RESEARCH PAPER

Acalypha Indica Attenuates Insulin Resistance In Stz-Induced Type 2 Diabetic Male Rats: Mechanistic Study

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Received: *20th Feb, 2026;* ***Revised:*** *4th Mar, 2026;* ***Accepted:*** *25th Mar, 2026;* ***Available Online:*** *10th Apr, 2026*

Abstract

Background: Type 2 diabetes mellitus (t2dm) is characterised by progressive peripheral insulin resistance manifesting as dysregulated skeletal muscle glucose metabolism, including depletion of glycogen stores, suppression of glycolytic enzyme activities, and constitutive overexpression of gluconeogenic enzymes. At the molecular level, failure of the ir/irs1/irs2-pi3k-akt signalling cascade leads to aberrant activation of gsk3β and pkc, transcriptional suppression of pparγ, and upregulation of srebp1c, collectively perpetuating hyperglycaemia and metabolic dysfunction. Acalypha indica linn. (euphorbiaceae), a herb widely used in ayurvedic and siddha medicine, possesses a diverse phytochemical profile including flavonoids, tannins, phenols, and polyphenolic compounds demonstrating established antioxidant, anti-inflammatory, and insulin-sensitising bioactivities. However, its mechanistic effects on skeletal muscle glycogen metabolism, glycolytic and gluconeogenic enzyme activities, and insulin signalling gene expression have not been systematically investigated.

Aim: The current investigation aimed to assess the dose-dependent effects of acalypha indica's ethanolic extract at 100, 200, and 400 mg/kg body weight on tissue glycogen content, glycolytic enzymes (hexokinase and pyruvate kinase), gluconeogenic enzymes (pepck and g6pase), and the mrna expression of key insulin signalling and metabolic genes (ir, irs1, irs2, gsk3β, pkc, pparγ, and srebp1c) in the gastrocnemius muscle of stz-induced t2dm male wistar rats, with metformin (50 mg/kg) as the reference standard.

Results: Stz-induced diabetic rats exhibited significantly depleted muscle glycogen content, markedly reduced hexokinase and pyruvate kinase activities, and substantially elevated pepck and g6pase activities compared to normal controls ($p < 0.05$). At the transcriptional level, ir, irs1, irs2, and pparγ mrna were significantly downregulated, while gsk3β, pkc, and srebp1c mrna were markedly overexpressed in the diabetic group. Treatment with acalypha indica produced consistent, dose-dependent reversal of all these alterations. Muscle

glycogen content was progressively restored, glycolytic enzyme activities were significantly elevated, and gluconeogenic enzyme activities were significantly suppressed across all treatment doses. The downregulated insulin signalling genes were progressively restored toward control levels while the overexpressed genes were correspondingly suppressed in a dose-dependent manner. At 400 mg/kg, all enzymatic and gene expression parameters were restored to levels which are similar to that of reference drug treated and healthy control groups, indicating near-complete normalisation of skeletal muscle glucose metabolic dysfunction.

Conclusion: Acalypha indica exerts potent, dose-dependent, and mechanistically integrated anti-diabetic effects in stz-induced t2dm rats, restoring skeletal muscle glycogen metabolism, reinstating glycolytic enzyme activity, suppressing gluconeogenic enzyme overactivity, and normalising the transcriptional landscape of insulin signalling and lipid metabolic gene networks. The 400 mg/kg dose demonstrated efficacy comparable to metformin across all measured endpoints, providing a comprehensive mechanistic approach for the traditional use of a. Indica in diabetes management and warranting further investigation into its active constituents and translational therapeutic potential.

Keywords: Acalypha Indica, Insulin Resistance, Gluconeogenic Enzymes, Glycolytic Enzymes, Insulin Signalling, Type 2 Diabetes Mellitus.

How To Cite This Article: Panneerselvam S, Viswaja K, Prathiba S, Rebecca Fs, Nivedha Rp, Rajagopal P, Shazia Fathima Jh, Veeraraghavan Vp, G S, Palanisamy Cp, Jayaraman S. Acalypha Indica Attenuates Insulin Resistance In Stz-Induced Type 2 Diabetic Male Rats: Mechanistic Study. Int J Drug Deliv Technol. 2026;16(26s):1027-1037. Doi: 10.25258/ijddt.16.26s.109

INTRODUCTION

Diabetes mellitus constitutes a multifactorial metabolic dysfunction defined by sustained hyperglycaemia arising from compromised pancreatic β-cell secretory function, attenuated insulin action, or both. As reported by the IDF 2025, more than 629 million adults were living with diabetes globally in 2025, with projections estimating this figure will reach approximately 853 million by 2050 (1). Among the various forms, T2DM constitutes nearly 90–95% of all diagnosed cases and is characterised by progressive peripheral insulin resistance, compensatory hyperinsulinaemia, and eventual pancreatic β-cell exhaustion (2). The skeletal muscle, mediating nearly 80% of insulin-stimulated peripheral glucose uptake under euglycaemic conditions, represents the primary site of peripheral insulin resistance and is therefore a critical target tissue in the mechanistic investigation of T2DM (3).

Insulin resistance in skeletal muscle manifests as a fundamental disruption of intracellular glucose metabolism, extending well beyond impaired glucose uptake to encompass dysregulation of glycogen synthesis, glycolytic enzyme activity, and gluconeogenic enzyme expression. Under euglycemic homeostatic conditions, ligand-receptor binding initiates downstream activation of the IR/IRS1/IRS2-PI3K-Akt signalling cascade, which coordinately promotes glycogen deposition through GSK3β inactivation, sustains glycolytic flux through transcriptional support of hexokinase (HK) and pyruvate kinase (PK), and suppresses gluconeogenesis through Akt-dependent phosphorylation and cytoplasmic sequestration of FoxO1 (4). In the insulin-resistant diabetic state, failure of this cascade leads to depletion of skeletal muscle glycogen stores, suppression of glycolytic enzyme activities, and constitutive stimulation of gluconeogenic enzymes namely PEPCK and G6Pase, which together drive excess endogenous glucose production and perpetuate fasting hyperglycaemia (5,6).

PEPCK governs the irreversible decarboxylation-phosphorylation of oxaloacetate to phosphoenolpyruvate, while G6Pase catalyses the hydrolytic cleavage of glucose-6-phosphate, releasing free glucose into systemic circulation as the rate-determining terminal reaction shared by both gluconeogenesis and glycogenolytic pathways (4). Both enzymes are transcriptionally regulated by the FoxO1-PGC-1α axis, which is normally suppressed by insulin-activated Akt but remains constitutively active in the insulin-resistant state, sustaining gluconeogenic gene expression independently of metabolic demand (6). Concurrently, aberrant activation of GSK3β a serine/threonine kinase normally inhibited by Akt perpetuates glycogen synthase inhibition and further amplifies insulin resistance by phosphorylating IRS1 at inhibitory serine residues, creating a self-reinforcing cycle of metabolic dysregulation (7,8). The overexpression of PKC isoforms driven by diacylglycerol accumulation under chronic hyperglycaemia further phosphorylates IRS1 at

inhibitory sites, attenuating PI3K-Akt signalling and compounding both glycolytic suppression and gluconeogenic overactivity (9).

At the transcriptional level, dysregulation of PPARγ and SREBP1c further characterises the metabolic reprogramming of T2DM skeletal muscle. PPARγ, whose expression depends on intact PI3K-Akt signalling, is suppressed in the insulin-deficient state, impairing insulin-sensitising and adiponectin-mediated glucose disposal pathways (10). SREBP1c, driven by a selectively preserved mTORC1-dependent branch of insulin signalling, remains aberrantly elevated despite global insulin resistance, perpetuating de novo lipogenesis and further aggravating ectopic lipid accumulation in metabolic tissues (11). The integrated consequence of these transcriptional and enzymatic derangements is a skeletal muscle that is simultaneously unable to utilise glucose and contributing to systemic glucose overproduction a mechanistic nexus that remains incompletely targeted by current pharmacotherapy.

Streptozotocin (STZ)-induced diabetic rat models are well-validated tools for replicating these skeletal muscle metabolic derangements, enabling the systematic evaluation of antidiabetic interventions at both enzymatic and molecular levels. The gastrocnemius muscle, as a major insulin-responsive skeletal muscle, is particularly suited for the concurrent assessment of glycogen content, glycolytic and gluconeogenic enzyme activities, and insulin signalling gene expression profiles in such experimental models (12).

Acalypha indica Linn. (family Euphorbiaceae), commonly known as "Kuppaimeni" in Tamil, is a widely distributed tropical herb with an established ethnomedicinal history in Ayurvedic and Siddha systems of medicine. Phytochemical investigations have identified a diverse repertoire of bioactive constituents including flavonoids, tannins, phenols, saponins, alkaloids, and the cyanogenic glucoside acalyphin, several of which exhibit free radical scavenging, anti-inflammatory, and insulin-sensitising effects (13). HR-LC-MS analysis has additionally identified polyphenolic secondary metabolites including ellagic acid and gallic acid derivatives, which have been reported to activate AMPK a known upstream suppressor of mTORC1, SREBP1c, and gluconeogenic gene networks in metabolic tissues (13). The flavonoid fraction of *A. indica* has additionally demonstrated α-glucosidase inhibitory activity and postprandial hypoglycaemic effects in STZ-diabetic rats (14), while its flavonoid constituents have demonstrated capacity to attenuate NF-κB-driven suppression of insulin signalling (15). Despite these promising profiles, the mechanistic effects of *A. indica* on skeletal muscle glycogen metabolism, glycolytic and gluconeogenic enzyme activities, and the transcriptional regulation of insulin signalling and lipid metabolic genes have not been systematically investigated. Accordingly, the current study was aimed to assess the dose-dependent effects of *A. indica's* ethanolic extract administered at different doses (100, 200, and 400 mg/kg b.w) on tissue glycogen content, hexokinase and pyruvate kinase activities, PEPCK and G6Pase activities, and the mRNA expression of IR, IRS1, IRS2, GSK3β, PKC, PPARγ, and SREBP1c in the gastrocnemius muscle of experimental rats, with Metformin as the reference standard. By integrating enzymatic and transcriptomic endpoints, this investigation provides a comprehensive mechanistic characterisation of the insulin-sensitising actions of *A. indica* at the skeletal muscle level, extending and complementing prior biochemical evaluations of this plant.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents employed in the current investigation were procured from Krishgen Biosystems (Mumbai, India), Sigma (St. Louis, MO, USA), Invitrogen (USA). TRIR reagent used for total RNA isolation was obtained from Invitrogen (USA). Reverse transcriptase was procured from New England Biolabs (NEB, USA), and Streptozotocin was sourced from Sigma (St. Louis, MO, USA). PCR utilized Promega's GoTaq Green Master Mix (Promega, USA) and primers from Eurofins Genomics (Bangalore, India).

Animals

The animals were maintained in accordance with the National Guidelines and Protocols approved by IAEC: BRULAC/SDCH/SIMATS/IAEC/03-2025/08.

Healthy adult male albino Wistar rats (Rattus norvegicus) aged 150-180 days, weighing 180-220g, were used in the current investigation. Animals were accommodated in sterilised polypropylene housing units at the BRULAC, SDC under standardised environmental parameters including temperature (21± 2°C), relative humidity (65± 5°C), and a 12h light/dark cycle. Rats were provided unrestricted access to a

standard pelleted diet (Lipton India, Mumbai, India) and clean drinking water.

Diabetes induction in experimental animals

Experimental diabetes was established via a single intraperitoneal injection of freshly reconstituted STZ (35 mg/kg b.w.) dissolved in 0.1 M sodium citrate buffer (pH 4.5). Following 72 hours of fasting blood glucose concentrations were determined, and animals exhibiting glycemia ≥ 200 mg/dl were classified as diabetic.

Study design

For the present investigation, the doses of *Acalypha indica* were determined using the existing literature evidences. The rats were allocated into six experimental groups, each comprising six animals as outlined below: Group I – Normal control rats administered vehicle only, Group II – T2DM rats developed through STZ induction, Group III – T2DM animals received oral administration of *Acalypha indica* (100mg/kg b.w) over a 45-days treatment period, Group IV – T2DM rats received *Acalypha indica* treatment (200mg/kg b.w) for 45 days, Group V – T2DM rats received *Acalypha indica* treatment (400mg/kg b.w) for 45 days, Group VI – T2DM rats received standard drug Metformin (50mg/kg b.w) treatment for 45 days. At the end of the treatment, animals were subjected to overnight fasting, followed by deep anaesthesia induced with ketamine-xylazine combination, and subsequently euthanised by cervical decapitation. Blood was obtained via retro-orbital venous plexus puncture, and serum was isolated by centrifugation and preserved at −80 °C until further analysis. Gastrocnemius muscle was rapidly dissected and used for further experimental assays.

Tissue glycogen

To quantify glycogen, Hassid 1957 method was employed (16). The amount of glycogen in the gastrocnemius tissue is indicated as mg/g wet tissue.

Glycolytic enzymes

Hexokinase (HK) enzymatic activity was quantified using the colorimetric method originally described by Brandstrup et al. (1957) (17). HK catalysing the phosphorylation of D-glucose utilising ATP as the phosphate donor to yield glucose 6-phosphate and ADP. In the presence of an o-toluidine reagent, any residual glucose in the sample underwent a reaction, leading to the formation of a color green. The sample's glucose concentration was assessed by measuring this color's intensity at 640 nm. HK enzyme activity was quantified as the quantity of glucose phosphorylated per hour/mg of protein. To measure tissue pyruvate kinase (PK) activity, the Valentine and Tanaka, 1966 method was employed (18). Pyruvate synthesis from phosphoenolpyruvate began. Dinitrophenyl hydrazine was been employed to estimate pyruvate release. µmol pyruvate formed/min/mg protein was the unit of measurement.

Gluconeogenic enzymes

The method of Chang and Lane (1966) was used to measure phosphoenolpyruvate carboxykinase (PEPCK) activity (19). PEPCK catalyzed the decarboxylation and phosphorylation of oxaloacetate (OAA) to phosphoenolpyruvate (PEP) in the presence of GTP or ATP and a divalent metal ion cofactor. The reaction product PEP was quantified spectrophotometrically or via coupled enzyme assays measuring NADH oxidation at 340 nm, or by colorimetric detection in some adaptations. Enzyme activity was expressed as µmol PEP formed/min/mg protein. The Zak et al. method (20) or Taussky-Shorr procedure was employed to measure glucose-6-phosphatase (G6Pase) activity. G6Pase hydrolyzed glucose-6-phosphate (G6P) to release inorganic phosphate (Pi) and glucose. Liberated Pi was quantified colorimetrically using acid-molybdate and Fiske-Subbarow or similar reagents, forming a phosphomolybdate complex measured at 660-820 nm. The enzyme activity was determined as µmol Pi released/min/mg protein.

Gene expression analysis

Total RNA isolation

Briefly, 100mg of fresh gastrocnemius muscle tissue from all groups were homogenized using 1ml of TRIR reagent. To this homogenate, chloroform (0.2 ml) was added, and the solution was subjected to vortexing for 15 seconds and kept at 4ºC for 5 mins. Then the mixture was subjected to centrifugation at 12500 rpm for 15mins. The top transparent aqueous layer was the total RNA and it was transferred into the clean microcentrifuge tube. To this aqueous phase, an equal volume of isopropyl alcohol was introduced and thoroughly mixed by vortex agitation for 15 seconds, & subjected to incubation at 4°C for 10 minutes. After cooling, the contents were then centrifuged at 9000

rpm for 10 mins. After centrifugation, the aqueous supernatant was carefully aspirated and the resultant RNA pellet was rinsed with 70% ethanol. Then the pellet was air dried and the RNA concentration in each sample was measured with Nanodrop lite UV-visible spectrophotometer (THERMO, AMP-10) and the obtained readings were reported in µg.

cDNA synthesis

Using the commercial cDNA synthesis kit from TAKARA, approximately 2µg of total RNA was reverse transcribed following the user guidelines. Obtained RNA and resulting cDNA were subsequently quantified by a Nanodrop lite UV-visible spectrophotometer to ensure suitability for downstream molecular analysis.

Real time PCR

A 45µl reaction mixture was prepared by combining 2X SYBR Green master mix, target specific primers along with β actin primers as an internal control and nuclease free water. This mixture was briefly centrifuged before adding 5µl of either positive control DNA, nuclease free water as a no-template control or cDNA template at a concentration of 500ng. The qRTPCR amplification was done on a Bio-Rad thermal cycler, initiated with a denaturation phase at 95ºC for 5 mins followed by 40 amplification cycles of 95ºC for 5 mins, 60ºC for 30 secs and 72ºC for 30 secs. Specificity and relative quantification of gene expression were validated through melting curve and amplification analyses ensuring accurate and reliable results.

STATISTICAL ANALYSIS

The experimental data from all the experimental groups were expressed as Mean ± Standard error of the mean. Inter-group statistical comparisons were done using one-way ANOVA followed by Tukey's multiple comparison post-hoc test, conducted in GraphPad Prism (version 8.2), and data with a p-value below 0.05 were deemed statistically significant.

RESULTS

Influence of *Acalypha indica* on tissue glycogen levels in the experimental animals

Gastrocnemius muscle glycogen concentration was markedly depleted in the diabetic group compared to normoglycemic controls ($p < 0.05$), consistent with the impaired glycogen synthesis characteristic of insulin-deficient states (Figure 1). Treatment with ethanolic extract of *Acalypha indica* at 100, 200, and 400 mg/kg body weight produced a dose-dependent and statistically significant restoration of muscle glycogen levels in diabetic rats ($p < 0.05$). The 400 mg/kg treatment group demonstrated glycogen levels most closely approaching those of the normal control group, with values compared to that of metformin-treated group ($p < 0.05$). The 100 mg/kg dose produced a modest but significant increase relative to the diabetic control, while the 200 mg/kg dose showed intermediate improvement, indicating a clear dose-response relationship across all treatment groups.

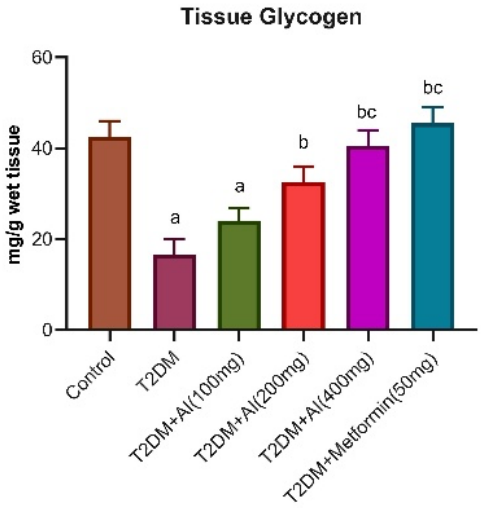

Figure 1: Effect of ethanolic extract of *Acalypha indica* on tissue glycogen concentration of diabetic rats. Results indicated as Mean±SEM. Significance at ($p<0.05$). a-differs significantly from control group. b-differs significantly from diabetes group. c-differs significantly from *A. indica* (100mg) treatment.

Effect of *Acalypha indica* on glycolytic and gluconeogenic enzymes levels in the diabetic rats

Hexokinase activity was significantly reduced in the STZ-induced diabetic group relative to normal controls ($p < 0.05$), reflecting the well-established suppression of glycolytic flux under conditions of severe insulin deficiency (Figure 2a). Administration of *A. indica* at all three doses produced a progressive and significant elevation of HK activity in diabetic rats ($p < 0.05$). The highest dose of 400 mg/kg elicited the greatest restoration of HK activity, achieving levels statistically comparable to the Metformin reference

group, while the 100 mg/kg and 200 mg/kg groups demonstrated incrementally lower but significantly elevated activities relative to the diabetic control. Pyruvate kinase activity was similarly diminished in the diabetic group compared to normal controls ($p < 0.05$), indicative of compromised downstream glycolytic capacity in insulin-resistant skeletal muscle (Figure 2b). Treatment with *A. indica* at doses of 100, 200, and 400 mg/kg b.w significantly enhanced PK activity in a dose-dependent manner ($p < 0.05$). At 400 mg/kg, PK activity levels restored nearly compared to that of control and metformin-treated groups. Statistically significant differences emerged between consecutive dose groups, verifying the dose-dependent glycolytic restoration achieved by *A. indica*.

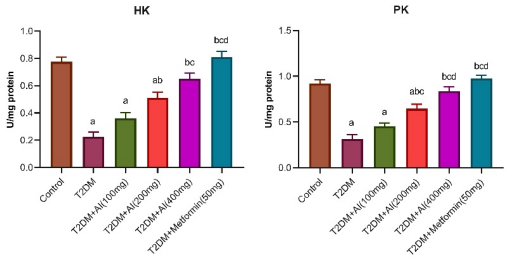

Figure 2 a & 2b: Shows the impact of ethanolic extract of *Acalypha indica* on glycolytic enzymes in the tissue of diabetic rats. Results indicated as Mean±SEM. Significance at ($p<0.05$). a-significantly different from control group. b-significantly different from diabetes group. c-significantly different from *A. indica* treatment (100mg). d- significantly different from *A. indica* treatment (200mg).

G6Pase activity was significantly elevated in the diabetic group compared to normal controls ($p < 0.05$), consistent with enhanced hepatic and peripheral glucose output driven by absent insulin suppression of this terminal gluconeogenic enzyme (Figure 3a). *A. indica* treatment at all three doses significantly reduced G6Pase activity relative to the diabetic control in a dose-dependent fashion ($p < 0.05$). The 400 mg/kg group demonstrated the greatest reduction, with G6Pase activity approaching levels comparable to the Metformin reference, while the 100 mg/kg and 200 mg/kg groups showed progressively intermediate reductions, further substantiating the dose-responsive anti-gluconeogenic profile of *A. indica*. PEPCK activity was constitutively overexpressed in the diabetic group relative to healthy controls ($p < 0.05$), reflecting the unrestrained gluconeogenic drive that characterises the insulin-deficient state (Figure 3b). Treatment with *A. indica* at 100, 200, and 400 mg/kg produced a dose-dependent and statistically significant suppression of PEPCK activity ($p < 0.05$). The 400 mg/kg dose group exhibited PEPCK activity which are similar to that of healthy control and standard drug treated groups, indicating near-complete normalisation of this key gluconeogenic enzyme at the highest tested dose.

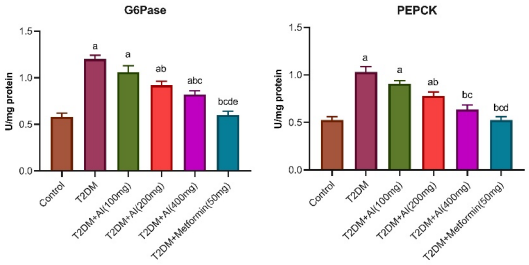

Figure 3a & 3b: Shows the influence of *Acalypha indica* on gluconeogenic enzymes in the tissue of diabetic rats. Results indicated as Mean±SEM. Significance at ($p<0.05$). a-significantly different from the control group. b-significantly different from the diabetes group. c-significantly different from *A. indica* treatment (100mg). d- significantly different from *A. indica* treatment (200mg). d- significantly different from *A. indica* treatment (400mg).

Effect of *Acalypha indica* on insulin signaling and metabolic gene expression in the experimental animals

The mRNA expression of seven key genes governing insulin receptor singaling and lipid metabolism (IR, IRS1, IRS2, SREBP1c, GSK3β, PKC & PPARγ) was assessed across all experimental groups. In the diabetic group, genes associated with insulin receptor signaling namely IR, IRS1, IRS2 were markedly downregulated relative to non-diabetic controls ($p < 0.05$), reflecting impaired insulin signal transduction. PPARγ mRNA was similarly suppressed in the DM group consistent with reduced transcription activity of this nuclear receptor under conditions of severe insulin deficiency. Conversely, genes governing aberrant kinase signalling and insulin desensitisation, including GSK3β and PKC were markedly overexpressed in the DM group ($p < 0.05$). SREBP1c mRNA was substantially upregulated in diabetic group relative to healthy controls ($p < 0.05$), indicative of dysregulated hepatic lipogenesis driven by selective insulin resistance. Treatment with *Acalypha indica* at 100, 200 and 400 mg/kg produced a consistent and dose dependent reversal of these alterations of the all the seven genes. The downregulated genes (IR, IRS1, IRS2, PPARγ) were progressively restored toward

control levels with increasing dose while the upregulated genes (GSK3β, PKC, SREBP1c) were correspondingly suppressed in a dose-dependent manner ($p < 0.05$) (figure 4 a-h). At the highest extract treated dose, results displayed expression levels comparable to those of metformin treated group for most genes, indicating near-equivalent efficacy to the reference drug. The reference drug metformin demonstrated the most complete normalization of all gene targets, with values approaching those of the healthy control group. These results suggest that *Acalypha indica* possess dose dependent modulatory effects on both the insulin signaling cascade and lipid metabolic gene networks disrupted by diabetes.

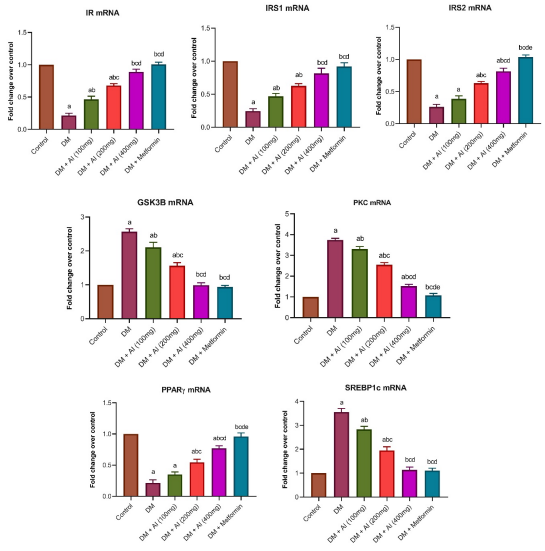

Figure 4 a-h: Effect of *Acalypha indica* on mRNA expression of key insulin signaling genes in streptozotocin induced diabetic rats. All experiments were done in triplicate and $p<0.05$ is considered as statistically significant.

DISCUSSION

This investigation demonstrated a marked depletion of glycogen content in the gastrocnemius muscle of diabetic rats compared to healthy controls, which was progressively restored following *A. indica* treatment in a dose-dependent manner, with the 400 mg/kg dose and Metformin group showing comparable and near-normal glycogen levels (figure 1). Comparable glycogen-restorative effects have been reported for flavonoid-rich plant extracts in STZ-diabetic rodents, further supporting the role of *A. indica*'s flavonoid constituents in reinstating anabolic glycogen metabolism (15).

The significant decline in HK and PK activities in the STZ-diabetic group aligns with observations of Oluba et al. (2018), who attributed reduced HK activity to impaired insulin-dependent GLUT4 translocation and limited intracellular glucose availability. The suppression of PK activity is consistent with Oluba et al. (2018), who reported diversion of glycolytic intermediates toward gluconeogenesis under insulin deficiency (12). The dose-dependent restoration of both enzymes by *A. indica* (figure 2 a & b) mirrors findings of Ramkumar et al. (2009) (21), who demonstrated that polyphenol-rich extracts reinstate glycolytic enzyme activity by attenuating oxidative stress and restoring insulin signalling, mechanisms well-supported by the antioxidant and IR/IRS1/IRS2 restoration properties of *A. indica* documented in this study.

The significant elevation of PEPCK and G6Pase in the STZ-diabetic group is consistent with Yoon et al. (2001), who demonstrated that loss of insulin-mediated FoxO1 suppression constitutively upregulates PEPCK transcription and activity, sustaining fasting hyperglycaemia (5). The suppression of both enzymes by *A. indica* in a dose-dependent manner aligns with findings on berberine and plant-derived AMPK activators that reduce PEPCK and G6Pase activity through AMPK-mediated inhibition of the CREB-PGC-1α-FoxO1 complex. The polyphenolic constituents of *A. indica*, particularly ellagic acid and gallic acid derivatives (13), plausibly mediate this effect through AMPK activation, and the near-normalisation of both enzymes at 400 mg/kg to Metformin-comparable levels confirms the potent anti-gluconeogenic efficacy of the extract (figure 3 a & b).

This study demonstrates that *Acalypha indica* (AI) exerts dose-dependent modulatory effects on key insulin signalling and metabolic genes in a streptozotocin (STZ)-induced diabetic rat model. The results collectively indicate that AI restores impaired insulin receptor signalling (IR, IRS1, IRS2), attenuates aberrant kinase activity (GSK3B, PKC), and corrects dysregulated lipid metabolic transcription (PPARγ, SREBP1c), with efficacy approaching that of Metformin at the highest dose tested (400 mg/kg). *A. indica* is an annual herb of the family Euphorbiaceae, traditionally employed in Ayurvedic and Siddha medicine for the management of diabetes, inflammation, and skin disorders (15). Phytochemical analyses of *A. indica* have consistently identified flavonoids, tannins, phenols, saponins, alkaloids, and the cyanogenic glucoside acalyphin as its principal bioactive constituents (13), several of which possess

antioxidant, anti-inflammatory, and insulin-sensitising properties that may collectively underlie the transcriptional effects observed in this study.

The downregulation of IR in diabetic group is consistent with well-documented reductions in insulin receptor transcription following STZ-induced insulin deficiency. In skeletal muscle of STZ-diabetic rats, insulin receptor content and downstream phosphorylation are significantly reduced, reflecting impairment at the very beginning of the insulin signalling cascade (22). This has also been confirmed at the transcriptional level in human and mouse skeletal muscle, where INSR mRNA is robustly downregulated as part of a compensatory desensitisation response to chronic hyperinsulinaemia (23). The pathological consequences of reduced IR expression impaired glucose uptake, attenuated Akt activation, and progressive peripheral insulin resistance are widely recognised in both animal models and clinical type 2 diabetes (24). The dose-dependent restoration of IR mRNA by AI treatment is consistent with findings for other plant-derived bioactive compounds such as cinnamaldehyde, which has been shown to restore the IRS1/PI3K/AKT2 signalling axis in STZ-diabetic rats (25), and berberine, which upregulates IR gene expression in insulin-resistant models (26). The flavonoid-rich fraction of *A. indica*, which has been shown to suppress postprandial hyperglycaemia through alpha-glucosidase inhibition in STZ-diabetic rats (14), may also contribute to improved insulin receptor signalling by reducing chronic glucose excursions that drive insulin receptor downregulation.

The co-restoration of IRS1 and IRS2 mRNA with AI treatment is particularly significant, as both adapter proteins serve as critical nodes for propagating the insulin signal toward glucose transporter translocation, glycogen synthesis, and suppression of hepatic gluconeogenesis (4). The regulation of IRS proteins is achieved through phosphorylation of more than 50 serine/threonine residues; heterologous kinases including JNK, IKKβ, and novel PKC isoforms, which are aberrantly activated in metabolic disease, phosphorylate IRS proteins at inhibitory serine residues, attenuating downstream PI3K-Akt signalling (27). More recent studies have delineated specific phosphorylation codes in IRS1/2 that are directly related with inflammatory mediator-mediated inhibition of insulin signal transduction, forming a feed-forward cycle of progressive insulin resistance (28). Ubiquitin ligases including SOCS1/3 and GSK3β further target IRS proteins for proteasomal degradation under chronic inflammatory and hyperglycaemic conditions (6). Mitochondrial reactive oxygen species generated under hyperglycaemic conditions activate NF-κB and its upstream kinase IKKβ, which not only phosphorylates IRS proteins at inhibitory serine residues but also suppresses IRS gene transcription at the promoter level, perpetuating insulin resistance through both post-translational and transcriptional mechanisms (29). The restoration of IRS1 and IRS2 mRNA by AI is mechanistically consistent with its well-characterised antioxidant and anti-inflammatory properties. Similarly, Sharma et al. (2024) (15) confirmed that *A. indica* flavonoids exert anti-inflammatory effects that reduce NF-κB-mediated suppression of insulin signalling, providing a plausible molecular basis for the IRS1/IRS2 restoration observed in the present study.

GSK3B functions as a constitutively active serine/threonine kinase whose catalytic activity is physiologically restrained through Akt-dependent phosphorylation at the Ser9 following insulin receptor activation. Overexpression and overactivity of GSK3B have been consistently demonstrated in skeletal muscle of obese rodent models and poorly controlled type 2 diabetic subjects, where it is strongly associated with impaired insulin-stimulated glycogen synthase activation and blunted glucose disposal (8). A comprehensive recent review by Wang et al. (2022) (7) further confirmed that GSK3B serves as a master negative regulator of glycogen synthesis, insulin signalling, and glucose metabolism across multiple metabolic tissues, and highlighted its capacity to directly phosphorylate IRS1 at inhibitory serine residues creating an amplifying loop of insulin resistance. This mechanistic link between GSK3B overactivity and IRS1 degradation is consistent with the co-upregulation of IRS1/IRS2 mRNA alongside GSK3B suppression observed in the present study. The dose-dependent reduction of GSK3B mRNA by AI treatment indicates a reconstitution of Akt-mediated inhibitory control over this kinase. Flavonoids isolated from *A. indica*, including kaempferol, glycosides, mauritianin, and nicotiflorin have been reported to inhibit GSK3B-mediated phosphorylation of downstream targets in metabolic disease models, consistent with the GSK3B suppression observed here. The normalization of GSK3B expression at 400 mg/kg AI to levels comparable with both controls and the Metformin group further supports the capacity of AI to broadly restore insulin signalling fidelity.

The substantial overexpression of PKC mRNA in the DM group aligns with extensive evidence that chronic hyperglycaemia-induced de novo synthesis of diacylglycerol (DAG) constitutively activates novel PKC isoforms in vascular and metabolic tissues (9). In skeletal muscle, DAG-mediated activation of PKCθ has been demonstrated in human subjects to directly phosphorylate IRS-1 at Ser1101, inhibiting IRS-1 tyrosine phosphorylation and Akt2 activation (30). The dose-dependent reduction of PKC mRNA by AI treatment may therefore exert benefits both directly on PKC transcription and indirectly by relieving DAG-PKC-mediated serine phosphorylation of IRS1/IRS2, consistent with the co-improvement of IRS transcripts observed in this study. The tannins and phenolic compounds abundant in *A. indica* have been shown to suppress DAG-PKC pathway activation by chelating calcium ions and reducing oxidative stress-driven DAG accumulation in hyperglycaemic conditions (9), providing a phytochemical basis for the PKC-suppressive effects of AI observed here.

The downregulation of PPARγ mRNA in the DM group is consistent with the known dependence of PPARγ transcription on intact insulin signalling via the PI3K-Akt pathway in insulin-sensitive peripheral tissues. Genetic deletion of PPARγ specifically in skeletal muscle (MuPPARγKO) in mice causes whole-body insulin resistance with an approximately 80% reduction in the insulin-stimulated glucose disposal rate during hyperinsulinaemic-euglycaemic clamp, underscoring the critical role of muscle PPARγ in maintaining systemic insulin sensitivity (10). Additionally, Norris et al. (2003) (31) demonstrated using an independent MuPPARγKO model that muscle-specific PPARγ deficiency produces increased adiposity and whole-body insulin resistance. A subsequent study by Amin et al. (2010) (32) showed that selective pharmacological activation of PPARγ in primary skeletal muscle cells induced endogenous adiponectin production and provided significant protection against high-fat diet-induced insulin resistance, reinforcing the functional importance of adequate PPARγ expression in peripheral tissues. The progressive restoration of PPARγ mRNA with increasing AI doses suggests that AI may partially reinstate insulin-like signalling sufficient to support PPARγ transcription, mechanistically consistent with the concomitant upregulation of IR, IRS1, and IRS2 mRNA observed in parallel. The flavonoid constituents of *A. indica*, including kaempferol and glycosides, have been reported to act as partial PPARγ agonists and transcriptional activators in adipocyte and muscle cell models, providing a plausible mechanism through which AI may restore PPARγ expression in the present study.

The marked elevation of SREBP1c mRNA in the diabetic group is consistent with the concept of selective hepatic insulin resistance. Brown and Goldstein (2008) described this paradox wherein the Akt branch of insulin signalling responsible for FoxO1-mediated suppression of gluconeogenesis becomes impaired, while an alternative mTORC1-dependent branch remains active and continues to drive SREBP1c transcription and de novo lipogenesis (11). Li et al. (2010) directly demonstrated in hepatocytes that mTORC1 is specifically required for insulin-stimulated induction of SREBP1c and lipogenic gene expression, whereas Akt-mediated FoxO1 phosphorylation and gluconeogenesis suppression occur through a distinct and separable pathway. Laplante and Sabatini (2010) further established that mTORC1 activation uncouples lipogenesis from gluconeogenesis in the insulin-resistant liver, explaining the simultaneous hyperglycaemia and hypertriglyceridaemia that characterise type 2 diabetes (33). Extending this model, Yecies et al. (2011) (34) demonstrated that both mTORC1-dependent and mTORC1-independent Akt pathways converge on SREBP1c activation in the liver, reinforcing the robustness of hepatic lipogenic programming under conditions of partial insulin resistance. The dose-dependent suppression of SREBP1c mRNA by AI treatment therefore suggests that AI may attenuate aberrant mTORC1-SREBP1c signalling. The polyphenolic constituents of *A. indica*, including ellagic acid and gallic acid derivatives identified by HR-LC-MS (13), have been shown to modulate AMPK activity a known upstream inhibitor of mTORC1 and SREBP1c in hepatic cell models, providing a plausible mechanistic link between *A. indica*'s phytochemical composition and its suppressive effect on SREBP1c transcription. These transcriptomic data presented here indicate that *Acalypha indica* exerts pleiotropic, dose-dependent effects on insulin signalling and metabolic gene networks in the diabetic state (figure 4a-h).

CONCLUSION

The present study demonstrates that ethanolic extract of *Acalypha indica* exerts significant, dose-dependent, and mechanistically integrated antidiabetic effects in

STZ-induced type 2 diabetic male Wistar rats, as evidenced by restoration of depleted skeletal muscle glycogen content, reinstatement of hexokinase and pyruvate kinase activities, suppression of constitutively elevated PEPCK and G6Pase activities, and comprehensive normalisation of insulin signalling and metabolic gene expression including upregulation of IR, IRS1, IRS2, and PPARγ alongside suppression of GSK3β, PKC, and SREBP1c. The 400 mg/kg dose consistently achieved outcomes comparable to the reference drug Metformin across all enzymatic and transcriptional endpoints, underscoring the therapeutic potency of the extract at higher doses. These findings provide a robust mechanistic foundation for the traditional use of *A. indica* in diabetes management and identify the IR/IRS/PI3K-Akt-FoxO1 and AMPK-mTORC1-SREBP1c axes as plausible molecular targets of its bioactive phytoconstituents. Future investigations should focus on bioassay-guided isolation of active compounds, molecular docking studies, and long-term safety evaluations to facilitate the translational development of *A. indica*-derived therapeutics for clinical application in type 2 diabetes.

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