Ethiopian Journal of Environmental Studies & Management 19(Suppl.): 921 – 935, 2026
ISSN:1998-0507 doi: https://ejesm.org/doi/v19iSuppl.9
Submitted: October 10, 2026 Accepted: November 12, 2026

MODULATION OF INSULIN SIGNALLING AND GLUCOSE METABOLISM-RELATED GENES BY *Spondias mombin* SAPONINS AND ETHANOLIC LEAF EXTRACT IN TYPE 2 DIABETIC RATS

***ELUEHIKE, N., BALOGUN, C.S., UWADIA, A. B. AND OMAGBEMI, O.**
Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Nigeria
*Corresponding author: nkeiruka.ezeugwu@uniben.edu

Abstract
This study assessed the effects of saponins and extract of S. mombin on insulin signaling pathway and glucose metabolism related genes (IR-Insulin Receptor, AKT-protein kinase B, 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 saponins and Group 5: 400mg/kg of extract. T2DM was induced by a combination of high fat diet followed by low-dose STZ (35 mg/kg.). 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. The result obtained showed that diabetes resulted in significant reduction in body weight, pancreas weight and reduced expression of IR, AKT, GLUT-4, PPAR-γ, and of DPP-4 as seen in the diabetic control. Treatment of the diabetic rats with glipizide, saponins and ethanol extract of S mombin leaves improved the expression of these genes when compared with the untreated diabetic rats. The comparable performance of the isolated saponins to the crude extract strongly implies that saponins are a principal phytoconstituent responsible for the plant's insulin-sensitizing and antidiabetic activities. These findings provide solid molecular evidence supporting the ethnopharmacological use of S. mombin in managing type 2 diabetes.

Keywords: *Type 2 diabetes, Spondias mombin, Saponins, High fat diet, Gene expression*

Introduction

Type 2 diabetes mellitus (T2DM) has become a major global health concern, largely due to the persistent increase in its prevalence and the challenges associated with its long-term management. The disease is characterized by chronic hyperglycemia arising primarily from peripheral insulin resistance and progressive dysfunction of pancreatic beta cells (American Diabetes Association, 2023). Although conventional antidiabetic drugs, including metformin and sulfonylureas, remain important in the management of T2DM, their long-term use may be limited by adverse effects,

This work is licensed to the publisher under the Creative Commons Attributions License 4.0

secondary treatment failure, and the progressive nature of the disease (Kharroubi and Darwish, 2015). These challenges have encouraged growing interest in medicinal plants as potential sources of bioactive compounds that may improve glucose homeostasis and modulate key pathways involved in insulin action.

Spondias mombin L. (Anacardiaceae), commonly known as hog plum or yellow mombin, is a medicinal plant widely used in traditional medicine in many tropical regions. Different parts of the plant have traditionally been employed in the management of inflammatory, infectious, and metabolic conditions. Phytochemical studies have shown that *S. mombin* contains a variety of bioactive secondary metabolites, including saponins, which have attracted considerable attention because of their potential antidiabetic and hypoglycemic effects (Ayoka *et al*., 2008).

Saponins are naturally occurring glycosides that have been reported to improve insulin sensitivity, influence lipid metabolism (Elekofehinti *et al.,* 2012), and reduce oxidative stress in experimental models (Elekofehinti *et al.,* 2012). These properties suggest that saponins from *S. mombin* may have therapeutic potential in the management of metabolic disturbances associated with T2DM. Several research reports are available to show that saponins from medicinal plants are capable of reducing blood levels in diabetic states (McAnuff *et al*., 2005; Bhavsar *et al.,* 2009; Zheng *et al.,* 2012). Saponins are promising compounds with potential to be developed into new drugs for treatment of diabetes (Elekofehinti *et al*., 2012; Kim *et al*.,2006). We have also established that Saponins from *Spondias mombin* leaves act as potential PPAR gamma agonist and DPP-4 inhibitor for type 2 diabetes treatment via molecular docking studies and ADMET profiling (Eluehike and Onoagbe, 2025).

Insulin resistance in T2DM is closely associated with disruption of the intracellular insulin signaling pathway. Key components of this pathway include the insulin receptor (IR), protein kinase B (AKT), and glucose transporter 4 (GLUT-4), which collectively play important roles in glucose uptake and utilization by peripheral tissues (Petersen and Shulman, 2018). Following insulin stimulation, activation of the IR-AKT signaling pathway promotes the translocation of GLUT-4 to the cell membrane, thereby facilitating glucose uptake. In addition, abnormalities in the activity or expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and dipeptidyl peptidase-4 (DPP-4) have been implicated in the development and progression of insulin resistance and impaired glucose regulation (Gao *et al.,* 2020). Although previous work demonstrated the antidiabetic and beta-cell regenerating effects of crude *S. mombin* extracts (Eluehike and Onoagbe, 2020), the specific molecular mechanisms by which its isolated saponins modulate gene expression within the insulin signaling pathway remain poorly defined.

This study utilizes a high-fat diet (HFD) and low-dose streptozotocin (STZ) rat model to mimic human T2DM features, including insulin resistance and partial beta-cell loss (Reed *et al.,* 2000). We hypothesize that isolated *S. mombin* saponins ameliorate hyperglycemia by upregulating key insulin-signaling genes (*IR*, *AKT*, *GLUT-4*, and *PPAR-γ*) while downregulating *DPP-4*. Ultimately, this investigation aims to clarify the molecular targets of *S. mombin* saponins

in restoring insulin sensitivity and addressing T2DM metabolic abnormalities.

Materials and Methods

Collection and Identification and Authentication 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 with number UBH_S 345.

Preparation of Leaf Extract from Spondias mombin

Freshly harvested leaves were thoroughly rinsed with distilled water to remove soil and other extraneous materials. The leaves were subsequently shade-dried at room temperature. The dried leaves were pulverized into a fine powder using a mechanical grinder. A 500 g quantity of the powdered leaves was extracted by maceration in 2 L of absolute ethanol at room temperature for 72 hours. The mixture was intermittently stirred during the extraction period to enhance the recovery of soluble constituents. Following maceration, the mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid ethanolic extract. The extract was transferred into an airtight container and stored at 4°C until required for the experimental procedures. Before administration, the concentrated extract was reconstituted with distilled water to obtain the appropriate experimental concentrations.

Isolation of Saponins from the Leaves of Spondias mombin

Hostettmann, (1991) procedure was used for the isolation of saponins from *Spondias mombin* leaves. Briefly 500g of the pulverized leaves was macerated in 2 liters of methanol. The extract was filtered and the resulting solution concentrated using a rotary evaporator. The extract was dissolved with distilled water and put in a separating funnel. Partitioning was done using hexane/water (2:4v/v). The hexane layer was discarded and the water layer was then partitioned using ethylacetate/n-butanol (2:4v/v). The n-butanol which contains the saponins was further purified using a silica gel column.

Research Design

Thirty adult male Wistar albino rats (150–200 g) sourced from the University of Benin were housed in standard cages under controlled conditions, with food and water *ad libitum* during a 2-week acclimatization period. Ethical approval was granted by the University's College of Medical Sciences Research Ethics Committee (CMS/REC/2026/894), and standard guidelines for laboratory animal care (NRC, 2011) were strictly followed

Induction of Diabetes

The procedure of Srinivasan, *et al*. (2005) was employed to induce type 2 diabetes. Briefly, high-fat diet was prepared with the following composition: powdered rat feed 365g/kg, lard 310g/kg, casein, 250g/kg, cholesterol 10g/kg, vitamin and mineral mix 60g/kg, n-methionine3g/kg, yeast powder 1g/kg, sodium chloride 1g/kg. These ingredients were thoroughly mixed to create a uniform pelletized diet. Groups 2-5 were placed on this HFD for two weeks, while Group 1 continued with the standard chow. 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 35 mg/kg body weight. STZ was freshly dissolved in a 0.1 M citrate buffer (pH 4.5) just prior to administration. Fasting blood glucose (FBG) levels were assessed 4 days post-injection using a glucometer (Accu-Chek Active). Rats exhibiting FBG levels exceeding 200 mg/were classified as diabetic and included in the study.

Experimental Design

A total of 30 rats were divided into 5 groups (n=6 per group)

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 saponins from *S. mombin* 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).

Rats received daily treatments via oral gavage using an orogastric tube for two weeks. Body weight was recorded at baseline (day 0) and on the sacrifice day to calculate weight change. Following the two-week treatment, rats were anesthetized with chloroform, and liver tissues were excised, blotted dry, weighed with a digital balance, and snap-frozen in liquid nitrogen for gene expression analysis.

Histology

The pancreas was also collected from all animals and a section of these tissues embedded in paraffin and examined for any pathological anomalies. 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).

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).

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

Genes	Forward primer	Reverse primer
AKT	TCACCTCTGAGACCGACACC	ACTGGCTGAGTAGGAGAACTGG
Insulin Receptor	CGGACCATGCCTGAAGCTAA	AATGCTTCCGGGAGACACAG
Glut 4	GGCCGGGACACTATACCCTA	GGTTCCCCATCTTCAGAGCC
DPP-4	GCAAGACGTGGGTAATGATG	AGCCTGGTTGGGTTTGTATG
PPARγ	CGAGCTGGGAGTAGCCTGA	GATCACCAGCAGAGGTCCAG
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

Data 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).

Results

Effect of saponins and ethanol extract of Spondias mombin leaves on body weight and pancreas weight in type 2 diabetic rats

Fig 1 and 2 shows the effect of saponins and ethanol extract of *Spondias mombin* leaves on body weight and pancreas weight in type 2 diabetic rats. A significant ($p \leq 0.05$) decrease was observed in the body and pancreas weight in the diabetic control rats when compared with the normal control. However, treatment with saponins and ethanol extract of *S. mombin* leaves resulted in a significant increase in the weight of the body and pancreas compared to the diabetic control groups.

Effect of saponins and ethanol extract of Spondias mombin leaves on expression level of Insulin receptor genes in type 2 diabetic rats

The effect of saponins and ethanol extract of *S. mombin* leaves on expression level of Insulin receptor genes is shown in figure 3. The result showed that induction of diabetes to the rats led to a significant ($p \leq 0.05$) decrease in the expression of insulin receptor genes, while treatment with saponins and extract led to a significant $p \leq 0.05$) increase in its expression.

Effect of saponins and ethanol extract of Spondias mombin leaves on expression level of protein kinase B (AKT) in type 2 diabetic rats

Fig 4 shows the effect of saponins and ethanol extract of *S mombin* leaves on expression level of protein kinase B (AKT) in type 2 diabetic rats. The Diabetic control (Group 3) had the lowest expression, notably below that of the Positive control group (Group 2), treatment with saponins and extract in Groups 4 and 5 showed significantly increased expression

Effect of saponins and ethanol extract of Spondias mombin on Glucose Transporter 4 (GLUT4) Expression

The expression level of Glucose Transporter 4 (GLUT4) genes is shown in fig 5. A significant ($p \leq 0.05$) decrease was recorded in the expression of GLUT4 genes in the diabetic control rats when compared with the normal control. However, treatment with saponins and

extract led to a significant p≤0.05) increase in its expression.

Effect of saponins and ethanol extract of Spondias mombin treatment on proliferator-activated receptor gamma (PPARγ)

Figure 6 shows that diabetic induction significantly reduced (p≤0.05) PPARγ expression, whereas treatment with *S. mombin* saponins, extracts, or standard drug significantly increased (p≤0.05) its expression.

Effect of saponins and ethanol extract of Spondias mombin treatment on Dipeptidyl Peptidases 4 (DPP4) Gene Expression

Figure 7 shows *DPP4* gene expression across treatment groups. Diabetic control rats exhibited a significant increase ($p \le 0.05$) in *DPP4* expression compared to normal controls. Conversely, treatment with *S. mombin* saponins, extracts, or standard drug significantly decreased 0.05$) *DPP4* expression relative to diabetic controls.

Effects of saponins and extract of Spondias mombin leaves on Histology of pancreas

The effects of saponins and extract of *Spondias mombin* leaves on Histology of pancreas is shown in figure 8. Histological examination of the Pancreas of normal control rats showed normal architecture revealing normal acini lined by cuboidal epithelial cells and normal islet of langerharn. The diabetic control pancreas was characterized by severe atrophy in pancreatic islets and fewer islet of langerharn. Treatment with standard drug, saponins and ethanol extract of *S mombin* resulted in pancreas with no observable pathological changes.

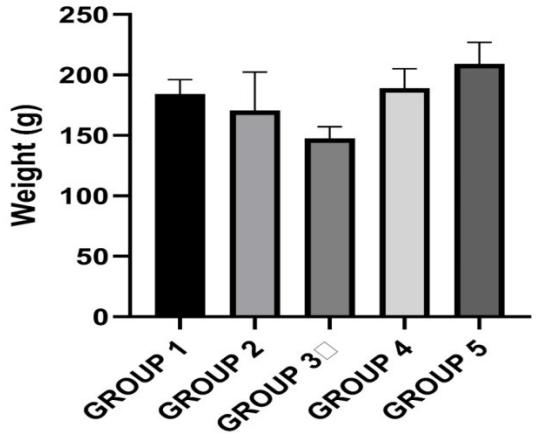


Fig 1: Effect of saponins and ethanol extract of *Spondias mombin* leaves on body weight in type 2 diabetic rats

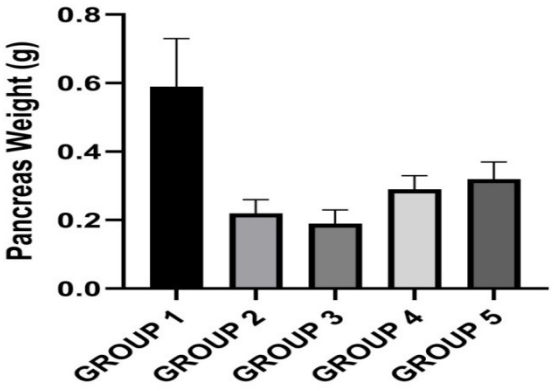


Fig 2: Effect of saponins and ethanol extract of *Spondias mombin* leaves on pancreas weight in type 2 diabetic rats

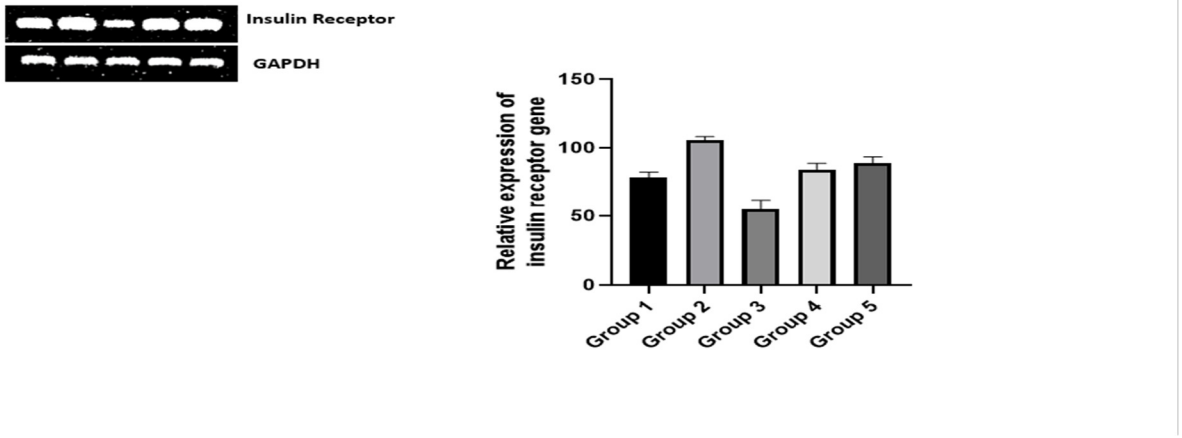


Fig 3: Effect of saponins and ethanol extract of *Spondias mombin* leaves on expression level of Insulin receptor genes in type 2 diabetic rats

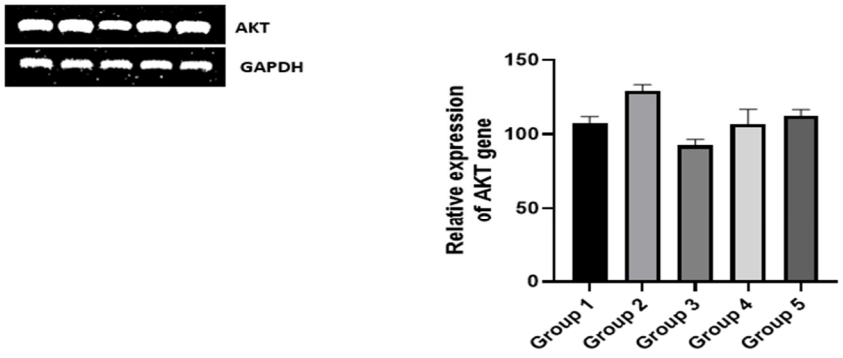


Fig 4: Effect of saponins and ethanol extract of *Spondias mombin* leaves on expression level of protein kinase B (AKT) in type 2 diabetic rats

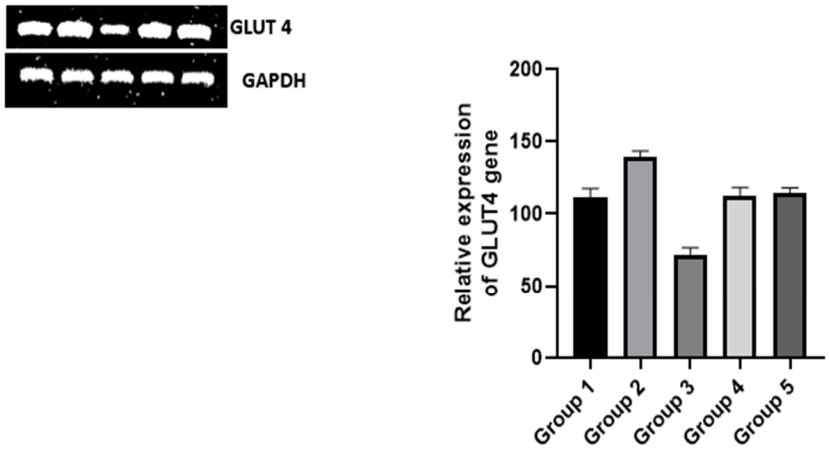


Fig 5: Effect of saponins and ethanol extract of *Spondias mombin* on Glucose Transporter 4 (GLUT4) Expression in type 2 diabetic rats

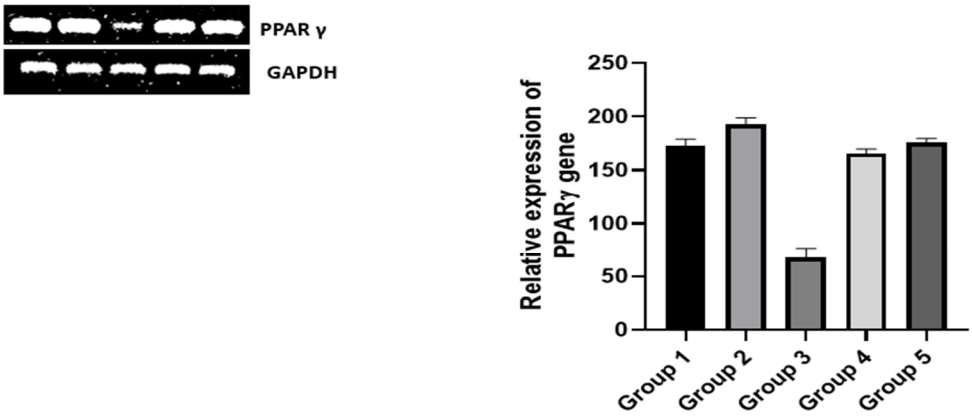


Figure 6: Effect of saponins and ethanol extract of *Spondias mombin* treatment on proliferator-activated receptor gamma (PPARγ) in type 2 diabetic rats

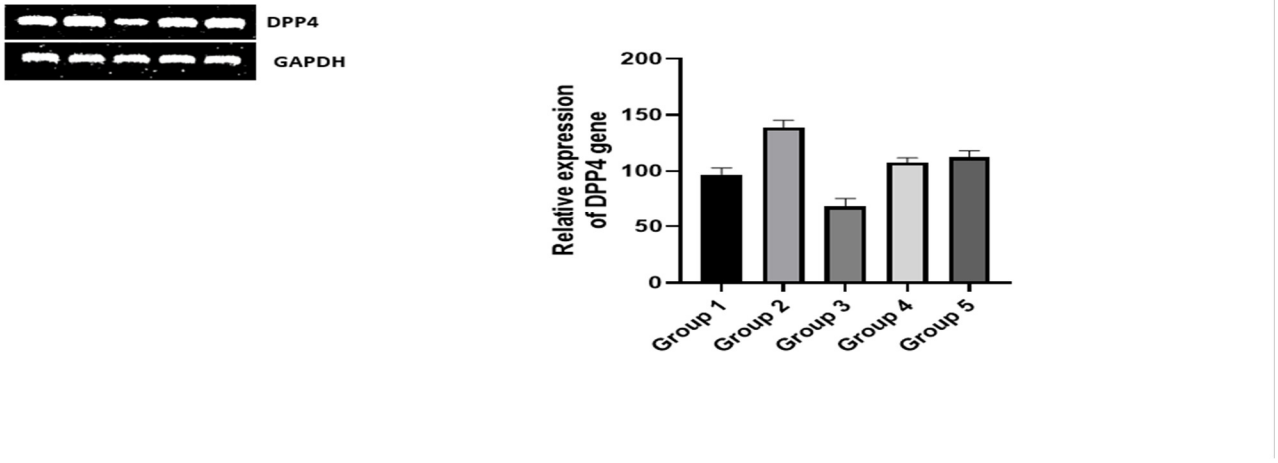
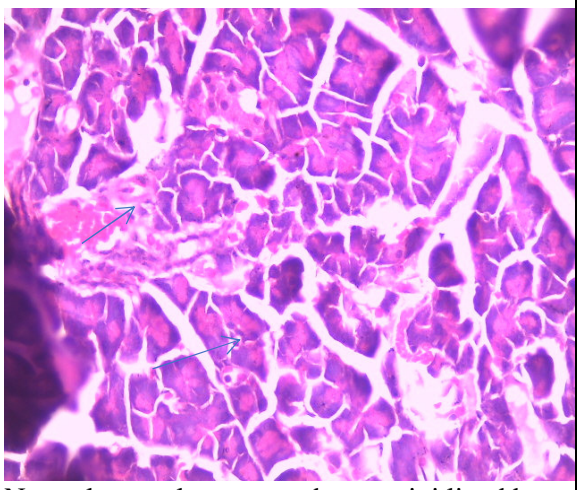
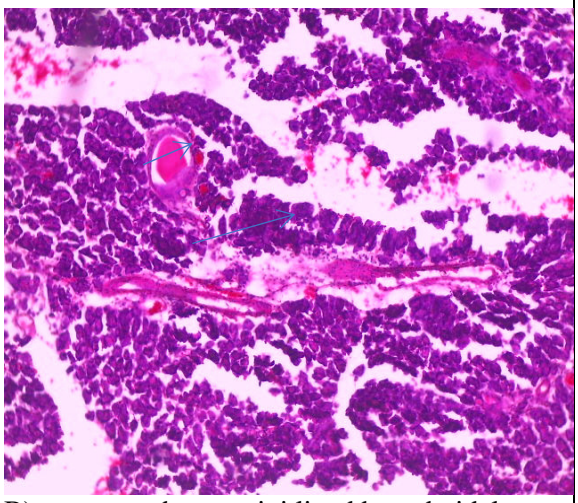


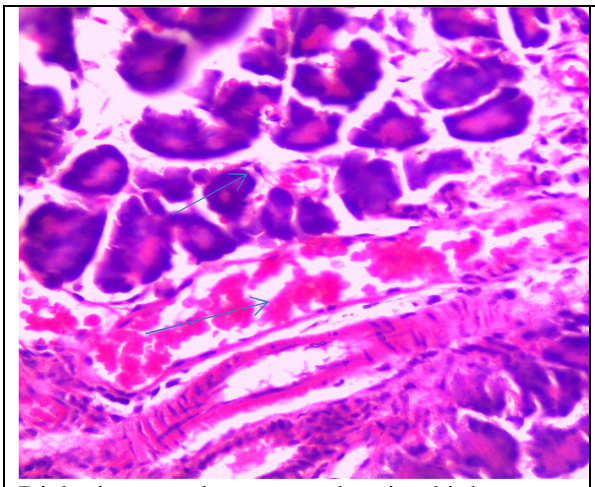
Fig 7: Effect of saponins and ethanol extract of *Spondias mombin* treatment on Dipeptidyl Peptidases 4 (DPP4) gene expression in type 2 diabetic rats



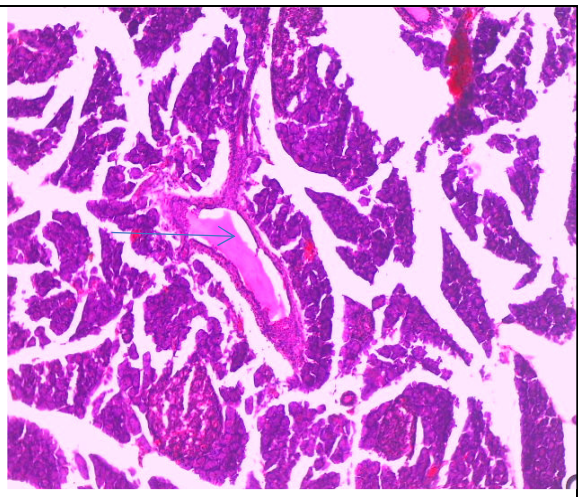
Normal control pancreas shows acini lined by cuboidal epithelial cells (black arrow), islet of langerharn and normal ducts(blue arrow)



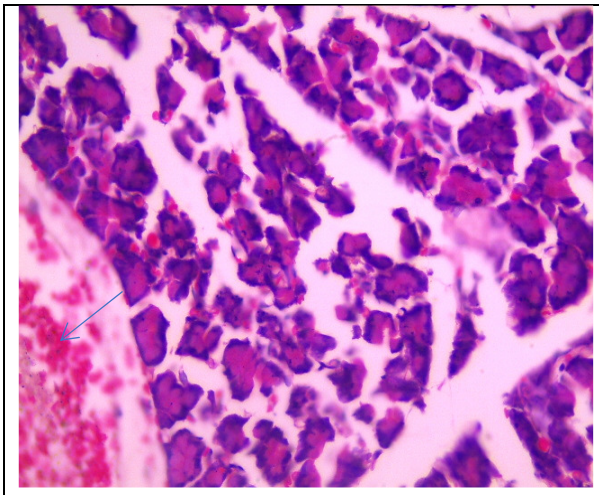
B) pancreas shows acini lined by cuboidal epithelial cells, islet of langerharn and normal ducts



Diabetic control pancreas showing high presence of inflammatory cells and pancreatitis



D) pancreas of saponin treated groups showing acini lined by cuboidal epithelial cells, islet of langerharn and normal ducts



(E) Extract treated rats showing normal pancreas

Fig. 8: Effects of saponins and ethanol extract of *Spondias mombin* in type 2 diabetic rats induced by high fat diet and Streptozotocin. A) Normal control B) Diabetic control C) Positive control D) Diabetic rats treated with saponins 200mg/kg body weight E) diabetic rats treated with 400mg/kg of ethanol extract of *Spondias mombin*

Discussion

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and progressive impairment of pancreatic beta-cell function. In experimental studies, these features can be reproduced using a combination of a high-fat diet (HFD) and low-dose streptozotocin (STZ), which induces metabolic stress associated with insulin resistance together with partial impairment of insulin secretion. In the present study, the potential antidiabetic effects of isolated saponins and ethanol extracts of *S mombin* leaves were investigated by assessing the relative expression of genes involved in

insulin signaling and glucose metabolism, namely the insulin receptor (IR), protein kinase B (AKT), glucose transporter 4 (GLUT4), peroxisome proliferator-activated receptor gamma (PPARγ), and dipeptidyl peptidase-4 (DPP4).

The insulin receptor represents an important starting point in the cellular response to insulin. Following insulin binding, activation of the receptor initiates a series of intracellular phosphorylation events that ultimately regulate glucose metabolism. One of the major downstream mediators of this pathway is AKT, which plays an important role in several metabolic actions of insulin, including the regulation of glucose uptake. In the present study, induction of T2DM with HFD/STZ resulted in a marked reduction in the expression of both IR and AKT in the diabetic untreated group (Group 3) compared with the normal control group (Group 1) (figure 3). The reduced expression of these genes suggests disruption of insulin signaling in the diabetic animals and is consistent with impaired insulin responsiveness. Interestingly, treatment with isolated saponins (Group 4) and the ethanol extract of *S. mombin* leaves (Group 5) significantly increased the expression of both IR and AKT compared with the diabetic untreated group. The expression levels approached those observed in the normal control and showed a pattern comparable to that of the positive control group (Group 2). This finding suggests that constituents of *S. mombin*, particularly its saponin fraction, may help restore components of the insulin signaling pathway at the transcriptional level. However, because gene expression does not necessarily reflect protein abundance or activity, these findings should be interpreted as evidence of a potential improvement in insulin signaling rather than definitive proof of increased insulin sensitivity. The restoration of IR and AKT expression observed following treatment may be particularly relevant to glucose metabolism because activation of the insulin-AKT pathway is closely linked to GLUT4-mediated glucose uptake in skeletal muscle and adipose tissue. Iheagwam *et al.* (2022) also reported that diabetes resulted in reduced expression of IR genes and AKT and treatment with *Terminalia catappa* aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats.

GLUT4 is the major insulin-responsive glucose transporter in these tissues, and its movement to the plasma membrane following insulin stimulation facilitates the uptake of circulating glucose. In the present study, HFD/STZ administration markedly reduced GLUT4 gene expression in Group 3 compared with the normal control group. This reduction may contribute to impaired peripheral glucose utilization and the hyperglycemic state observed in the diabetic animals. Treatment with both isolated saponins (Group 4) and *S. mombin* ethanol extract (Group 5) significantly increased GLUT4 expression relative to the diabetic untreated group (figure 4). A similar restorative pattern was observed in the positive control group (Group 2). The simultaneous increase in AKT and GLUT4 expression in the treated groups is noteworthy because these molecules occupy closely related positions within the insulin signaling pathway. The findings therefore suggest that *S. mombin* may improve peripheral glucose handling, at least partly, through modulation of the AKT/GLUT4 pathway.

This observation is consistent with previous report on the antidiabetic mechanisms of medicinal plants (El-Abhar and Schaalan, 2014, Amjad *et al.,* 2025). Several mechanisms have been proposed to account for the glucose-lowering effects of medicinal plants, including enhancement of glucose uptake, stimulation of GLUT4 translocation, insulinomimetic and insulinotropic activities, modification of glycogen metabolism, and modulation of incretin activity (El-Abhar and Schaalan, 2014; Kumar *et al.,* 2009; Ben *et al.,* 2007). *Momordica charantia* has been reported to enhance cellular glucose uptake and increase the expression of components of the PI3K pathway and GLUT4 (Kumar *et al.*, 2009; Ben *et al.,* 2007). Similarly, a recent narrative review reported that several medicinal plants exert antidiabetic effects by enhancing glucose uptake through mechanisms involving GLUT4 translocation (Rabiatul *et al.,* 2023). The present findings are also in agreement with reports indicating that saponins obtained from medicinal plants can exert antidiabetic effects through modulation of AKT phosphorylation and enhancement of GLUT4 activity or translocation (Chen *et al*., 2022; Jing *et al*., 2018). Taken together, these observations provide a possible molecular basis for the improvement in glucose regulation observed following treatment with *S. mombin* saponins and ethanol extract.

Another important regulator of glucose and lipid metabolism examined in this study was PPARγ. PPARγ is a member of the nuclear receptor superfamily and plays a central role in adipocyte differentiation and the regulation of lipid metabolism (Desvergne and Wahli, 1999; Ntambi and Young-Cheul, 2000). Activation of PPARγ has also been associated with improved insulin signaling and glucose uptake, particularly through its effects on adipose tissue function, adiponectin secretion, and circulating free fatty acids (Ciaraldi *et al.,* 1995). Consequently, alterations in PPARγ expression may have important implications for insulin sensitivity and metabolic homeostasis. In the present study, PPARγ expression was markedly reduced in the diabetic untreated group (Group 3) compared with the normal control (figure 6). The reduction in PPARγ expression may reflect disturbances in lipid metabolism and adipose tissue function associated with the diabetic state. In contrast, treatment with isolated saponins (Group 4) and *S. mombin* ethanol extract (Group 5) substantially increased PPARγ expression toward the level observed in the normal control group. This response suggests that the metabolic effects of *S. mombin* may extend beyond the classical insulin signaling pathway and may involve modulation of transcriptional regulators of lipid and glucose metabolism. The increase in PPARγ expression following treatment may be particularly relevant in the context of insulin resistance because altered lipid metabolism and elevated circulating free fatty acids can interfere with insulin signaling. By restoring PPARγ expression, *S. mombin* constituents may potentially contribute to improved lipid handling and insulin responsiveness. Findings from this study therefore corroborates the results of our *in silico* study (Eluehike and Onoagbe, 2025) that several saponins of *S mombin* leaves are capable of activating PPARγ hence supporting the use of *S mombin* as plant-derived therapeutic agents for T2DM management. Also, Belgum *et al.*, (2017) in their study demonstrated that medicinal plants with potential binding

property towards the specific target protein, PPARγ, can be a potential target to develop drugs that can interact and control diabetes.

An interesting finding was observed with respect to DPP4 expression. Unlike the other genes examined, DPP4 expression showed a reduction in the diabetic untreated group (Group 3), followed by an increase after treatment with the positive control, isolated saponins, and *S. mombin* ethanol extract (Groups 2, 4, and 5) (figure 7). DPP4 is an enzyme involved in the degradation of incretin hormones, particularly glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Increased DPP4 enzymatic activity can therefore reduce incretin action and contribute to impaired glucose regulation. This finding is not in agreement with our previous *in silico* investigation as the saponins were shown to inhibit as DPP4 inhibitor (Eluehike and Onoagbe, 2025).

Histological evaluation revealed that treatment with standard drug, saponins and ethanol extracts of *S. mombin* leaves improved the pathological alterations observed in the diabetic control rats (figure 8). In our previous report we have established that treatment with extracts of *S. mombin* leaves causes regeneration of pancreatic beta cells destroyed by streptozotocin induction (Eluehike and Onoagbe, 2020).

Conclusion

In summary, the administration of isolated saponins and ethanol extracts of *Spondias mombin* leaves demonstrated powerful transcriptional modulation of the insulin signaling pathway in T2DM rats. Both treatments successfully ameliorated insulin resistance by upregulating the expression of the Insulin Receptor, AKT, GLUT4, and PPAR gamma. The comparable performance of the isolated saponins (Group 4) to the crude extract (Group 5) strongly implies that saponins are a principal phytoconstituent responsible for the plant's insulin-sensitizing and antidiabetic activities. These findings provide solid molecular evidence supporting the ethnopharmacological use of *Spondias mombin* in managing type 2 diabetes.

References

American Diabetes Association (2023). Standards of Care in Diabetes (2023). *Diabetes Care*, 46(Supplement_1).

Amjad, A., Khan, A.U., Raza, Q. and Tahir, S.K. (2025). *Praecitrullus fistulosus* Extract Exhibits Antidiabetic Potential by Augmenting Insulin-Signaling Cascade, GLUT-4 and IRS-1, in Streptozotocin–Nicotinamide-Induced Diabetic Rats. *Foods*, 14: 3764.

Ayoka, G.A., Ideho, M.O., Ezenyi, M.O. and Agbaje, O.A. (2008). Preliminary phytochemical screening and the anti-diabetic effect of *Spondias mombin* in rats. *Journal of Ethnopharmacology*, 116(3): 463-467.

Begum, S.M.F.M., Fathima, S.Z., Priya, S., Sundararajan, R. and Hemalatha, S. (2017). Screening Indian Medicinal Plants to Control Diabetes – An *in silico* and *in vitro* Approach. *Gen. Med.,* (Los Angeles) 5: 289.

Ben W.C.R., Avtar S., Atwal, T. J. and Stan K. (2007). Water extracts from *Momordica charantia* increase glucose uptake and adiponectin

secretion in 3T3 L1 adipose cells *Journal of Ethnopharmacology,* 112(1): 77–84

Bhavsar, S.K., Singh, S., Giri, S., Jain, M.R. and Santani, D.D. (2009). Effect of saponins from Helicteres isora on lipid and glucose metabolism regulating genes expression. *Journal of Ethnopharmacology*, 124: 426-433.

Chen, Z., Jiayuan, L., Mingjie J., Yangyang C and Yihong Bao (2022*). Polygonatum sibiricum saponin* Exerts Beneficial Hypoglycemic Effects in Type 2 Diabetes Mice by Improving Hepatic Insulin Resistance and Glycogen Synthesis-Related Proteins. *Nutrients*, 14(24): 5222

Ciaraldi, T.P., Abrams, L., Nikoulina, S., Mudaliar, S. and Henry, R. R. (1995). Glucose transport in cultured human skeletal muscle cells. Regulation by insulin and glucose. *Journal of Clinical Investigation*, 96(6): 2820–2827.

Desvergne B and Wahli. W. (1999). Peroxisome Proliferator-Activated Receptors: Nuclear Control of Metabolism. Endocrine Reviews. 20(5): 649–688.

El-Abhar, H.S. and Schaalan, M.F. (2014). Phytotherapy in diabetes: Review on potential mechanistic perspectives. *World Journal of Diabetes,* 5: 176–197.

Elekofehinti, O.O., Adanlawo, I.G., Saliu, J.A. and Sodehinde, S.A. (2012). Saponins from *Solanum anguivi* fruits exhibit hypolipidemic potential in *Rattus norvegicus. Der Pharma Lettre*, 4(3): 811–814.

Elekofehinti, O.O., Adanlawo, I.G., Komolafe, K. and Ejelonu, O.C. (2012). Saponins from *Solanum anguivi* fruits exhibit antioxidant potential in Wistar rats. *Annals of Biological Research*, 3(7): 3212-3217.

Elekofehinti, O.O., Lawal, A.O., Ejelonu, O.C. Molehin O.R., and Famusiwa, C.D. (2020). Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of Annona muricata Annonaceae: *in silico* and *in vivo* studies. *Journal of Diabetes Metabolic Disorders,* (2020). https://doi.org/10.1007/s40200-020-00491-7.

Elekofehinti, O.O. Onunkun, A.T. and Olaleye, M.T. (2020). *Cymbopogon citratus* (DC.) Stapf mitigates ER-stress induced by streptozotocin in rats via down-regulation of GRP78 and up-regulation of Nrf2 signaling *Journal of Ethnopharmacology,* 262: 113130.

Eluehike, N. and Onoagbe, I.O. (2020). Preliminary nutrient determination and regeneration of pancreatic islet cells by extracts of *Spondias mombin* leaves in streptozotocin-induced diabetic rats. *Avicenna Journal of Medical Biochemistry.* 8(1):1-14.

Eluehike, N. and Onoagbe, I.O. (2025). Saponins from *Spondias mombin* leaves as potential PPAR$_\Upsilon$ agonist and DPP-4 inhibitor for type 2 diabetes treatment using molecular docking studies and ADMET profiling. *Journal of Basic and applied Medical Sciences*, 5(2): 122-133.

Gao, H., Zhen-Yu, G. and Guang-Yao, S. (2020). PPAR-γ and DPP-4: The complex interplay in the

management of Type 2 Diabetes. *Frontiers in Endocrinology*, 11: 584.

Hostettmann, K. (Ed.) (1991). Method in plant Biochemistry, assays for bioactivity. Academic press, London. 6:142-148.

Iheagwam, F.N., Iheagwam, O.T., Onuoha, M.K. Ogunlana, O.O. and Chinedu, S.N. (2022). *Terminalia catappa* aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats. *Scientific Report.* 12, 10711.

Jing, X., Wang, S., Feng, T., Chen, Y. and Yang, G. (2018). Hypoglycemic and hypolipidemic effects of total saponins from *Stauntonia chinensis* in diabetic *db/db* mice *Journal of Cell Molecular Medicine*, 22(12): 6026–6038

Kim, S. H., Hyun, S.H. and Choung, S. Y. (2006). Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacology*, 104(1-2): 119-123.

Kharroubi, A.T. and Darwish, H.M. (2015). Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*, 6(6): 850.

Kumar, R.S., Balaji, T.S. and Uma, P.K. (2009). Sehgal Fruit extracts of *Momordica charantia* potentiate glucose uptake and up-regulate Glut-4, PPARγ and PI3K. *Journal of Ethnopharmacology,* 126(3): 533-537

McAnuff, M.A., Harding, W.W., Omoruyi, F.O., Jacobs, H., Morrison, E.Y. and Asemota, H.N. (2005). Hypoglycemic effects of steroidal sapogenins isolated from Jamaican bitter yam, Dioscorea polygonoides. *Food Chemical Toxicology*, 43(11): 1667-1672.

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals*. 8th ed., The National Academies Press.

Ntambi, J.M. and Young-Cheul, K. (2000). Adipocyte Differentiation and Gene Expression. The *Journal of Nutrition,* 130(12): 3122S–3126S

Petersen, M.C. and Shulman, G.I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological Reviews*, 98(4): 2133-2223.

Reed M.J., Meszaros K., Entes L.J., Claypool M.D., Pinkett J.G., Gadbois T.M. and Reaven G.M. (2000). A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism*, 49(11): 1390-1394.

Rabiatul, A., Ayu, S., Sapto, Y. and Nurkhasanah (2023). potential plants with antidiabetic activity in glut-4 translocation: A narrative review. *Jurnal Ilmu Farmasi dan Farmasi Klinik (JIFFK)*, 1(1): 43-52. ISSN: 1693-7899 e-ISSN: 2716-3814

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C.L. and Ramarao, P. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacology Research*, 52(4): 313-320.

Zheng, T.G., Shu, Z., Yang, S., Mo, Y. Zhao and Mei, Z. (2012). Antidiabetic effect of total saponins from *Entada phaseoloides* (L.) merr. In type 2 diabetic rats. *Journal of Ethnopharmacology*, 139: 814-821.