*., 2023). The fruit possesses a thin but tough skin enclosing a juicy, acidic pulp and a large, lignified endocarp that is pentagonal and covered with tubercles (Ogunro *et al*., 2023).

Regarding habitat, *S. mombin* is a strictly tropical plant found in rainforest lowlands, although it has adapted to thrive in more arid zones (Ogunro *et al*., 2023). It is widely distributed across the tropical Americas, including Mexico, Peru, Brazil, and the West Indies, and has been naturalized in parts of tropical Africa, such as Nigeria, Ghana, and Ivory Coast, as well as parts of Asia (Nwidu *et al*., 2018; Ogunro *et al*., 2023).

Figure 0.1: Spondias mombin tree (Ogunro *et al.,* 2023)

2.5.3 Ethnomedicinal Uses

Spondias mombin holds significant value in traditional medicine across various tropical regions, where almost every part of the tree; including the leaves, bark, fruit, and roots are utilized for therapeutic purposes (Ogunro *et al*., 2023). In Nigerian folk medicine, the plant is extensively employed for the treatment of psychiatric disorders and reproductive health issues (Iwu, 1993; Ogunro *et al*., 2023).

The leaves are particularly renowned for their application in reproductive health. They are used to facilitate childbirth, induce lactation, and reduce postpartum hemorrhage (Ogunro *et al*., 2023; Maria *et al*., 2022). Furthermore, leaf decoctions serve as antiseptic vaginal washes to treat postpartum infections (Ogunro *et al*., 2023). The leaves are also used in the management of diabetes mellitus in southwestern Nigeria, a practice supported by local traditional healers (Swathi and Lakshman, 2022; Ogunro *et al*., 2023).

Gastrointestinal disorders are frequently treated with *S. mombin*. Infusions and decoctions of the leaves and bark are used to manage diarrhea, dysentery, dyspepsia, gastralgia, colic, and constipation (Ogunro *et al*., 2023; Swathi and Lakshman, 2022). In various African regions, the bark and leaves are potent remedies for fever, cough, sore throat, and gonorrhea (Maria *et al*., 2022; Ogunro *et al*., 2023). Additionally, the bark is utilized for its analgesic and anti-

inflammatory properties to treat arthritis, rheumatism, and muscle pain (Ogunro *et al*., 2023). The plant is also applied externally for the treatment of wounds, infected cuts, rashes, and dermatitis due to its antimicrobial and cicatrizing properties (Ogunro *et al*., 2023).

2.5.4 Phytochemical Constituents

The therapeutic efficacy of *S. mombin* is attributed to its rich diversity of secondary metabolites. Phytochemical screening of the leaf and stem extracts has revealed the presence of tannins, saponins, alkaloids, flavonoids, phenols, and anthraquinones (Nwidu *et al*., 2018; Ajaegbu *et al*., 2022; Gobinath *et al*., 2022). Quantitative analysis has demonstrated significant concentrations of total phenols and flavonoids in methanolic leaf extracts, which are correlated with biological activities (Gobinath *et al*., 2022; Amos-Tautua *et al*., 2025).

Phenolic compounds are abundant in the leaves, with high-performance liquid chromatography (HPLC) profiling identifying specific constituents such as chlorogenic acid, geraniin, gallic acid, and ellagic acid (Maria *et al*., 2022; Ajaegbu *et al*., 2022). Ellagic acid and chlorogenic acid have been specifically linked to the plant's anti-inflammatory and antioxidant capacities (Maria *et al*., 2022).

In the context of diabetes management, the flavonoid content of *S. mombin* is of particular importance. Flavonoids such as quercetin, kaempferol, rutin, and isoquercitrin (quercetin-3-O-glucoside) have been isolated from the leaves (Akinmoladun *et al*., 2021; Ogunro *et al*., 2023). Notably, isoquercitrin and quercetin are linked to insulin pathways; research suggests these compounds may potentiate the secretion of insulin from pancreatic beta-cells and enhance glucose uptake by peripheral tissues (Gobinath *et al*., 2022). Furthermore, novel compounds such

as mombintane I and II, isolated from the leaves, have demonstrated glucose-lowering capabilities (Ogunro *et al*., 2023). Other bioactive compounds identified include phytol, a diterpene with anti-inflammatory properties, and various fatty acid methyl esters such as hexadecanoic acid methyl ester (Amos-Tautua *et al*., 2025).

2.5.5 Pharmacological Activities

Antioxidant Activity

The antioxidant potential of *S. mombin* has been extensively documented. Methanolic and ethanolic extracts of the leaves and stems exhibit strong free radical scavenging activity, as evidenced by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays (Nwidu *et al*., 2018; Amos-Tautua *et al*., 2025). In vivo studies involving carbon tetrachloride (CCl4)-induced hepatotoxicity in rats demonstrated that pretreatment with *S. mombin* extracts significantly elevated the levels of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and cellular glutathione (GSH) (Nwidu *et al*., 2018). The plant's ability to reduce lipid peroxidation, measured by thiobarbituric acid reactive substances (TBARS), further confirms its capacity to mitigate oxidative stress (Nwidu *et al*., 2018; Akinmoladun *et al*., 2021). This activity is largely attributed to the high content of phenolic acids and flavonoids, which act as electron donors to neutralize reactive oxygen species (Ajaegbu *et al*., 2022).

Antidiabetic and Hypoglycemic Activity

Spondias mombin demonstrates significant hypoglycemic effects, validating its traditional use in diabetes management. In streptozotocin (STZ)-induced diabetic rats, methanolic leaf extracts

significantly reduced blood glucose levels in a dose-dependent manner (Gobinath *et al*., 2022). The extract's efficacy was comparable to standard drugs like glibenclamide. Mechanistically, the extract has been shown to increase plasma insulin levels, suggesting a potential to regenerate pancreatic beta-cells or stimulate insulin secretion (Gobinath *et al*., 2022; Moke *et al*., 2023). Additionally, specific compounds isolated from the plant, such as 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate, have been identified as alpha-amylase inhibitors, which reduce postprandial hyperglycemia by retarding carbohydrate digestion (Ogunro *et al*., 2023). The plant also exhibits antihyperlipidemic effects by normalizing lipid profiles—reducing total cholesterol, triglycerides, and low-density lipoproteins (LDL) while increasing high-density lipoproteins (HDL) in diabetic models (Gobinath *et al*., 2022).

Anti-inflammatory Activity

The anti-inflammatory properties of *S. mombin* have been validated using various experimental models. In carrageenan-induced paw edema models, methanolic and hydroethanolic leaf extracts significantly inhibited edema formation, indicating an ability to modulate the acute inflammatory response (Amos-Tautua *et al*., 2025; Maria *et al*., 2022). The extract also reduced vascular permeability induced by acetic acid (Amos-Tautua *et al*., 2025). This activity is linked to the inhibition of leukocyte migration to the site of inflammation, a specific effect attributed to the presence of ellagic acid and chlorogenic acid (Maria *et al*., 2022). Furthermore, *S. mombin* extracts have shown efficacy in treating oral mucositis and stabilizing erythrocyte membranes against stress-induced hemolysis (Maria *et al*., 2022).

Hepatoprotective Activity

Spondias mombin provides significant protection against liver damage induced by xenobiotics. In studies involving CCl4-induced hepatotoxicity, pretreatment with leaf and stem extracts prevented the elevation of serum markers of hepatocellular injury, specifically alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Nwidu *et al*., 2018). Histopathological examinations revealed that the extracts preserved the cytoarchitecture of the liver, reducing necrosis and fibrosis (Nwidu *et al*., 2018). The hepatoprotective mechanism is believed to involve membrane stabilization and the enhancement of the hepatic antioxidant defense system, thereby preventing oxidative damage to hepatocytes (Nwidu *et al*., 2018).

Antimicrobial Activity

The extracts of *S. mombin* exhibit broad-spectrum antimicrobial activity. Methanolic and aqueous extracts have demonstrated inhibitory effects against a range of pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* (Maria *et al*., 2022; Swathi and Lakshman, 2022). The antimicrobial action is attributed to the presence of tannins and phenolic compounds, which can disrupt bacterial cell walls and metabolic processes. Additionally, silver nanoparticles synthesized using *S. mombin* seed extracts have shown efficacy against multidrug-resistant bacteria, suggesting potential applications in developing novel antimicrobial agents (Maria *et al*., 2022).

2.6 Experimental Studies on *Spondias mombin*

2.6.1 Antidiabetic Potential and Insulin Enhancement

The therapeutic potential of *Spondias mombin* in the management of diabetes mellitus has been extensively evaluated using various experimental models, revealing significant hypoglycemic

properties attributed to its diverse phytochemical constituents. Research indicates that the plant exerts antidiabetic effects through multiple mechanisms, including the enhancement of insulin secretion, the regeneration of pancreatic beta-cells, and the inhibition of carbohydrate-hydrolyzing enzymes.

In streptozotocin (STZ)-induced diabetic rat models, the methanolic extract of *S. mombin* leaves demonstrated a capacity to reverse hyperglycemia in a dose-dependent manner. Gobinath *et al*. (2022) reported that oral administration of the extract at doses of 125, 250, and 500 mg/kg significantly elevated plasma insulin levels. Histopathological analysis in the same study revealed a pronounced regeneration of the beta-cells of Langerhans in the pancreas, particularly at the 500 mg/kg dose, suggesting that the extract not only stimulates insulin secretion but may also facilitate the structural recovery of insulin-producing tissues (Gobinath *et al*., 2022). This regenerative capability is critical, as STZ pathology involves the destruction of beta-cells via reactive oxygen species (ROS) production and DNA alkylation.

Furthermore, comparative studies involving standard antidiabetic drugs have reinforced the efficacy of *S. mombin*. In a study evaluating the co-administration of *S. mombin* aqueous leaf extract and metformin, the plant extract exhibited superior glycemic control compared to metformin alone in certain parameters, and the combination therapy demonstrated synergistic potential in mitigating hyperglycemia (Akwu *et al*., 2022). Additionally, the ethyl acetate fraction of *S. mombin* stem bark was found to restore glucose homeostasis in STZ-induced diabetic rats, performing comparably to metformin in reducing blood glucose levels over a 14-day treatment period (Omoboyowa *et al*., 2025).

Beyond direct insulin enhancement, the inhibition of carbohydrate-digesting enzymes prevents postprandial hyperglycemia. *In vitro* assays have demonstrated that leaf and stem bark extracts of *S. mombin* specifically the ethyl acetate and n-butanol fractions exhibit inhibitory activity against α-amylase and α-glucosidase (Sinan *et al*., 2021; Omoboyowa *et al*., 2023). This mechanism delays carbohydrate digestion and glucose absorption, complementing the insulin-secretagogue properties of the plant.

2.6.2 Antioxidant and Hepatoprotective Effects

Oxidative stress is a central pathogenomic factor in diabetes, leading to cellular damage and complications such as hepatotoxicity. *Spondias mombin* has demonstrated robust antioxidant and hepatoprotective activities in experimental models, largely attributed to its high phenolic and flavonoid content.

In models of high-fat diet (HFD) induced obesity and dyslipidemia, supplementation with *S. mombin* fruit pulp significantly enhanced hepatic antioxidant status. Lucena *et al*. (2022) observed increased activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), alongside an elevation in total antioxidant capacity (TAC) in the livers of treated rats. Concurrently, the treatment reduced hepatic lipid peroxidation, measured via malondialdehyde (MDA) levels (Lucena *et al*., 2022). Similar findings were reported in STZ-induced diabetic rats, where treatment with *S. mombin* leaf extract restored depleted levels of reduced glutathione (GSH), SOD, and catalase (CAT), while significantly lowering serum MDA levels (Akwu *et al*., 2022).

The hepatoprotective efficacy of the plant is further evidenced by the normalization of liver function markers. Diabetic rats typically exhibit elevated serum levels of aspartate

aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) due to hepatocellular leakage. Treatment with *S. mombin* extracts consistently reduced these enzymes to near-normal levels, indicating preservation of hepatic membrane integrity (Gobinath *et al*., 2022; Omoboyowa *et al*., 2025). Histological examinations confirm these biochemical findings; liver sections from treated animals showed reduced fatty accumulation, preserved hepatocyte morphology, and diminished periportal inflammation compared to untreated diabetic controls (Akwu *et al*., 2022; Gobinath *et al*., 2022).

2.6.3 Dose-Dependent Efficacy and Safety Profiles

While the therapeutic benefits of *S. mombin* are evident, the safety profile is contingent upon dosage. Experimental data suggests a non-linear relationship between dose and toxicity, necessitating the establishment of safe therapeutic windows.

In efficacy studies, dose-dependent improvements in glycemic control and lipid profiles were observed between 125 mg/kg and 500 mg/kg, with higher doses generally yielding superior metabolic outcomes without acute toxicity (Gobinath *et al*., 2022). Acute toxicity tests indicated no mortality at doses up to 2000 mg/kg in certain leaf extract preparations (Gobinath *et al*., 2022). Similarly, Akwu *et al*. (2022) determined an LD50 of 800 mg/kg for the aqueous leaf extract, utilizing therapeutic doses of up to 600 mg/kg with beneficial effects on hepatorenal histology.

However, toxicological evaluations of the root extract caution against high-dose administration. Ogeyemhe and Atoyebi (2025) reported that while doses up to 600 mg/kg preserved hepatic architecture, higher doses of 800 mg/kg and 1000 mg/kg induced hepatocellular injury, characterized by ballooning degeneration and microvesicular steatosis. This suggests that while the plant is relatively safe at low-to-moderate doses, high concentrations particularly of the root

extract may induce hepatotoxicity, emphasizing the need for precise dosing in therapeutic applications.

2.6.4 Gene Expression and Molecular Mechanisms

Recent investigations have utilized gene expression analysis and molecular docking to elucidate the molecular targets of *S. mombin*. These studies highlight the plant's ability to modulate critical pathways involved in glucose metabolism and inflammation.

In a Wistar rat model, Eluehike *et al*. (2022) demonstrated that the methanolic leaf extract of *S. mombin* significantly upregulated the expression of the *peroxisome proliferator-activated receptor-gamma* (PPAR-γ) gene. PPAR-γ is a nuclear receptor pivotal for insulin sensitization and lipid homeostasis. The extract also upregulated *phosphofructokinase-1* (PFK-1), a rate-limiting enzyme in glycolysis, thereby enhancing glucose utilization. Conversely, the extract downregulated the expression of pro-inflammatory cytokines, specifically *interleukin-1-beta* (IL-1β) and *tumor necrosis factor-alpha* (TNF-α), which are implicated in insulin resistance (Eluehike *et al*., 2022). *In silico* molecular docking identified alpha-sitosterol as a key ligand within the extract, exhibiting high binding affinity for PPAR-γ, GLUT-2, and inflammatory cytokine receptors (Eluehike *et al*., 2022).

Furthermore, in a high-sucrose diet-induced Type 2 diabetes model using *Drosophila melanogaster*, stem bark fractions of *S. mombin* were found to upregulate the mRNA expression of insulin signaling genes, including *insulin-like peptide 2* (ILP-2), *insulin receptor* (InR), and

ecdysone-inducible gene 2 (IMPL2) (Omoboyowa *et al*., 2023). This genetic modulation was accompanied by the restoration of glucose homeostasis and locomotor activity, suggesting that the bioactive constituents identified via HPLC as flavonoids like quercetin and rutin act directly on insulin signaling pathways (Omoboyowa *et al*., 2023).

2.6.5 Identified Gaps in Literature

Despite the promising data, gaps remain in the translational understanding of *S. mombin*. While gene expression studies have been conducted in STZ-induced rats (often mimicking Type 1 diabetes or late-stage beta-cell failure) and invertebrate models (*Drosophila*), there is a paucity of comprehensive gene-level data in mammalian models specifically designed to mimic Type 2 diabetes (e.g., high-fat diet/low-dose STZ models). Specifically, the modulation of downstream insulin signaling molecules (such as IRS-1, PI3K, and Akt/PKB) in insulin-sensitive tissues (skeletal muscle and adipose tissue) of mammal remains underexplored. Furthermore, while the root extract has shown toxicity at high doses, the chronic toxicological profile of the stem bark and leaf fractions at effective gene-modulating doses requires further validation to ensure long-term safety.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials and Apparatus Used

i. Ethanol

ii. Distilled water

iii. Citrate buffer

iv. Whatman filter paper

v. Mechanical grinder

vi. Oral gavage

vii. Syringes

viii. Electronic weigh balance

ix. Glucometer (Accu-Check Active)

x. Standard chow

xi. High fat-diet (Rat feed (1825 g) - Butter (1600 g) - Soy protein (1250 g) - Vitamin and mineral mix (300 g) - Yeast powder (5 g) - Salt (NaCl, 5 g) - Methionine (15 g))

xii. Quick-RNA MiniPrep™ Kit (Zymo Research)

xiii. DNase I (NEB, Cat: M0303S)

xiv. A&E Spectrophotometer (A&E Lab, UK)

xv. cDNA synthesis kit (ProtoScript II, New England BioLabs)

xvi. OneTaqR2X Master Mix (NEB)

xvii. Agarose gel electrophoresis apparatus

xviii. Image J software

3.2 Chemicals and Reagents Used

i. Streptozotocin

ii. Glipizide

iii. Ketamine

iv. Xylazine

v. Saline solution

vi. Primers (Inqaba Biotec, Hatfield, South Africa)

3.3 Collection and Confirmation of Plant Material

Fresh leaves of *Spondias mombin L.* (commonly known as hog plum) were obtained from a local botanical garden in the Iguomon area of Benin City, Nigeria. Professor H.A Akinnbosun, a taxonomist from the Department of Botany, University of Benin, identified and confirmed the plant. A voucher specimen was archived in the herbarium for future reference.

3.3.1 Preparation of Leaf Extract from *Spondias mombin*

The harvested leaves were thoroughly rinsed with distilled water to eliminate any dirt and debris, then air-dried in a shaded area at room temperature for 7-10 days to prevent sunlight exposure and maintain bioactive compounds. Once dried, the leaves were ground into a fine powder using a mechanical grinder. A total of 500 g of the powdered leaves was macerated in 2 L of 70% ethanol (v/v) at room temperature for 72 hours, with occasional stirring. The mixture was then filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator to yield a semi-solid ethanolic extract. The extract was stored in an airtight container at 4°C until needed. It was reconstituted in distilled water for administration to achieve the desired concentrations.

3.4 Animal Studies

3.4.1 Animal Subjects

Adult male Wistar albino rats weighing between 150-200 g were sourced from the Department of Anatomy Animal House Facility at the University of Benin. The rats were kept in standard polypropylene cages under controlled environmental conditions, with access to standard rodent chow and water *ad libitum* for a 2-week acclimatization period. Each rat was marked for identification using non-toxic markers (such as permanent ink) on specific areas like the back, head, forelimbs, hindlimbs, tail, or abdomen to track them throughout the study.

3.4.2 Preparation of High-Fat Diet (HFD)

To induce obesity and insulin resistance, a high-fat diet was prepared with the following composition: 1825 g of rat feed, 1600 g of butter, 1250 g of soy protein, 300 g of a vitamin and mineral mixture, 5 g of yeast powder, 5 g of salt (NaCl), and 15 g of methionine. These ingredients were thoroughly mixed to create a uniform pelletized diet. Groups 2-5 were placed on this HFD for 2 weeks, while Group 1 continued with the standard chow.

Table 3.1. Composition of HFD

Ingredients	**Diet(g/kg)**
Powdered NPD	1825
Butter	1600
Soy protein	1250
Vitamin and Mineral Mix	300
Yeast powder	5
Sodium chloride	5

3.4.3 Induction of Type 2 Diabetes Mellitus

After the 2-week HFD period, diabetes was induced in rats from Groups 2-5 through a single intraperitoneal injection of streptozotocin (STZ) at a dosage of 40 mg/kg body weight. STZ was freshly dissolved in a 0.1 M citrate buffer (pH 4.5) just prior to administration. The dosage was calculated based on each rat's body weight, while Group 1 received an equivalent volume of

citrate buffer as the control. Fasting blood glucose (FBG) levels were assessed 4 days post-injection using a glucometer (Accu-Chek Active). Rats exhibiting FBG levels exceeding 200 mg/dL (11.1 mmol/L) were classified as diabetic and included in the study. Some mortality was noted following induction, and only the surviving rats proceeded with the experiment.

3.4.4 Experimental Design

A total of 30 rats were divided into 5 groups (n=6 per group, accounting for mortality):

i. Group 1: Normal Control (non-diabetic, received standard chow and distilled water).
ii. Group 2: Positive Control (diabetic, treated with glipizide at 5 mg/kg body weight orally).
iii. Group 3: Diabetic Control (diabetic, received distilled water).
iv. Group 4: Treatment Group (diabetic, treated with *S. mombin* leaf ethanolic extract at 200 mg/kg body weight orally).
v. Group 5: Treatment Group (diabetic, treated with *S. mombin* leaf ethanolic extract at 400 mg/kg body weight orally).

Treatments were administered daily via oral gavage for 2 weeks using an oro gastric tube. Doses were adjusted weekly based on changes in body weight to ensure accuracy.

3.4.5 Measurement of Body Weight and Organ Weight

Body weights were recorded weekly using a digital electronic balance (precision ±0.01 g). These measurements occurred at baseline (post-acclimatization), after 2 weeks of HFD, 4 days post-STZ induction, and after weeks 1 and 2 of treatment. The percentage change in body weight was calculated with the formula:

[(Final weight - Initial weight)/Initial weight] × 100.

The organs (liver and pancreas) were also removed and weight.

3.4.6 Sacrifice and Tissue Collection

At the conclusion of the 2-week treatment period, the rats were anesthetized with chloroform. Blood was collected via cardiac puncture for potential further analysis. The animals were sacrificed through cervical dislocation. Liver tissues were excised, rinsed with saline, dried with a blot, weighed using a digital balance, and snap-frozen in liquid nitrogen for subsequent gene expression analysis.

3.5 Gene Expression Study

3.5.1 Isolation of Total RNA

Total RNA was isolated from liver tissue samples using the Quick-RNA MiniPrep™ Kit (Zymo Research). DNA contaminants were removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using an A&E Spectrophotometer (A&E Lab, UK).

3.5.2 cDNA Conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti *et al*., 2020).

3.5.3 PCR Amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of genes of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa). PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse), and Ready Mix Taq PCR master mix. Under the following conditions: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 60 s) and ending with final extension at 72 °C for 10 min. The amplicons were resolved on 1.0% agarose gel. The

GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "Image J" software (Elekofehinti *et al*., 2020).

Primer Sequences

AKT

Forward: TCACCTCTGAGACCGACACC

Reverse: ACTGGCTGAGTAGGAGAACTGG

Insulin Receptor

Forward: CGGACCATGCCTGAAGCTAA

Reverse: AATGCTTCCGGGAGACACAG

GLUT 4

Forward: GGCCGGGACACTATACCCTA

Reverse: GGTTCCCCATCTTCAGAGCC

DPP-4

Forward: GCAAGACGTGGGTAATGATG

Reverse: AGCCTGGTTGGGTTTGTATG

PPARγ

Forward: CGAGCTGGGAGTAGCCTGA

Reverse: GATCACCAGCAGAGGTCCAG

GAPDH

Forward: AGACAGCCGCATCTTCTTGT

Reverse: CTTGCCGTGGGTAGAGTCAT

3.6 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Differences were considered significant at $p < 0.05$. Analysis was conducted using GraphPad Prism software version 9.0 (GraphPad Software, USA).

3.7 Ethical Considerations

Ethical approval was obtained from the University of Benin, College of Medical Sciences Research Ethics Committee before commencement of the study. The ethical clearance number is CMS/REC/2026/894

CHAPTER FOUR

RESULTS

This chapter presents the findings from the study, including body weight changes, organ weights (pancreas and liver), and semi-quantitative RT-PCR analysis of mRNA expression levels for key genes involved in insulin signaling, glucose transport, and metabolic regulation in a diabetic model. Gene expression was normalized to the housekeeping gene GAPDH. Data are presented as relative expression levels, with figures including gel electrophoresis bands and corresponding bar graphs depicting mean values ± standard error (SEM) from replicate experiments. Groups are defined as follows: Group 1 (Normal Control: non-diabetic, received standard chow and distilled water), Group 2 (Positive Control : diabetic, treated with glipizide at 5 mg/kg body weight orally), Group 3 (Diabetic Control: diabetic, received distilled water), Group 4 (Treatment Group: diabetic, treated with *S. mombin* leaf ethanolic extract at 200 mg/kg body weight orally), and Group 5 (Treatment Group: diabetic, treated with *S. mombin* leaf ethanolic extract at 400 mg/kg body weight orally).

4.1 Effects of Treatment on Body Weight

Body weights were monitored throughout the study, with final measurements taken at the end of the treatment period. As shown in Figure 4.1, the normal control (Group 1) exhibited a body weight of 184.17 ± 11.92 g. The diabetic control (Group 3) showed a marked reduction in body weight (147 ± 9.56 g), indicative of diabetes-induced weight loss. The positive control (Group 2) treated with glipizide had a partial recovery (170.79 ± 31.55 g). Treatment with *S. mombin* extract at 200 mg/kg (Group 4) and 400 mg/kg (Group 5) restored body weights to 189.11 ±

15.99 g and 209.28 ± 17.74 g, respectively, demonstrating a dose-dependent improvement and surpassing the glipizide group.

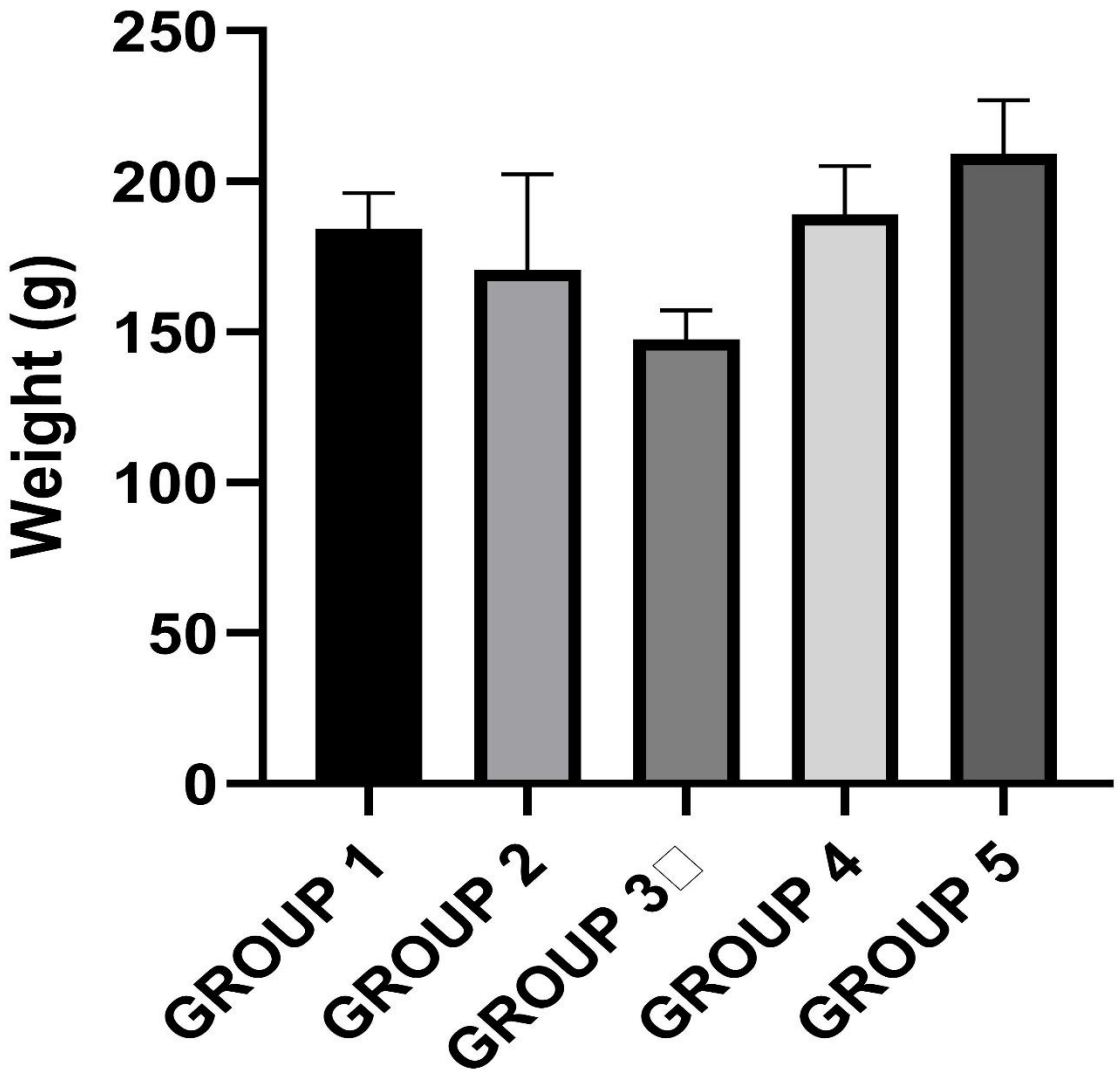

Figure 4.1: Bar graph of final body weights (g) across groups.

4.2 Effect of Treatment on Organ Weights

Organ weights were measured post-sacrifice to assess the impact of diabetes and treatments on pancreas and liver.

4.2.1 Pancreas Weight

Figure 4.2 illustrates pancreas weights. The normal control (Group 1) had the highest pancreas weight (0.58 ± 0.14 g). Diabetes induction led to a significant reduction in the diabetic control (Group 3, 0.19 ± 0.04 g). Glipizide treatment (Group 2) showed partial restoration (approximately 0.22 ± 0.04 g). *S. mombin* extract at 200 mg/kg (Group 4) and 400 mg/kg (Group 5) increased pancreas weights to 0.29 ± 0.04 g and 0.32 ± 0.05 g, respectively when compared to the diabetic control.

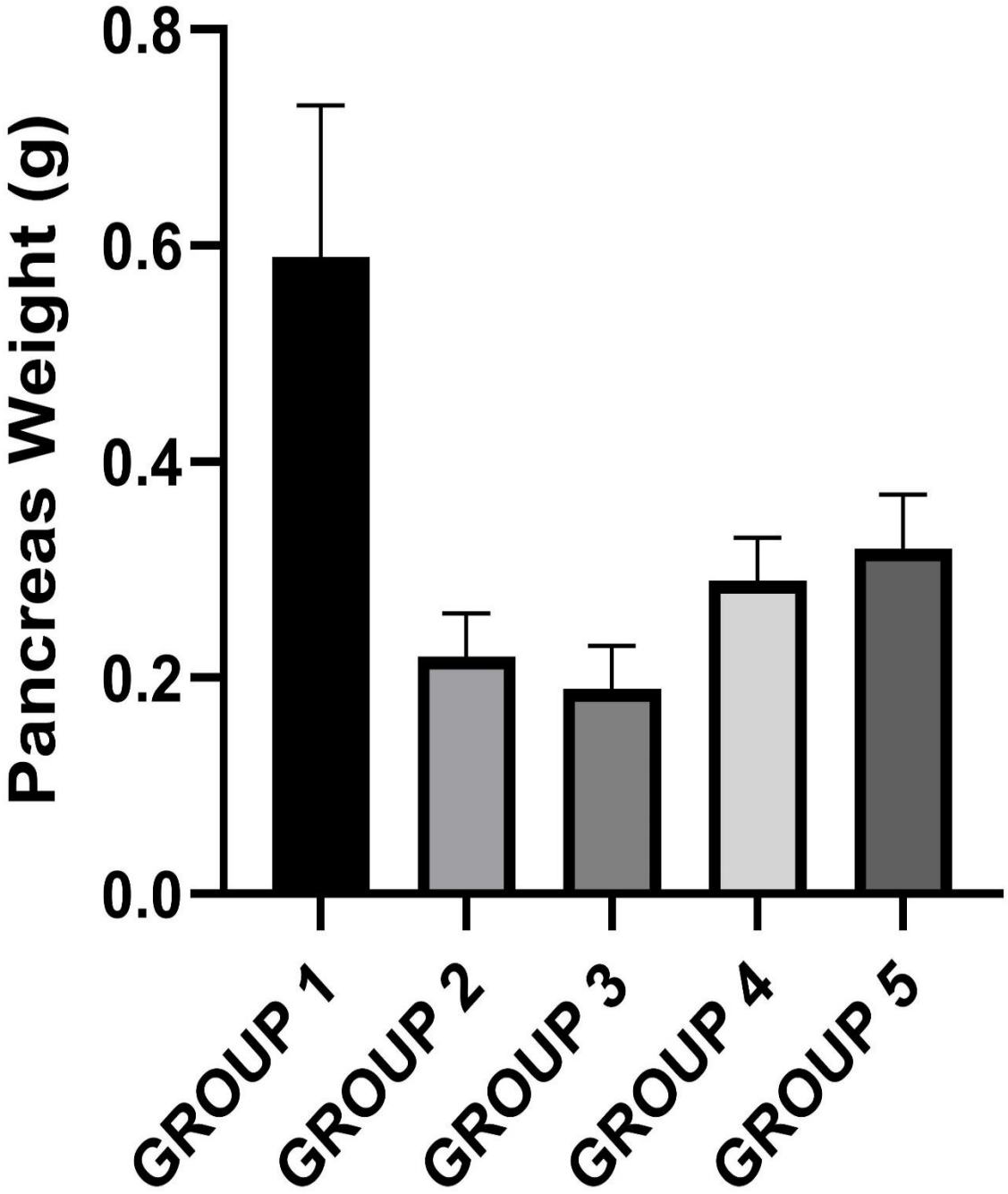

Figure 4.2: Bar graph of pancreas weights (g) across groups.

4.2.2 Liver Weight

As depicted in Figure 4.3, liver weights varied across groups. The normal control (Group 1) had a liver weight of approximately 7.33 ± 0.53 g. The diabetic control (Group 3) showed an increase (approximately 7.36 ± 0.90 g), possibly due to hepatic steatosis. Glipizide (Group 2) slightly reduced this to approximately 7.36 ± 0.90 g. Treatment with *S. mombin* extract at 200 mg/kg (Group 4) further lowered liver weight to approximately 6.42 ± 0.35 g, while the 400 mg/kg dose (Group 5) increased it to 8.47 ± 0.57 g, suggesting differential dose effects on hepatic pathology.

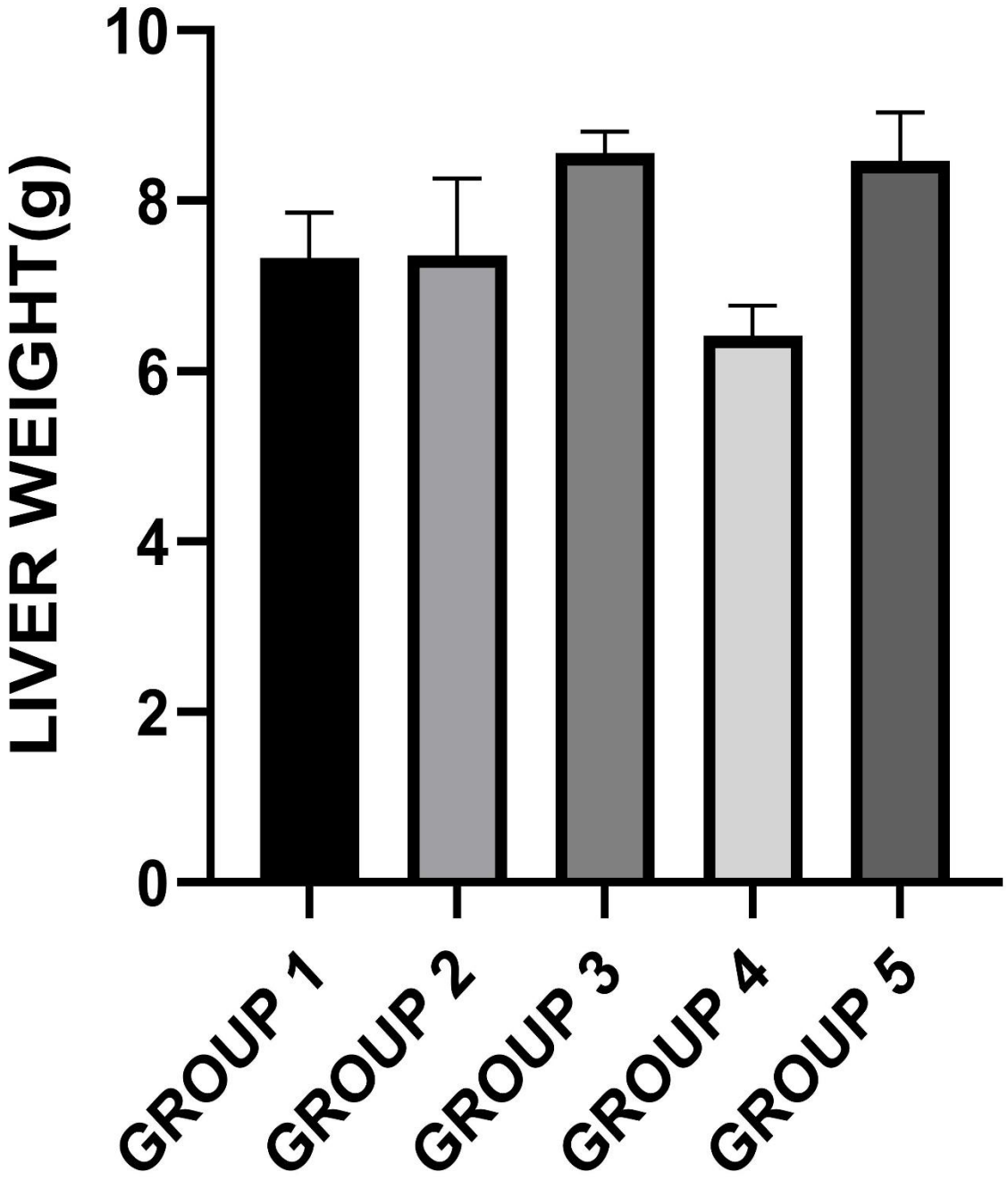

Figure 4.3: Bar graph of liver weights (g) across groups.

4.3 Effect of Treatment on Gene Expression of Insulin Signaling Markers

The insulin signaling pathway was assessed through mRNA levels of the Insulin Receptor (INSR) and AKT genes.

4.3.1 Insulin Receptor Gene Expression

Figure 4.4 displays the gel electrophoresis and quantitative bar graph for INSR expression. The Diabetic Control (Group 3) showed a significant reduction in INSR expression compared to the Normal Control (Group 1) and Positive Control (Group 2) groups. Treatment with the *S. mombin* leaf ethanolic extract at 200 mg/kg (Group 4) and 400 mg/kg (Group 5) led to a partial restoration, with Group 5 exhibiting higher levels than Group 4, indicative of a dose-dependent effect.

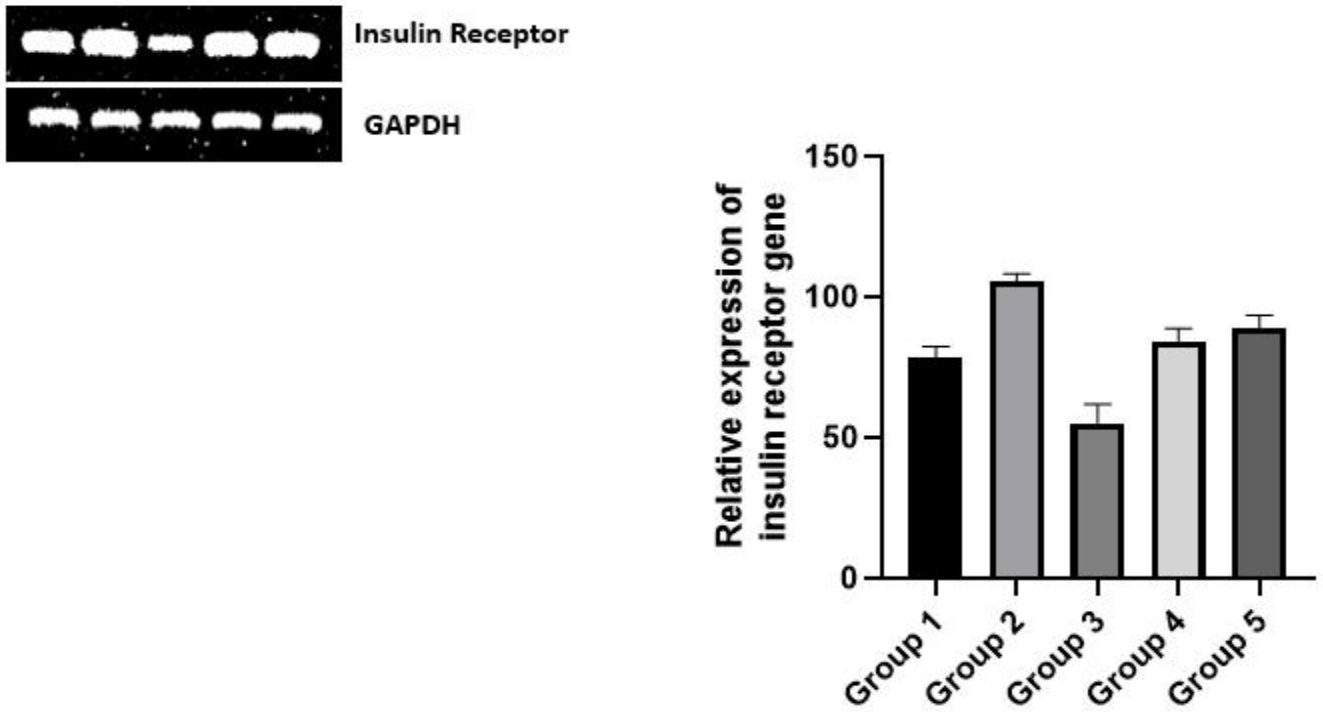

Figure 4.4: Gel electrophoresis and relative expression of the Insulin Receptor gene.

4.3.2 AKT Gene Expression

As illustrated in Figure 4.5, AKT expression mirrored the pattern observed for INSR. The Diabetic control (Group 3) had the lowest expression, notably below that of the Positive Control group (Group 2). Extract treatments in Groups 4 and 5 show significantly increased expression relative to Group 2, approaching levels seen in the control groups, with a trend toward greater recovery at the higher dose.

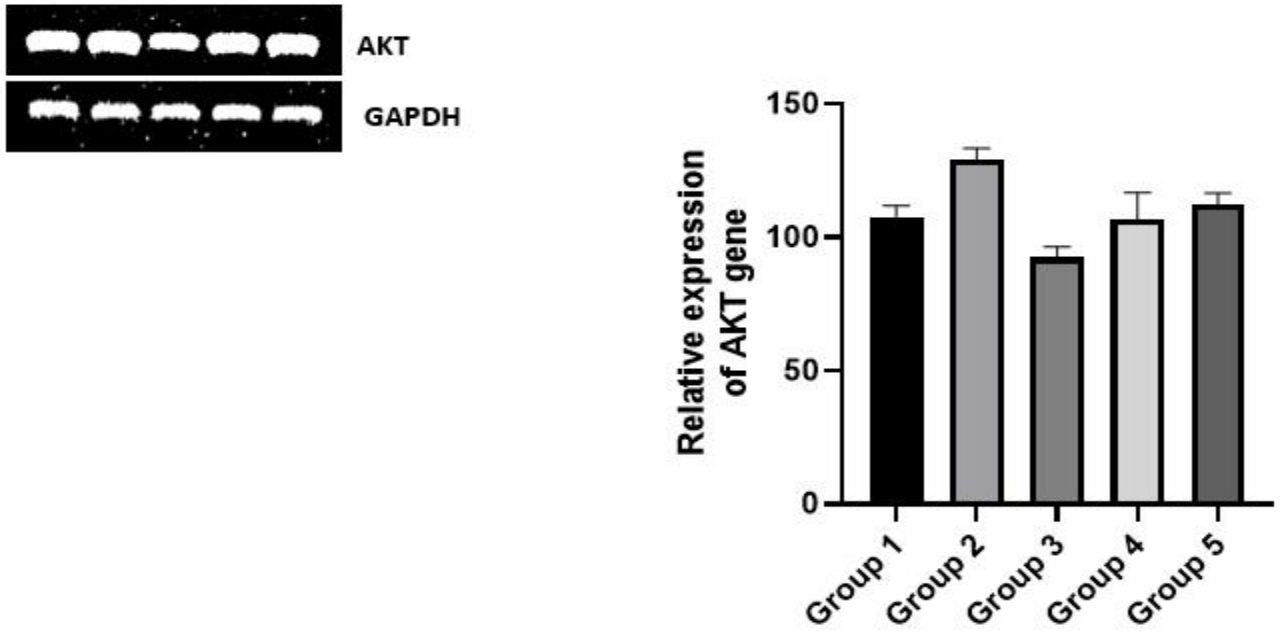

Figure 4.5: Gel electrophoresis and relative expression of the AKT gene.

4.4 Effect of Treatment on Glucose Transporter 4 (GLUT4) Expression

GLUT4 mRNA levels, crucial for glucose uptake, are shown in Figure 4.6. Expression was substantially suppressed in the Diabetic Control (Group 3) relative to Groups 1 and 2. Groups 4 and 5 demonstrated upregulation compared to Group 3, suggesting enhanced glucose transport potential, with the 400 mg/kg dose showing slight or little superior efficacy.

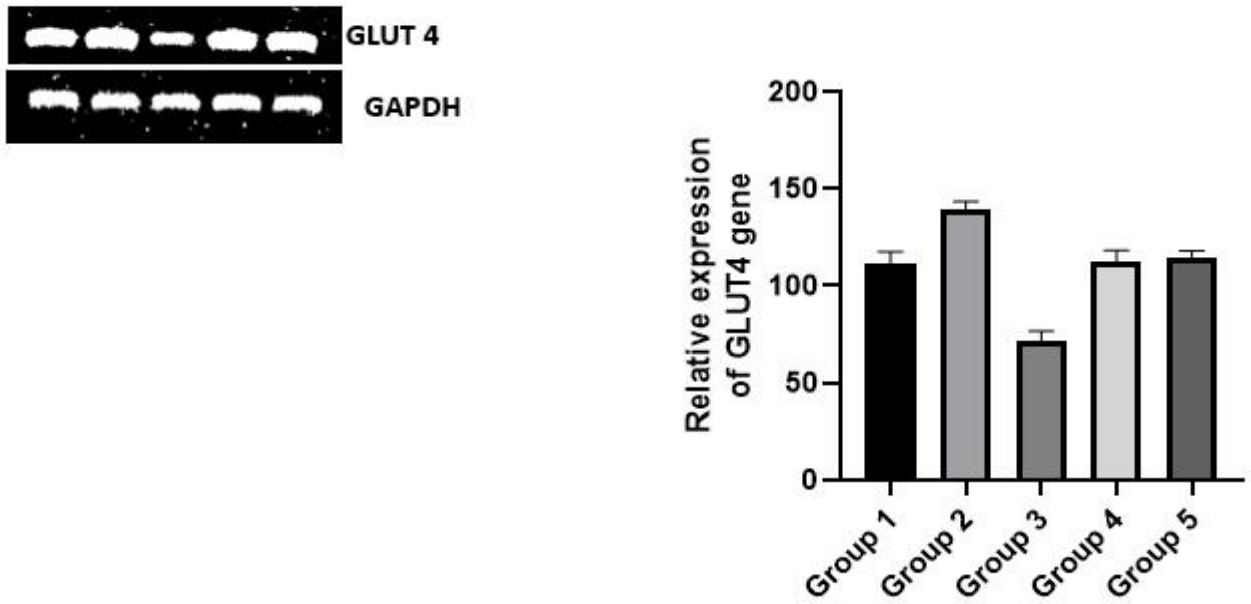

Figure 4.6: Gel electrophoresis and relative expression of the GLUT4 gene.

4.5 Effect of Treatment on Metabolic Regulators (PPARγ and DPP4)

4.5.1 Peroxisome Proliferator Activated Receptor (PPARγ) Gene Expression

Figure 4.7 presents PPARγ expression, a regulator of lipid and glucose metabolism. A pronounced decrease was observed in the Diabetic Control (Group 3) compared to all other groups. Extract administration in Groups 4 and 5 restored expression to levels comparable to the Normal Control (Group 1), with evidence of dose dependency.

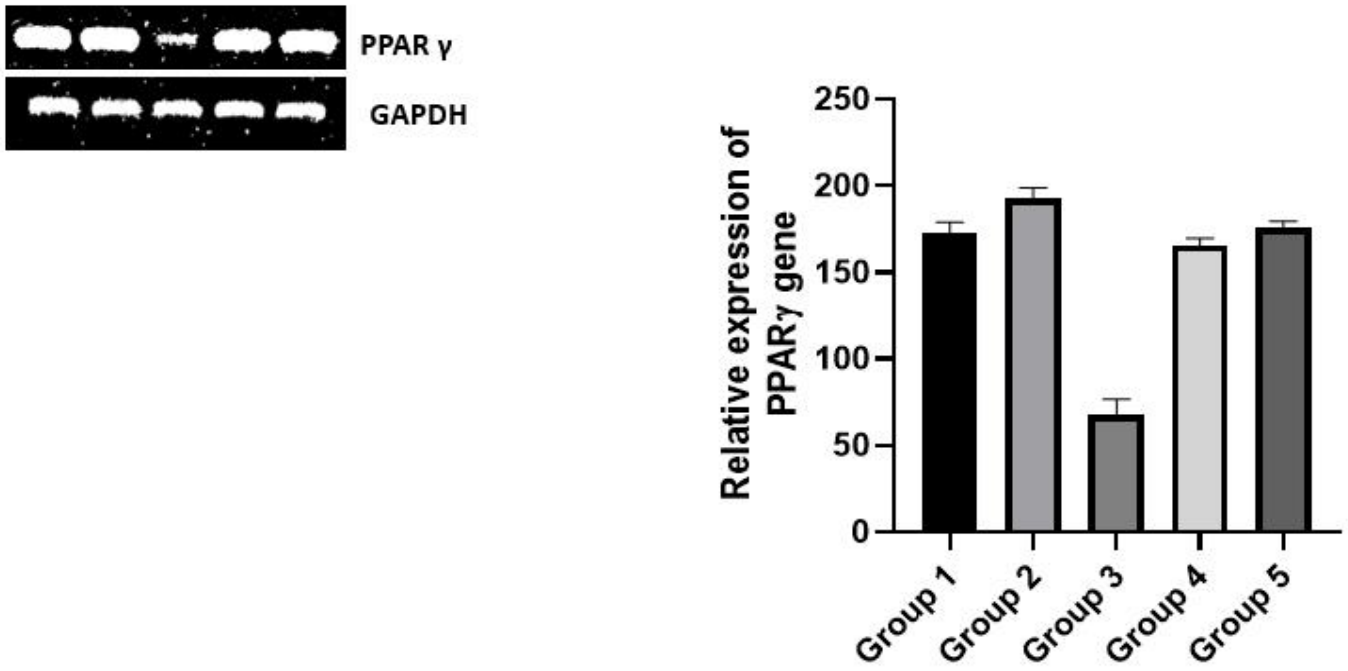

Figure 4.7: Gel electrophoresis and relative expression of the PPARγ gene.

4.5.2 Dipeptidyl Peptidases 4 (DPP4) Gene Expression

DPP4 expression data are depicted in Figure 4.8. The Diabetic Control (Group 3) displayed reduced levels relative to the Positive Control group (Group 2). Treatment groups (Groups 4 and 5) exhibited increased expression compared to Group 3, aligning closer to control baselines, particularly at the higher extract dose.

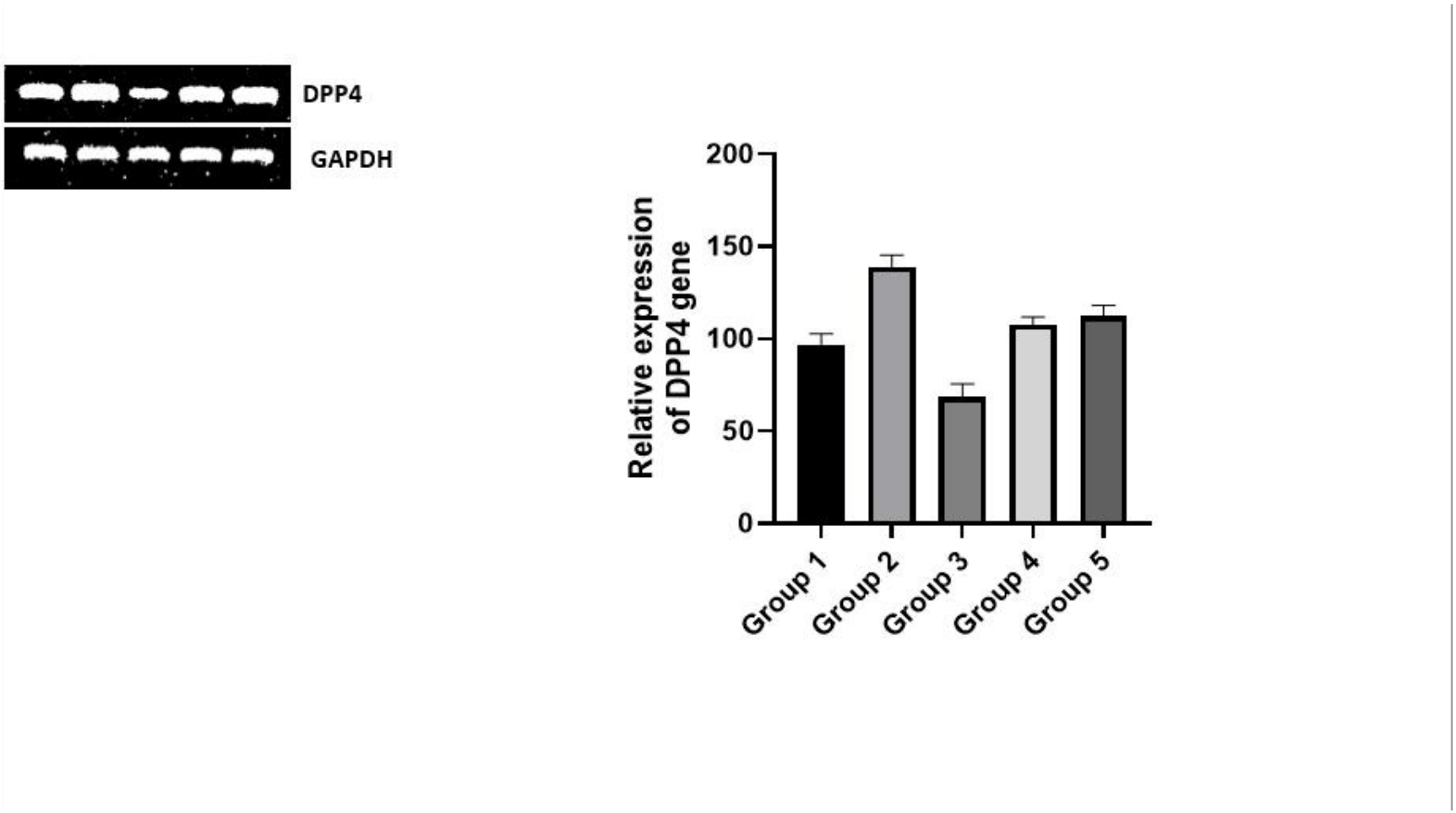

Figure 4.8: Gel electrophoresis and relative expression of the DPP4 gene.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigates the therapeutic potential of ethanolic leaf extract of *Spondias mombin* in modulating body weight, organ weights, and the expression of key insulin signaling and metabolic genes in a high-fat diet (HFD) and streptozotocin (STZ)-induced type 2 diabetes mellitus (T2DM) rat model. The results demonstrate that *S. mombin* extract exerts dose-dependent ameliorative effects on diabetes-induced physiological and molecular alterations, often comparable to or surpassing the standard antidiabetic drug glipizide. These findings align with the ethnomedicinal use of *S. mombin* for diabetes management, as highlighted in the literature review (Rafiu *et al*., 2025; Gobinath *et al*., 2022), and provide mechanistic insights into its hypoglycemic activity through gene modulation, building on the pathophysiological mechanisms discussed in Chapter 2, such as insulin resistance and β-cell dysfunction (Goyal *et al*., 2023; Galicia-Garcia *et al*., 2020). Diabetes induction via HFD-STZ led to significant body weight loss in the diabetic control group, consistent with the catabolic state induced by hyperglycemia, insulin deficiency, and increased gluconeogenesis, as described in the literature review's section on T2DM pathophysiology (Galicia-Garcia *et al*., 2020; Yau *et al*., 2025). Treatment with *S. mombin* extract at 200 mg/kg and 400 mg/kg restored body weight in a dose-dependent manner, exceeding the recovery observed with glipizide. This suggests that the extract may enhance nutrient utilization and reduce muscle wasting, possibly through improved insulin sensitivity and reduced oxidative stress, as reported in previous studies with *S. mombin* (Gobinath *et al*., 2022; Akwu *et al*., 2024) and paralleling the antioxidant and anti-inflammatory

effects of medicinal plants outlined in the literature review (Hegazy *et al*., 2025; Mthiyane *et al*., 2022).

Pancreatic atrophy, evidenced by reduced pancreas weight in diabetic rats, reflects β-cell destruction and dysfunction typical of T2DM, as elaborated in the literature review under pathophysiological mechanisms (Yau *et al*., 2025; Goyal *et al*., 2023). The extract's dose-dependent restoration of pancreas weight indicates protective effects on β-cells, potentially via antioxidant mechanisms that mitigate STZ-induced toxicity, consistent with the role of oxidative stress in β-cell failure discussed in Chapter 2 (Caturano *et al*., 2025; Tegegne *et al*., 2024) and supported by *S. mombin*'s documented antioxidant capacity (Moke *et al*., 2023; Lucena *et al*., 2022). Similarly, the increased liver weight in diabetic rats may indicate hepatic steatosis or inflammation, a common complication linked to dysregulated lipid metabolism in T2DM (Akwu *et al*., 2022; Huang *et al*., 2025), which was normalized by the extract, particularly at 400 mg/kg. These organ weight changes corroborate histological improvements reported in prior *S. mombin* studies (Nwidu *et al*., 2018; Gobinath *et al*., 2022), highlighting its hepatoprotective role as reviewed in the literature on medicinal plants' multi-target approaches (Rahman *et al*., 2022; Shi *et al*., 2023). The downregulation of insulin receptor (INSR) and AKT gene expression in the diabetic control group underscores impaired insulin signaling, a hallmark of T2DM where oxidative stress and inflammation disrupt the PI3K/AKT pathway, as detailed in the literature review's section on gene dysregulation (Zhao *et al*., 2023; Galicia-Garcia *et al*., 2020). *S. mombin* extract upregulated these genes dose-dependently, suggesting enhanced insulin receptor activation and downstream signaling, which could facilitate glucose uptake and glycogen synthesis. This aligns with molecular docking studies showing *S. mombin* phytocompounds like alpha-sitosterol binding to insulin-related targets (Eluehike *et al*., 2022) and mirrors the

enhancement of glucose uptake mechanisms by plant bioactives described in Chapter 2 (Pattanaik *et al*., 2024; Hegazy *et al*., 2025). Compared to glipizide, which primarily stimulates insulin secretion via KATP channels (Sahin *et al*., 2024; Mohajan and Mohajan, 2024), the extract's effects indicate a broader insulin-sensitizing mechanism, potentially involving flavonoids and polyphenols, as emphasized in the literature review on phytotherapy (Rahman *et al*., 2022; Ansari *et al*., 2024).

GLUT4 expression, critical for peripheral glucose transport, was suppressed in diabetic rats, contributing to hyperglycemia, in line with the molecular defects in insulin signaling pathways discussed in the literature review (Wang *et al*., 2025; Zhao *et al*., 2023). The extract's upregulation of GLUT4, especially at higher doses, implies improved translocation to the plasma membrane, akin to the actions of AMPK-activating phytochemicals in other plants like *Moringa oleifera* (Hegazy *et al*., 2025; Mthiyane *et al*., 2022) and consistent with the mechanisms of antidiabetic plants enhancing glucose uptake (Clemente-Suárez *et al*., 2025; Shi *et al*., 2023). This could explain the extract's hypoglycemic efficacy observed in prior in vivo models (Gobinath *et al*., 2022; Omoboyowa *et al*., 2023). PPARγ, a key regulator of insulin sensitivity and lipid metabolism, was downregulated in diabetic rats, exacerbating insulin resistance, as noted in the literature review (Tegegne *et al*., 2024; Alnuaimi *et al*., 2024). *S. mombin* extract restored PPARγ expression to near-normal levels, supporting its role as a PPARγ agonist, similar to thiazolidinediones but with potentially fewer side effects (Alnuaimi *et al*., 2024; Pattanaik *et al*., 2024). This modulation may involve bioactive compounds like quercetin, which activate PPARγ and reduce inflammation, aligning with the gene-specific modulation by plant metabolites reviewed in Chapter 2 (Hegazy *et al*., 2025; Eluehike *et al*., 2022). DPP4 expression was reduced in the diabetic group, which might seem counterintuitive given DPP4's role in

degrading incretins (Tegegne *et al*., 2024; Florentin *et al*., 2022), but this could reflect compensatory mechanisms or tissue-specific dysregulation in advanced T2DM, as discussed in the literature on incretin-based therapies (Yin *et al*., 2022; Tang *et al*., 2025). The extract's upregulation suggests enhanced incretin stability, aligning with DPP4 inhibitory effects of plant metabolites (Tang *et al*., 2025; Tegegne *et al*., 2024), and may contribute to improved insulin secretion.

These findings suggest the extract targets multiple pathways, offering a multi-faceted approach superior to glipizide's primarily secretagogue action, addressing limitations of conventional therapies like side effects and therapeutic inertia highlighted in the literature review (Mohajan and Mohajan, 2024; Almigbal *et al*., 2023). The observed gene modulations are likely driven by *S. mombin*'s rich phytochemical profile, including flavonoids, tannins, and saponins, which exert antioxidant, anti-inflammatory, and enzyme-inhibitory effects, as reviewed in Chapter 2 (Ogunro *et al*., 2023; Sinan *et al*., 2021; Ansari *et al*., 2023). For instance, the upregulation of Nrf2-related antioxidants (as inferred from hepatoprotection) could mitigate oxidative stress, preserving insulin signaling, consistent with the role of oxidative stress in T2DM complications (Caturano *et al*., 2025; Banaszak *et al*., 2024). This is in agreement with ethnomedicinal validations of *S. mombin* for diabetes (Rafiu *et al*., 2025; Iwu, 1993) and parallels antidiabetic plants like *Vernonia amygdalina* and *Moringa oleifera* (Adekemi *et al*., 2024; Mthiyane *et al*., 2022; Akpan and Okugbo, 2025). The dose-dependent superiority over glipizide highlights *S. mombin*'s potential as a complementary therapy, addressing gaps in conventional management such as cost, accessibility, and side effects (Padhi *et al*., 2020; Chantzaras and Yfantopoulos, 2025).

However, discrepancies exist; while some studies report toxicity at high doses of root extracts (Ogeyemhe and Atoyebi, 2025), our leaf extract showed no adverse effects at up to 400 mg/kg, supporting its safety (Gobinath *et al*., 2022; Akwu *et al*., 2022). Limitations include the semi-quantitative nature of RT-PCR, which may not capture protein-level changes, and the short treatment duration (2 weeks), warranting chronic studies to align with long-term complications discussed in the literature review (Sami *et al*., 2025; Dal Canto *et al*., 2019). Future research should explore tissue-specific expressions (e.g., muscle, adipose) and clinical trials to validate translational potential, building on the identified gaps in *S. mombin* literature (Omoboyowa *et al*., 2023; Eluehike *et al*., 2022).

5.2 Conclusion

This study successfully achieved its aim of evaluating the effects of *Spondias mombin* leaf extract on insulin signaling pathway genes in experimental T2DM rats. The extract demonstrated dose-dependent restoration of body weight, organ weights, and upregulation of key genes (INSR, AKT, GLUT4, PPARγ, DPP4,), often outperforming glipizide. These findings provide scientific validation for the ethnomedicinal use of *S. mombin* and underscore its potential as a natural, multi-target antidiabetic agent that enhances insulin sensitivity, glucose uptake, and metabolic regulation while mitigating oxidative stress and inflammation.

By addressing gaps in molecular mechanisms, this research contributes to the growing evidence for phytotherapy in diabetes management, particularly in resource-limited settings where conventional therapies are inaccessible or associated with adverse effects. Future investigations should focus on isolating active compounds, elucidating protein-level interactions, and conducting long-term human trials to facilitate the integration of *S. mombin* into clinical

protocols for T2DM. Ultimately, this plant holds promise as a safe, affordable adjunct to existing treatments, potentially reducing the global burden of diabetes and its complications.

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