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Original Research Article

Effect of *Terminalia catappa in* High-Fat Diet/STZ-Induced Insulin Resistance: Deciphering Possible Role of GLUT4

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ABSTRACT

Insulin resistance (IR) is reduced responsiveness of peripheral tissues, such as skeletal muscle, liver, and adipose tissue, to insulin action. Since skeletal muscle is most liable for the majority of glucose uptake, any disturbance in GLUT4 translocation becomes a major contributor to impaired glucose homeostasis. The current study examined the therapeutic potential of Terminalia catappa (TC) leaf extract in reducing insulin resistance with a focus on GLUT4 expression in skeletal muscle. The extract was first analysed using LC-MS to identify its phytochemical constituents, and the major compounds were further subjected to in silico docking using Schrodinger Glide to assess their interaction with the GLUT4 protein (PDB ID: 7WSN). The Male Sprague-Dawley rats were given a high-fat diet for eight weeks before receiving an intraperitoneal injection of streptozotocin (35 mg/kg) to induce insulin resistance. The dose of 400 and 800 mg/kg of TC extract were given daily orally. When compared to the HFD+STZ group, treatment with TC significantly lowered fasting blood glucose levels, enhanced circulating insulin concentrations, and decreased HOMA-IR values. In the oral glucose tolerance test, treated animals also showed improved glucose clearance. Increased GLUT4 expression in skeletal muscle was also found by Western blot analysis, suggesting that insulin signalling pathways have been restored. All of these findings point to *Terminalia catappa* potential as a natural treatment option for metabolic diseases by suggesting that it reduces insulin resistance by boosting GLUT4-mediated glucose uptake and enhancing metabolic homeostasis.

Keywords: Glucose Transporter-4, Insulin Resistance, High-fat Diet, Streptozotocin, *Terminalia catappa*

Introduction

Insulin resistance is related to the disruption of various cellular pathways, which causes the reduced sensitivity of the peripheral tissues like skeletal muscles, liver and adipose tissue to insulin.[1] In the earlier stages, the body can compensate for decreased insulin sensitivity by an increase in the insulin secretion, but over time, there is a decrease in the beta cell functioning and the development of insulin deficiency, which leads to hyperglycaemia.[2]

Skeletal muscle has a crucial role in maintaining blood glucose homeostasis, as it represents the principal site of glucose transport and utilisation of glucose but in insulin resistance cases, there is significantly reduced responsiveness of skeletal muscle to insulin.[3] As the skeletal muscle is the main site for glucose uptake, it is therefore considered the main cause of whole-body insulin resistance.[4] The impairment in the GLUT4 translocation causes insulin resistance. GLUT4 represents the predominant insulin-dependent glucose transporter responsible for mediating the uptake of extracellular glucose in insulin-sensitive tissues.[5]

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GLUT4, primarily found in muscle and fat tissues, is crucial for glucose uptake. Normally, when insulin binds to its receptor on cell surfaces, it triggers a series of phosphorylation events within the insulin signalling pathway. Consequently, GLUT4 is mobilised to the cell surface, resulting in increased intracellular glucose entry. In insulin resistance, this binding fails to initiate GLUT4 translocation, preventing glucose transport into cells and causing elevated blood glucose levels.[6] So, GLUT4 plays a vital role in managing insulin resistance.

Plants have been used for centuries to treat various diseases. Plant-based medical formulations and their active constituents have long been part of medical practices for managing blood glucose levels and improving metabolic abnormalities. One of such plants is *Terminalia catappa,* which belongs to the family Comborteace. It is a tropical tree widely distributed in coastal and humid regions. *Terminalia catappa* leaves, rich in flavonoids and tannins, have been traditionally employed for medicinal purposes for many years. The leaf extract of *Terminalia catappa* has medicinal properties, including antimicrobial,[7, 8] anti-inflammatory,[7] hepatoprotective[9], hypoglycaemic[10,11] antioxidant[7, 12] and anti-cancer[13, 14] properties. In this study, we aimed to find out the potential benefits of the methanolic leaf extract of *Terminalia catappa* and its molecular mechanism in managing insulin resistance in rats.

Materials and Methods

Chemicals and reagents

Streptozotocin was purchased from Sigma, cholesterol and, casein from Hi-Media, and lard was purchased from a commercial vendor. The Rat insulin ELISA kit was from Elab Sciences, and the CHOD-POD kit was from Coral Clinical Systems.

Plant collection and extraction

The leaves of *Terminalia catappa* (TC) were collected from the surrounding areas of Rushikonda, Visakhapatnam, Andhra Pradesh,

India (17°46'57"N, 83°23'06"E), during the month of June. The authentication was done at the Department of Botany, Andhra University. The Voucher specimen No of *Terminalia catappa* is (AUV25463). The leaves were shade-dried and blended into a coarse powder. The 800 g of leaf powder was macerated with intermittent stirring for 3 days with methanol in (1:5w/v), filtered using Whatman No. 1 filter paper, and the filtrate was evaporated using a rotary evaporator and stored in a desiccator.

Liquid Chromatography-Mass Spectroscopy

Chromatographic separation was carried out by using a linear ion trap quadrupole LC–MS/MS system (QTRAP 5500+, AB Sciex, USA) equipped with a reversed-phase analytical column (150 mm × 2.1 mm, 2.7 µm). With a constant flow rate of 0.5 mL/min and a sample injection volume of 5 µL, the column temperature was kept at 40 °C. Mobile phase A (distilled water with 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol supplemented with 5 mM ammonium formate and 0.1% formic acid). 20% of mobile phase B was used to start the gradient elution program, and over the course of 25 minutes, it was progressively increased to 100%. Before the subsequent injection, this composition was maintained at 100% mobile phase B for ten more minutes, and then it was re-equilibrated to the starting conditions (20% mobile phase solvent B) for ten more minutes.[15, 16]

Molecular docking

Ligand Preparation

Natural compounds isolated from the methanolic extract of *Terminalia catappa* were identified using LC-MS/MS analysis. The selected phytochemicals included: Vitexin-2"-O-rhamnoside, Isoquercetin, Vitexin, Cis-resveratrol-O-glucuronide, D-mannose, 1,4-D-Xylobiose, Trans-resveratrol 3-O-glucuronide, Kaempferol, and Gallic acid. The 2D structures of these ligands were drawn and converted into 3D using the Lig Prep module of the Schrödinger suite (Schrödinger Release 2023-1, Schrödinger, Inc., USA)[17, 18.] Ligands were energy-minimised using the OPLS4 force field, with proper ionisation states at pH 7.0 ± 0.5.

Protein Preparation

The Research Collaboratory for Structural Bioinformatics Protein Data Bank provided the three-dimensional structure of human glucose transporter-4 (GLUT4; PDB ID: 7WSN), crystallised in complex with cytochalasin B. The Protein Preparation Wizard module in Maestro (Schrödinger, Inc., USA) was used to refine the protein's structure. Water molecules that were more than 5 Å away from heteroatoms were eliminated, hydrogen atoms were added, the proper bond orders were assigned, and disulfide bridges were created during preparation. After reconstructing incomplete side chains, the Schrödinger Suite's OPLS4.4 force field was used for restrained energy minimisation (Schrödinger, Inc., USA).

Receptor Grid Generation

A receptor grid was generated at the active site where cytochalasin B was bound. The binding site was defined using the co-crystallised ligand, and Van der Waals radii scaling was applied with a partial charge cut off of 0.25. We conducted molecular docking studies using natural compounds identified from the methanolic extract of *Terminalia catappa*. These were analysed to evaluate their potential to bind with the GLUT4 protein (PDB ID: 7WSN), a crucial receptor involved in glucose transport. Glide XP (Extra Precision) docking was performed using the Glide module. All ligands were flexibly docked into the defined binding pocket of GLUT4. Docking scores (XP GScore) and 2D interaction diagrams were generated and analysed to understand the binding affinity and key interactions.

Experimental Animals

All Experiments were conducted according to the Committee for the Control and Supervision of Experimental Animals (CCSEA) guidelines, with an ethical approval reference No. of IAEC/GU-1287/PRR-F/2/December 2023. Male Sprague Dawley rats of weight 150-180 gm used in the study were purchased from Vyas Labs, Hyderabad. The animals were placed in polypropylene cages with corn cob bedding and changed regularly to maintain hygiene. Rats had free access to standard laboratory chow and filtered drinking water ad libitum. The animals were maintained at 22±2 °C and humidity was maintained at 60-70% with a 12 h light / 12 h dark cycle. The animals were acclimatised for a week prior to the experimental period. The insulin resistance model was developed by using High fat Diet (HFD).[19]The composition of the HFD 19 was Powdered normal pellet (365 mg/kg), Cholesterol (10 g), Casein (23 g), protein and mineral mixture(10 mg), Methionine(2 g), Sodium Chloride (2 g). The HFD was given daily for 8 weeks; the rats with HFD were given Streptozotocin (STZ) (dissolved in citrate buffer, pH-4.4) at a dose of 35mg/kg body weight given intraperitoneally. Rats were considered diabetic when the fasting blood glucose levels >250mg/dl.[20] Then, the rats were randomly allocated into four groups and each group contained 6 animals: Normal control group (i.e. received normal saline), HFD+STZ group (i.e. received HFD and STZ), then, low dose TC group (i.e. received TC 400mg/kg) and high dose TC group (i.e. received TC 800mg/kg) with HFD+STZ. The TC was given for 21 days, i.e. from the 10th week to the 12th week. Figure 1 shows the experimental design.

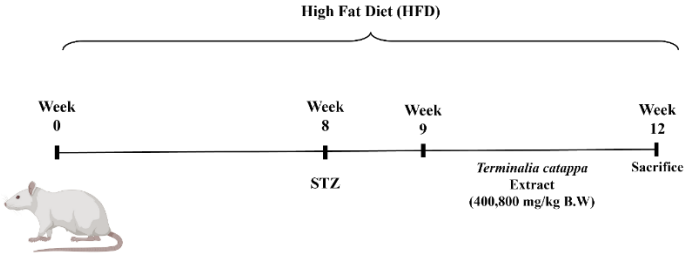

Figure 1: Experimental design

Collection of blood samples

After experimental procedures, Fasting blood samples were collected from the retro-orbital plexus and centrifuged at 5000 rpm for 10 minutes to separate the serum. The obtained serum was used for the analysis of glucose, insulin, and lipid profile parameters.

Collection of tissue samples

On the 12th week of the experiment, the rats were euthanised using carbon dioxide asphyxiation as per CCSEA guidelines. Pancreas were collected and fixed in 10% formalin for 24 hours for histopathological examination, while the skeletal muscle was stored at −80°C for further molecular analyses

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed after the development of insulin resistance, i.e., post-HFD and post-STZ administration. To assess OGTT, fasting blood glucose levels were measured in all groups. An oral dose of glucose (2 g/kg body weight) was administered, and fasting blood glucose levels were recorded at 0, 30, 60, 90, and 120 minutes post-administration using a glucometer (Accu-check active , Roche Diabates Care, India).[21]The area under the curve (AUC) for glucose was calculated to evaluate overall glucose exposure over time, using the trapezoidal rule.[22]

Assessment of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Homeostatic Model Assessment of Pancreatic β-Cell Function (HOMA-β), Quantitative Insulin Sensitivity Check Index (QUICKI)

Insulin resistance was assessed using the HOMA-IR index. Insulin concentrations were determined using a commercial ELISA kit. The formula (equation 1) for HOMA-IR[23] is given below

$$HOMA - IR = \frac{Glucose\ (mmol/L) \times Insulin\ (\mu U/mL)}{22.5}$$ Equation 1

HOMA-β was determined using the formula (i.e. equation 2).[24]

$$HOMA - \beta = \frac{20 \times insulin\ (\mu IU/mL)}{(fasting\ glucose\ (mmol/L) - 3.5)}$$ Equation 2

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QUICKI was determined according to the following formula (equation 3).[25]

$$QUICKI = \frac{1}{[(log\ fasting\ blood\ glucose\ (mg/dL) + (log\ insulin\ (\mu IU/mL)]}$$

Equation 3

Lipid profile

The total cholesterol, Triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were quantified in serum using CHOD-POD-based enzymatic assay kits (Coral Clinical Systems, India) in compliance with the manufacturer's instructions. The Friedewald equation was used to calculate the levels of low-density lipoprotein cholesterol (LDL-C), i.e. equation 4.[26]

$$LDL - C\ (mg/dL) = TC - HDL - C - \left(\frac{TG}{5}\right)$$ Equation 4.

Western Blot

The skeletal muscle tissue was lysed by using RIPA buffer containing 1% protease inhibitor. Protein samples were resolved using 10% SDS–SDS-polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene fluoride (PVDF) membrane.[27] To avoid nonspecific binding, the membrane was blocked with 5% skimmed milk. Following blocking, the membrane was incubated with primary antibodies overnight, i.e. Anti-GLUT4 (Invitrogen, Cat PA5-80022; rabbit polyclonal, 1:1000 dilution) and Anti-β-actin (Invitrogen, Cat: PA1-16777; rabbit polyclonal, 1:2000 dilution). Later, secondary antibody (Goat Anti-Rabbit IgG, Sigma, Cat# AP307P; 1:5000 dilution) was added to the membrane and incubated for 1 hour. Detection was performed using enhanced chemiluminescence (ECL) with luminol as the substrate. Bands were visualised using an imager (Fusion Solo Smart Imager, Vilber, France) and estimated using ImageJ software.

Histopathology

The Pancreas were collected and stored in 10% formalin solution. After that, they were treated with descending alcoholic concentrations, and paraffin tissue embedding was done. The tissue was sliced into 4µm using a microtome, and deparaffinization was done by xylene, and slides were treated with a graded series of alcohols and later stained with Mayer's haematoxylin and eosin.[28] Histological slides of the Pancreas tissue were observed under a Leica microscope (DM100, Leica Microsystems, Germany).

Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison test were used to statistically compare the experimental groups. GraphPad Prism software (version 8.0) was used for all statistical analyses, and differences were considered statistically significant at $p < 0.05$.

Results and Discussion

LC-MS characterisation

The LC-MS analysis revealed the various components present in the methanol leaf extract of *Terminalia catappa*. The major compounds detected were vitexin, kaempferol, gallic acid and iso-quercetin, as shown in Table 1 followed by the total ion chromatogram of the methanol extract of *Terminalia catappa* shown in Figure 2 and Supplementary Data S1.

In silico study

The molecular docking study of the selected natural compounds from the methanol leaf extract of *Terminalia catappa* with the GLUT4 receptor (PDB ID: 7WSN) revealed that several of these compounds, particularly flavonoid glycosides like Vitexin-2"-O-rhamnoside and Isoquercetin, showed strong potential for interaction (Table 2, Figure 3a-3b; Figure 4a -b). The 2D ligand interaction diagrams for other active phytoconstituents can be found at the Supplementary Data S2.

The therapeutic effects observed in this study may be linked to the phytochemical constituents of *Terminalia catappa*, including flavonoids, tannins, and phenolic compounds, which have been previously reported.[29]

Table 1: Major constituents found in the extract of *Terminalia catappa*

S/N	RT	Mass	Component
1	1	175.07	Methyl. Beta-D-glucopyranoside
2	4.41	285.01	Kaempferol
3	9.19	431.0542	vitexin
4	10.28	431.15	vitexin
5	10.92	463.14	Isoquercetin
6	13.17	577.21	vitexin-2-O-rhamnoside
7	25.77	281.081	1,4-D-xylobiose
8	26.7	283.19	D-Mannose 6-phosphate
9	33.42	427.721	Resveratrol 3-O-D-glucuronide
10	37.86	169.051	Gallic acid

LC-MS/MS analysis of the extract confirmed the presence of multiple bioactive compounds, which may act synergistically to improve insulin signalling and metabolic outcomes. Among them, Vitexin-2"-O-rhamnoside showed the highest binding affinity, with a docking score of -16.008 kcal/mol, significantly outperforming the native bound ligand cytochalasin B with a docking score of -11.938 kcal/mol. This suggests a strong and stable interaction with the active site of GLUT4. It formed several hydrogen bonds with polar amino acid residues like ASN176, GLN177, GLN298, ASN304, and ASN427. Additionally, it engaged in Pi-Pi stacking interactions with aromatic residues such as TRP404 and PHE405. Its high polarity and numerous hydroxyl groups are likely responsible for its excellent binding performance. Isoquercetin also showed a notable docking score of -12.884 kcal/mol. It formed hydrogen bonds with ASN431, ASN427, and SER96. The presence of sugar groups in its structure likely enhances both its solubility and its ability to form multiple interactions within the GLUT4 binding pocket.

The glucuronide derivatives of resveratrol- cis- and trans-resveratrol glucuronides and D-mannose showed moderate binding scores of -10.236, -10.66 and -9.478 kcal/mol, respectively. These results suggest that glucuronidation may play a role in improving receptor interactions. These compounds mainly formed hydrogen bonds with residues like GLN298, ASN304, and TRP428. In contrast, simpler bioactive compounds such as kaempferol and gallic acid exhibited lower docking scores, i.e. -8.759 and -7.461 kcal/mol, respectively.

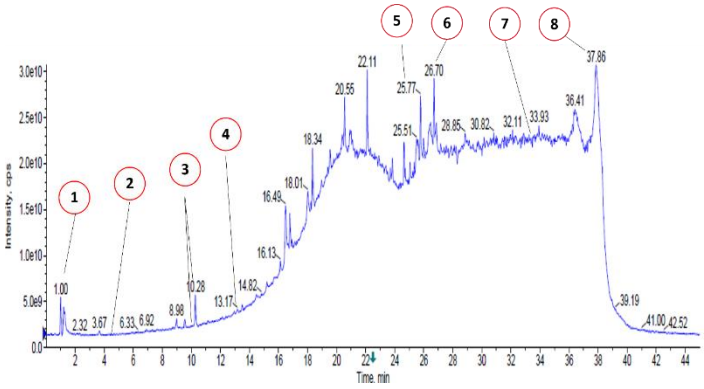

Figure 2: Total ion chromatogram of the methanol leaf extract of *Terminalia catappa,* where 1= Methyl. Beta-D-glucopyranoside, 2=Kaempferol, 3=Vitexin, 4=Vitexin-2-O-rhamanoside, 5=1,4-D-xylobiose, 6=D-Mannose-6-phosphate, 7=Resveratrol 3-O-D-glucuronide, 8= Gallic acid.

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Table 2: Docking score and key interactions of the phytoconstituents of *Terminalia catappa*

S/ No.	Compound	XP GScore	Key Interacting Residues	Interactions
1	Vitexin-2"-O-rhamnoside	-16.008	ASN176, GLN177, GLN298, ASN427, TRP404, GLN299, ASN304 GLH 396	H-bonds, Pi-Pi stacking
2	Isoquercetin	-12.884	ASN431, ASN427, TRP404, SER96, MET420, TRP428	H-bonds, Pi stacking
3	Vitexin	-11.239	ASN431, SER96, SER153, TRP428, TRP404	H-bonds, Pi-Pi stacking
4	Cis-resveratrol-O-glucuronide	-10.66	GLN298, GLN299, TRP404, ASN304, MET420	H-bonds, Pi stacking
5	D-mannose-6-Phosphate	-10.236	GLN298, ASN304, TRP404, GLH396.	H-bonds
6	1,4-D-Xylobiose	-10.061	GLN177, GLN298, GLN298, TRP404, GLH396	H-bonds
7	Trans-resveratrol 3-O-glucuronide	-9.478	ASN176, ASN176, TRP428, SER96	H-bonds, Pi stacking
8	Kaempferol	-8.759	SER153, MET420	H-bonds
9	Gallic acid	-7.461	TRP428, SER96, SER96	H-bonds
10	Cytochalasin B	-11.938	GLN298, GLN 299 TRP 404	H-bonds

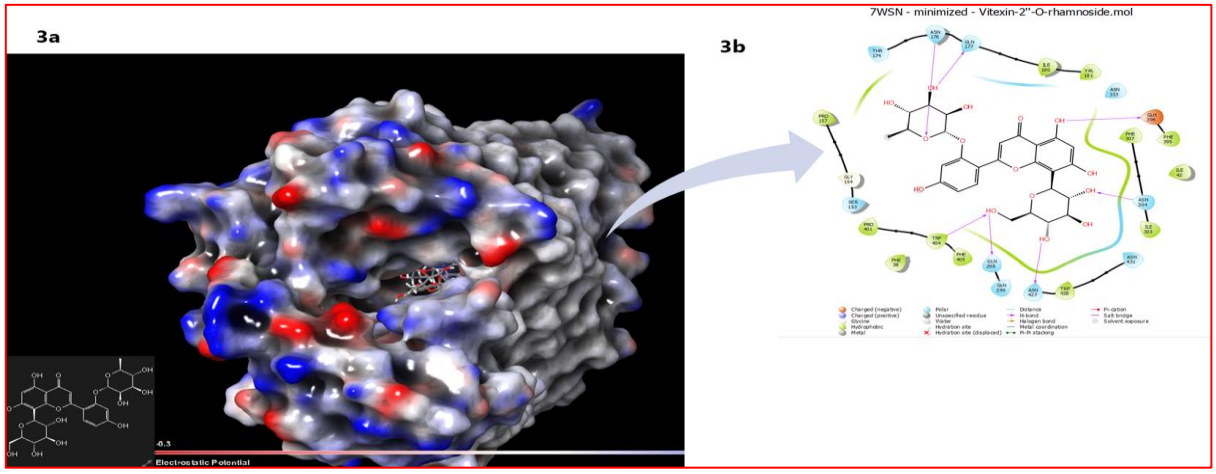

Figure 3: Electrostatic potential surface diagram of the GLUT4 receptor (PDB ID: 7WSN) in complex with Vitexin-2"-O-rhamnoside (A). Figure 2D ligand interaction diagram showing the hydrogen bonding and key interactions between Vitexin-2"-O-rhamnoside and the GLUT4 receptor (B).

Terminalia catappa methanol leaf extract alleviated fasting blood glucose levels and insulin resistance in HFD+STZ rats and increased insulin sensitivity

Treatment with *Terminalia catappa* 800mg/kg methanol leaf extract significantly (191.17± 12.12, p<0.001 vs HFD+STZ group) improved fasting blood glucose and insulin sensitivity. There was a significant elevation of fasting blood glucose levels in the HFD+STZ rats (298.13±21.22, p<0.001) when compared to the normal control shown in Figure 5A. Insulin concentrations were (0.9 ± 0.08 ng/mL) in the control group, which was markedly increased in the HFD+STZ group (1.39 ± 0.11 ng/mL, p<0.001), indicating a hyperinsulinemia condition in the later. Treatment with a High dose of TC decreased the insulin concentrations (1.1 ± 0.15 ng/mL) (p<0.001 vs HFD+STZ group) (Figure 5B). HOMA-IR values were high in HFD+STZ (p<0.001 vs control), indicating severe insulin resistance (Figure 5C). HOMA-IR was significantly reduced in all the TC800mg/kg group, reflecting an improvement in insulin sensitivity (12.51±0.63 vs 24.09±0.97 in HFD+STZ, P<0.001). Compared to the control group, HOMA-β in the HFD+STZ group was significantly decreased ($p < 0.001$). Whereas in the high-dose TC treatment group, a respective increase ($p < 0.001$) compared to the HFD+STZ group (Fig. 5D) was noted. QUICKI index in HFD+STZ rats was significantly decreased (0.2459± 0.001585 $p <$ 0.001 vs control rats).

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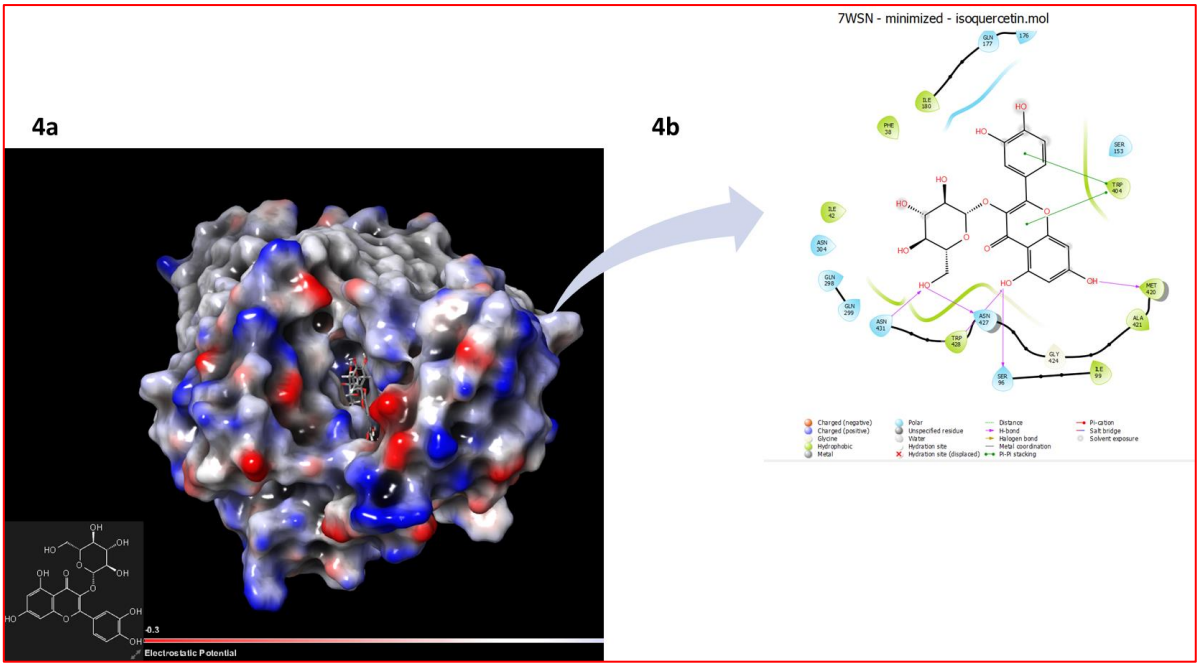

Figure 4: Electrostatic potential surface diagram of the GLUT4 receptor (PDB ID: 7WSN) in complex with Isoquercetin (A).2D ligand interaction diagram showing hydrogen bonding and key interactions between Isoquercetin and the GLUT4 receptor (B).

Furthermore, compared to the HFD+STZ group, the QUICKI index was significantly increased after treatment with TC high dose (0.2643±0.0019; $p < 0.001$) in comparison to the HFD+STZ group (Figure 5E).

Terminalia catappa improved OGTT in rats

The OGTT revealed a substantial increase in glucose excursion in the disease control group [HFD+STZ (Figure 6A)], as evidenced by a significantly elevated area under the curve (AUC) compared to the normal control shown in Figure 6B). This suggests impaired glucose clearance, a hallmark of Insulin Resistance. *Terminalia catappa* treatment produced a marked improvement in glucose tolerance as indicated by notable reduction in AUC (18426 ± 236.7, p<0.001) in comparison to HFD+STZ group.

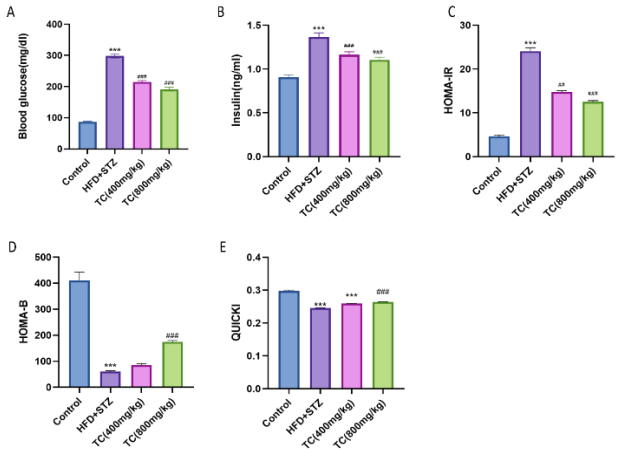

Figure 5: Effects of *Terminalia catappa* methanol leaf extract treatment on glucose tolerance and insulin sensitivity in insulin-resistant rats (A) Fasting Blood glucose, (B) Insulin levels (C) HOMA-IR, (D)HOMA-B, (E)QUICKI. Values were expressed as the mean± SEM (n=6), *P ≤ 0.05, **p ≤ 0.01, ***P ≤ 0.001 versus control and # P≤;0.05, ##p ≤ 0.01, ### P ≤ 0.001 versus HFD+STZ.

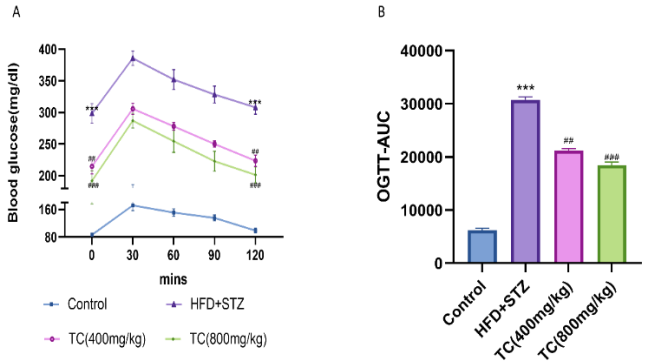

Figure 6: *Terminalia catappa* methanol leaf extract improved glucose tolerance and insulin sensitivity in insulin-resistant rats (A) OGTT, (B) AUC of OGTT, there is a significant increase in the area under the curve of OGTT. Values are expressed as the mean ± SEM (n=6), *P < 0.05, **P < 0.01, ***P < 0.001 versus NC and #P<0.05, ##P<0.01, ###P < 0.001 versus HFD+STZ.

Terminalia catappa effect on lipid profile

There was elevated Total cholesterol in the HFD+STZ group (111.2 ± 5.79 mg/dl in control vs 136.7 ± 7.09 mg/dl in HFD+STZ-treated rats, p<0.01) and triglyceride levels (123.3 ± 8.58 in control vs 185.7 ± 12.15 mg/dl in HFD+STZ-treated rats, p<0.01) (Figure 7 A-B). Treatment with *Terminalia catappa* methanol leaf extract significantly decreased the Triglycerides and Total cholesterol levels in HFD+STZ-treated rats. On the other hand, there were elevated LDL values in the HFD+STZ group, and that was decreased on treatment with TC 800 mg/kg (45.50 ± 3.32mg/dl vs 32.17 ± 3.54 mg/dl, p<0.05). The HDL levels in the TC 800 mg/kg group in comparison to HFD+STZ rats were similar (41.50 ± 2.935 vs 41.50 ± 2.75, p<0.05) (Figure 7 C-D).

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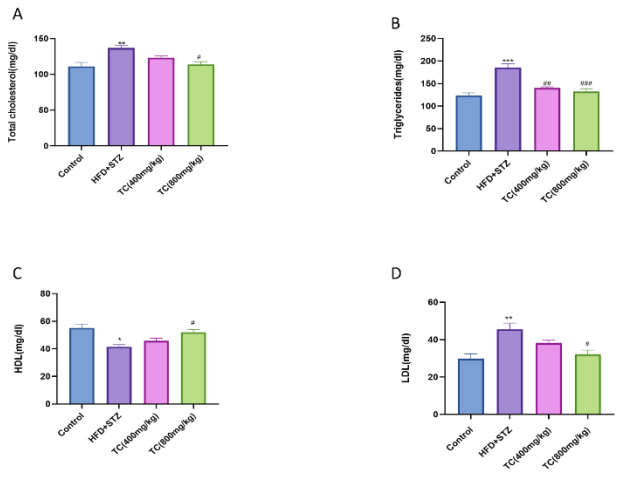

Figure 7: Effect of treatment with *Terminalia catappa* methanol leaf extract on lipid profile (A) Total cholesterol, (B) Triglycerides, (C) HDL levels, (D) LDL levels. Each bar represents the mean ± SEM (n = 6), *P < 0.05, **P < 0.01, ***P < 0.001versus Control and #P<0.05, ##P<0.01, ###P < 0.001 versus HFD+STZ.

Terminalia catappa improved GLUT4 protein expression

The GLUT4 expression in Skeletal muscle in control group, HFD+STZ group and HFD+STZ rats treated with the extract was determined by using western blotting. The bands were explicitly obtained for GLUT4 and β-Actin on the blots (Figure 8). There was a reduction in the GLUT4 expression in the HFD+STZ group (0.5064± 0.024, p<0.01) compared to the control. The TC 800mg/kg treatment modulated the expression of GLUT4 (0.8487± 0.297 p<0.01) in comparison to the HFD+STZ group.

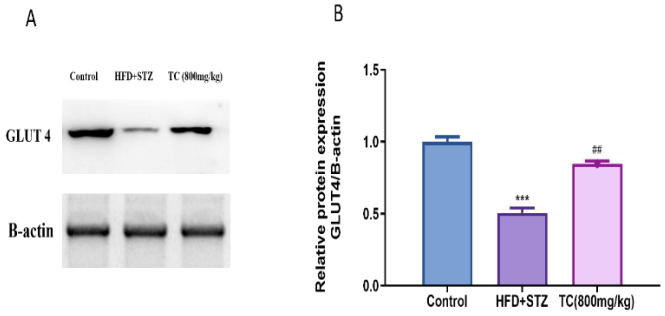

Figure 8: Effect of treatment on GLUT4 expression. (A) Relative expression of GLUT4 (B) western blot. Each bar represents the mean ± SEM (n = 6), *P < 0.05, **P < 0.01, ***P < 0.001versus Control and #P <0.05, ##P <0.01, ###P < 0.001 versus HFD+STZ.

Since skeletal muscle is the main location for the transportation and utilisation of glucose, it is essential for blood glucose regulation. However, in cases of insulin resistance, there is reduced responsiveness of skeletal muscle to insulin.[4, 30, 31] The transfer of GLUT4 from inside cell vesicles to the plasma membrane is the most crucial stage in regulating glucose uptake.[32] At the molecular level, Western blot analysis revealed a significant downregulation of GLUT4 protein expression in the skeletal muscle of insulin-resistant rats. However, treatment with TC restored GLUT4 expression to normal levels. This suggests that *Terminalia catappa* may be partially attributed to its ability to modulate insulin-responsive glucose transporter mechanisms. GLUT4 is critical for insulin-mediated glucose uptake, and its expression and translocation are essential for maintaining insulin sensitivity in muscle tissues.[32] Restoration of GLUT4 levels by TC reinforces the mechanistic basis of its insulin-sensitising properties.

Histopathology of Pancreas

There was no morphological change in the control group, which displayed normal pancreatic architecture. In the pancreas, acinar cells and the exocrine component were tightly packed. Whereas in the HFD+STZ group, the pancreatic tissue exhibited marked vascular congestion. There was noticeable infiltration of inflammatory cells within the acini and intralobular ducts. Small vacuoles were seen in nearly every acinar cell, and the cells were observed to be enlarged in the rats receiving HFD+STZ. However, treatment with *Terminalia catappa* methanol leaf extract repaired the alterations, as shown with the reduced infiltration of intralobular ducts and reduced vascular congestion and normal morphology, as shown in Figure 9.

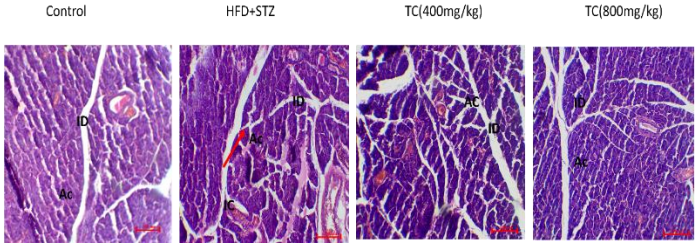

Figure 9: Pancreas Histopathology (H&E-stained section scale bar-200µm). Ac-acinar cells, ID-Intra lobular duct, IC - Inflammatory cells, Red arrow indicates cellular vacuolation.

Conclusion

This study highlights the promising potential of *Terminalia catappa* leaf extract in improving insulin resistance. In a rat model, the extract significantly lowered fasting blood glucose and insulin levels, improved glucose tolerance, and helped normalise lipid profiles-pointing to broad metabolic benefits. One of the key findings was the increased expression of GLUT4 in skeletal muscle, which suggests that the extract helps restore proper insulin signalling. These in vivo results are further supported by molecular docking studies. Flavonoid glycosides identified by LC-MS/MS—particularly Vitexin-2"-O-rhamnoside and Isoquercetin—exhibited strong binding affinities toward the GLUT4 receptor. Together, these in vivo and in silico results underscore the potential of *Terminalia catappa* as a natural, plant-based therapeutic agent for managing insulin resistance and associated metabolic disorders. However, further studies involving detailed mechanistic validation, pharmacokinetic profiling, and clinical translation are required to confirm its therapeutic applicability.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology. 2005;42(5):987-1000.
2. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. Avicenna J Med. 2020;10(04):174-88.
3. Hulett NA, Scalzo RL, Reusch JE. Glucose uptake by skeletal muscle within the contexts of type 2 diabetes and exercise: an integrated approach. Nutrients. 2022;14(3):647.
4. Merz K, Thurmond D. Role of skeletal muscle in insulin resistance and glucose uptake. Compr. Physiol. 10, 785–809. 2020.
5. Wang T, Wang J, Hu X, Huang XJ, Chen GX. Current understanding of glucose transporter 4 expression and functional mechanisms. World J of Biol Chem. 2020;11(3):76.
6. van Gerwen J, Shun-Shion AS, Fazakerley DJ. Insulin signalling and GLUT4 trafficking in insulin resistance. Biochem Soc Trans. 2023;51(3):1057-69.
7. Kumar VD, Kokila GS, Sarvatha A, Pradeepa D. Phytochemical profiles, in vitro antioxidant, anti inflammatory and antibacterial activities of aqueous extract of *Terminalia catappa* L. leaves. J Pharm Sci Res. 2021;13(6):340-6.
8. Courtney R, Cock IE. Comparison of the antibacterial activity of Australian Terminalia spp. extracts against *Klebsiella pneumoniae*: A potential treatment for ankylosing spondylitis. Inflammopharmacology. 2022;30(1):207-23.
9. Ero EO, Osadolor HB, Oyakhilome LI. Effect of Aqueous Leaf Extract of *Terminalia catappa* (Indian Almond) on the Liver of Alloxan-Induced Diabetic Wistar Rat. Int J Res Sci Innov. 2023;10(05):79-87.
10. Ben EE, Beshel JA, Owu DU, Palacios J, Nwokocha M, Bórquez J, Simirgiotis MJ, Nwokocha CR. Identification of phytochemicals and assessment of hypoglycemic and haematological potentials of *Terminalia catappa* Linn leaf extract in alloxan-induced diabetic Wistar rats. Cardiovasc. Hematol. Agents Med. Chem. 2024;22(2):139-50.
11. Agrawal OD, Kulkarni YA. *Terminalia catappa* aqueous extract reduces hyperglycaemia and oxidative stress in diabetic-hypercholesterolemic rats. J. Ayurveda Integr. Med. 2025;16(1):101025.
12. Chyau CC, Ko PT, Mau J-L. Antioxidant properties of aqueous extracts from *Terminalia catappa* leaves. LWT-Food Science and Technology. 2006;39(10):1099-108.
13. Chung HH, Hsieh MJ, Hsieh YS, Chen PN, Ko CP, Yu NY, Lin CW, Yang SF. The inhibitory effects of *terminalia catappa* l. Extract on the migration and invasion of human glioblastoma multiforme cells. Pharmaceuticals. 2021;14(11):1183.
14. Zarredar H, Khamaneh AM, Amoodizaj FF, Shanehbandi D, Seyedrezazadeh E, Jadid HS, Asadi M, Zafari V, Khalili Y, Soleimani Z. *Terminalia catappa* extract (TCE) reduces proliferation of lung and breast cancer cell by modulating miR-21 and miR-34a expressions. Asian Pac. J Cancer Prev.. 2021;22(4):1157.
15. Yilmaz MA. Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC–MS/MS method validation. Ind Crops Prod. 2020;149:112347.
16. Bouagnon JJR, Konan Y, Sinan KI, Konan F, Bolou GE-K, Koffi LR, Yeo D, N'Guessan JD, Zengin G, Djaman AJ. In vitro research to evaluate the antioxidant effects, inhibiting enzymes, methicillin-resistant *Staphylococcus aureus* strains of *Terminalia catappa* extracts. Sci Afr. 2024;23:e02058.
17. Glide, Schrödinger Release 2023-2, Glide, Schrödinger, LLC, New York, NY, 2023.
18. Mondal S, Panigrahi N, Sancheti P, Tirkey R, Mondal P, Almas S, Kola V. Evaluation of Toxicological, Diuretic, and Laxative Properties of Ethanol Extract from Macrothelypteris torresiana (Gaudich) Aerial Parts with in silico Docking Studies of Polyphenolic Compounds on Carbonic Anhydrase II: An Enzyme Target for Diuretic Acti. Pharmacognosy Res. 2018;10(4).
19. Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 2005;52(4):313-20.
20. Kaikini AA, Dhodi D, Muke S, Peshattiwar V, Bagle S, Korde A, Sarnaik J, Kadwad V, Sachdev S, Sathaye S. Standardization of type 1 and type 2 diabetic nephropathy models in rats: Assessment and characterization of metabolic features and renal injury. J Pharm Bioallied Sci. 2020;12(3):295-307.
21. Veerapur V, Prabhakar K, Kandadi M, Srinivasan K, Unnikrishnan M. Antidiabetic effect of Dodonaea viscosa aerial parts in high fat diet and low dose streptozotocin-induced type 2 diabetic rats: A mechanistic approach. Pharm. Biol. 2010;48(10):1137-48.
22. Sakaguchi K, Takeda K, Maeda M, Ogawa W, Sato T, Okada S, Ohnishi Y, Nakajima H, Kashiwagi A. Glucose area under the curve during oral glucose tolerance test as an index of glucose intolerance. Diabetol Int. 2016;7(1):53-8.
23. Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch Endocrinol Metab. 2016;60:138-42.
24. Andonova M, Dzhelebov P, Trifonova K, Yonkova P, Kostadinov N, Nancheva K, Ivanov V, Gospodinova K, Nizamov N, Tsachev I. Metabolic markers associated with progression of type 2 diabetes induced by high-fat diet and single low dose streptozotocin in rats. Vet Sci. 2023;10(7):431.
25. El-Shafei R, El-Adl M, Ali H, Nomier Y. Ameliorative effect of Arabic gum Acacia and mori extracts in streptozotocin-induced diabetic rats: implications of Cas-3 and TGF-β. Eur Rev Med Pharmacol Sci. 2023;27(7).
26. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. J Am Med. Assoc. 2013;310(19):2061-8.
27. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. nature. 1970;227(5259):680-5.
28. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book: Elsevier health sciences; 2018.
29. Kim Y, Lee SB, Cho M, Choe S, Jang M. Indian almond (*Terminalia catappa* linn.) leaf extract extends lifespan by improving lipid metabolism and antioxidant activity dependent on AMPK signaling pathway in Caenorhabditis elegans under high-glucose-diet conditions. Antioxidants. 2023;13(1):14.
30. Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. Biomed Res Int. 2010;2010(1):476279.
31. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;126(1):12-22.
32. Watson RT, Kanzaki M, Pessin JE. Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. Endocr. Rev. 2004;25(2):177-204.