RESEARCH PAPER

Effect Of Lupeol On Ampk-Mediated Signaling In Gracilis Muscle Of Hfd-Induced Type 2 Diabetic Rats

Reddy Prasad[1], Doni R Praveen Kumar[2], Thirunavukkarasu Jayaraman[3], Ponnulakshmi Rajagopal[4], C. S. Janaki[5], Ramya Murali[6], Selvaraj Jayaraman[6*]

[1] Ph.D. Scholar, Department Of Pharmacology, Saveetha Medical College, Saveetha Institute Of Medical And Technical Sciences, Thandalam, Chennai-602105, India.

[2] Assistant Professor, Department Of Anatomy, Government Medical College, Paderu, 531024, Andhra Pradesh, India.

[3] Professor, Department Of Pharmacology, Saveetha Medical College, Saveetha Institute Of Medical And Technical Sciences, Thandalam, Chennai-602105, India.

[4] Scientist Grade-Iii, Department Of Central Research Laboratory, Meenakshi Ammal Dental College And Hospital, Meenakshi Academy Of Higher Education And Research (Deemed To Be University), Chennai-600 095, India.

[5] Professor, Department Of Anatomy, Bhaarath Medical College & Hospital, Bharath Institute Of Higher Education And Research (A Unit Of Biher), Chennai, Tamil Nadu, India.

[6] Centre Of Molecular Medicine And Diagnostics (CommanD), Department Of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute Of Medical & Technical Sciences, Saveetha University, Chennai-600 077, India.

Corresponding Author: Dr. Selvaraj Jayaraman, Professor, Centre Of Molecular Medicine And Diagnostics (CommanD), Department Of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute Of Medical & Technical Sciences, Saveetha University, Chennai-600 077, India. Email: selvarajj.sdc@saveetha.com

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Abstract

Background: Type 2 diabetes mellitus (t2dm) is characterized by insulin resistance, impaired glucose uptake, oxidative stress, and metabolic dysregulation in skeletal muscle. The camkkβ–ampk–sirt1 signaling axis plays a central role in regulating energy homeostasis, mitochondrial function, and lipid metabolism. Lupeol, a pentacyclic triterpenoid, has demonstrated antidiabetic potential; however, its role in ampk-mediated signaling in skeletal muscle remains unclear.

Objective: This study aimed to investigate the effect of lupeol on ampk-mediated signaling pathways in the gracilis muscle of high-fat diet (hfd)-induced t2dm rats.

Methods: T2dm was induced in rats using a high-fat diet. Animals were divided into four groups: control, hfd-t2dm, hfd-t2dm treated with lupeol, and hfd-t2dm treated with metformin. Oxidative stress markers (lpo, oh•), antioxidant enzymes, and mrna expression of insulin signaling, ampk-related genes, and inflammatory/lipogenic markers were analyzed.

Results: Hfd-induced diabetic rats exhibited increased oxidative stress, reduced antioxidant defenses, impaired insulin signaling, and downregulation of ampk-associated genes. Lupeol treatment significantly decreased lpo and oh• levels while restoring sod, cat, and gsh levels. It upregulated insulin signaling genes (ir, irs-1, akt, glut-4), indicating improved glucose uptake. Furthermore, lupeol enhanced the expression of camkkβ, cpt-1, ppar-γ, and sirt1, suggesting activation of the ampk pathway and improved mitochondrial function. Additionally, lupeol reduced the expression of srebp-1c and tnf-α, indicating suppression of lipogenesis and inflammation. Metformin showed slightly greater effects but with comparable trends.

Conclusion: Lupeol ameliorates skeletal muscle insulin resistance in hfd-induced t2dm by reducing oxidative stress, restoring antioxidant capacity, improving insulin signaling, activating the camkkβ–ampk–sirt1 axis, and suppressing lipogenesis and inflammation. These findings highlight lupeol as a promising multi-targeted therapeutic agent for the management of t2dm.

Keywords: Lupeol, Ampk Signaling, Insulin Resistance, Skeletal Muscle, Type 2 Diabetes Mellitus, Oxidative Stress.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin resistance and/or impaired insulin secretion. The global prevalence of T2DM has increased dramatically, largely driven by sedentary lifestyles and high-fat diet (HFD) consumption, making it a major public health concern worldwide. Prolonged exposure to excess dietary lipids contributes to obesity, metabolic dysregulation, and the development of insulin resistance, which is a hallmark of T2DM pathogenesis (1). Skeletal muscle plays a central role in glucose homeostasis, accounting for approximately 70–80% of insulin-stimulated glucose uptake. Impairment in insulin signaling pathways within skeletal muscle is a key contributor to systemic insulin resistance. In T2DM, defects in the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), and downstream AKT signaling cascade led to reduced translocation of glucose transporter-4 (GLUT-4) to the plasma membrane, thereby limiting glucose uptake (2,3). Furthermore, lipid accumulation, mitochondrial dysfunction, and increased reactive oxygen species (ROS) production in skeletal muscle exacerbate insulin resistance, creating a self-perpetuating cycle of metabolic impairment (2).

Adenosine monophosphate-activated protein kinase (AMPK) is a critical cellular energy sensor that maintains metabolic homeostasis by regulating glucose and lipid metabolism. Activation of AMPK enhances glucose uptake, promotes fatty acid oxidation through carnitine palmitoyltransferase-1 (CPT-1), and modulates lipid metabolism via peroxisome proliferator-activated receptor gamma (PPARγ) (4). Additionally, AMPK interacts with sirtuin 1 (SIRT1) to regulate mitochondrial biogenesis and cellular energy balance. The upstream kinase calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) plays an essential role in AMPK activation under metabolic stress conditions (5). Dysregulation of the CaMKKβ–AMPK–SIRT1 axis has been strongly implicated in the pathogenesis of T2DM, making it an attractive therapeutic target (4,5). Oxidative stress and inflammation are key pathological features of T2DM, particularly in skeletal muscle. HFD-induced diabetes is associated with excessive ROS generation, increased lipid peroxidation, and elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and NF-κB. Concurrently, antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), are significantly compromised (6). This interplay between oxidative stress and inflammation, often termed "oxinflammation," disrupts insulin signaling and promotes lipogenesis through upregulation of sterol regulatory element-binding protein-1c (SREBP-1c), further aggravating metabolic dysfunction (2).

Natural bioactive compounds have gained considerable attention as potential therapeutic agents for managing T2DM due to their multi-targeted mechanisms and minimal side effects. Lupeol, a pentacyclic triterpenoid found in various fruits, vegetables, and medicinal plants, has been widely reported for its antioxidant, anti-inflammatory, antihyperglycemic, and antilipidemic properties (7). Experimental studies have demonstrated that lupeol improves insulin sensitivity, reduces oxidative stress by scavenging ROS, and suppresses inflammatory mediators such as TNF-α and NF-κB in diabetic models (8,9). Moreover, lupeol has been shown to inhibit carbohydrate-digesting enzymes such as α-glucosidase, thereby reducing postprandial hyperglycemia (10). Despite these promising findings, the specific role of lupeol in modulating AMPK-mediated signaling pathways in skeletal muscle under diabetic conditions remains poorly understood. Therefore, the present study aimed to investigate the effect of lupeol on AMPK-mediated signaling in the gracilis muscle of HFD-induced T2DM rats. This study focuses on evaluating its impact on insulin signaling pathways, oxidative stress markers, antioxidant defense systems, and key genes involved in the CaMKKβ–AMPK–SIRT1 axis, lipid metabolism, and inflammation. Understanding these mechanisms may provide valuable insights into the therapeutic potential of lupeol in the management of T2DM.

MATERIALS AND METHODS

Chemical and reagents

Lupeol (≥98% purity) was procured from a certified supplier (Sigma-Aldrich, USA). Streptozotocin (STZ), high-fat diet (HFD) components, and standard antidiabetic drug metformin were obtained from standard commercial sources. ELISA kits for the estimation of oxidative stress markers, and antioxidant markers were purchased from validated manufacturers following the manufacturer's protocols. All other chemicals and reagents used were of analytical grade.

Experimental animals

Adult male Wistar albino rats (180–220 g) were used for the study. Animals were housed under standard laboratory conditions (temperature: 22 ± 2°C; humidity: 50–60%; 12-hour light/dark cycle) with free access to food and water. All experimental procedures were conducted in accordance with institutional ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC no: 006/2016).

Induction of Type 2 Diabetes

Type 2 diabetes mellitus (T2DM) was induced using a combination of high-fat diet (HFD) feeding and low-dose streptozotocin (STZ). Rats were fed with HFD for 4 weeks to induce insulin resistance. Following this, a single low dose of STZ (35 mg/kg body weight, intraperitoneally) dissolved in citrate buffer (pH 4.5) was administered. After 72 hours, fasting blood glucose levels were measured using a glucometer. Rats with fasting blood glucose levels ≥250 mg/dL were considered diabetic and included in the study.

Experimental Design

Animals were randomly divided into four groups (n = 6 per group): Group I (Control): Normal rats fed with standard pellet diet; Group II (HFD-T2DM): Diabetic rats induced by HFD + STZ; Group III (HFD-T2DM + LUP): Diabetic rats treated with lupeol (40 mg/kg body weight/day, orally); Group IV (HFD-T2DM + MET): Diabetic rats treated with metformin (100 mg/kg body weight/day, orally).Treatment was continued for 45 days.

Assessment of Oxidative Stress Markers

Lipid peroxidation (LPO) in gracilis muscle tissue was estimated using ELISA, based on the quantification of malondialdehyde (MDA) as an index of membrane lipid damage. The results were expressed as nmol of MDA formed per mg protein. Hydroxyl radical (OH•) generation was determined and the values were expressed as units per liter (U/L).

Estimation of Antioxidant Enzymes

The activities of key antioxidant enzymes were measured in gracilis muscle homogenates using standard protocols. Superoxide dismutase (SOD) activity was determined and expressed as pg/mL. Catalase (CAT) and Reduced glutathione (GSH) activity was estimated and expressed as ng/L. These parameters were used to evaluate the antioxidant defense status in control and experimental groups.

Gene Expression Analysis

RNA Isolation and cDNA Synthesis

Total RNA was isolated from gracilis muscle tissues using TRIR reagent (Invitrogen) according to the manufacturer's instructions. Briefly, approximately 100 mg of fresh tissue was homogenized in 1 mL of TRIR reagent, followed by the addition of chloroform and centrifugation at 12,000 ×g for 15 min at 4°C. The aqueous phase was collected, and RNA was precipitated using isopropanol, washed with 75% ethanol, and dissolved in RNase-free water. RNA concentration and purity were determined spectrophotometrically. Complementary DNA (cDNA) was synthesized from 2 µg of total RNA using a reverse transcription kit (Eurogentec, Belgium) as per the manufacturer's protocol.

Quantitative Real-Time PCR (qRT-PCR)

Gene expression analysis was performed using SYBR Green-based real-time PCR (Takara). The reaction mixture consisted of 2× SYBR Green master mix, gene-specific forward and reverse primers, and nuclease-free water. Amplification was carried out using a Stratagene MX3000P system (Agilent Technologies) under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C

for 5 s, annealing at 60°C for 20 s, and extension at 72°C for 40 s. The mRNA expression levels of insulin signaling genes (IR, IRS-1, AKT, GLUT-4), AMPK pathway-related genes (CaMKKβ, CPT-1, PPAR-γ, SIRT1), and inflammatory/lipogenic markers (TNF-α and SREBP-1c) were quantified. β-actin was used as the internal control. Relative gene expression was calculated using comparative Ct (ΔΔCt) method based on amplification and melt curve analysis.

Statistical analysis

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the control and treatment groups using a computer -based software (Graph Pad Prism version 5). In Duncan's test, the significance was considered at the level of $p<0.05$.

RESULTS

Effect of Lupeol on oxidative stress markers in the gracilis muscle of HFD-Induced Type 2 Diabetic Rats

HFD-induced T2DM rats showed a significant increase in oxidative stress markers compared to control animals. Lipid peroxidation (LPO) levels were markedly elevated in the diabetic group (126±8 nmol/L) compared to control (75±5 nmol/L), indicating enhanced membrane lipid damage. Similarly, hydroxyl radical (OH•) levels were significantly increased (59±3 U/L vs. 30±2 U/L). Treatment with Lupeol significantly reduced LPO (90±5 nmol/L) and OH• levels (40±2 U/L) compared to diabetic rats, indicating attenuation of oxidative stress. Metformin treatment showed a comparable reduction (LPO: 80±6 nmol/L; OH•: 36±2 U/L), restoring values closer to normal.

Parameters	CONTROL	HFD-T2DM	HFD-T2DM+ LUP	HFD-T2DM + MET
LPO (nmol/L	75 ±5	126± 8[a]	90 ± 5[ab]	80 ± 6[bc]
OH* (U/L)	30 ±2	59 ±3	40	36

Table 1: Effect of lupeol on oxidative stress markers in the gracilis muscle of type-2 diabetic adult male rat. Each bar represents mean ± SEM. Significance at $p< 0.05$, a-compared with control, b-compared with diabetic control.

Effect of Lupeol on antioxidant enzymes in the gracilis muscle of HFD-Induced Type 2 Diabetic Rats

The diabetic group exhibited a significant decline in both enzymatic and non-enzymatic antioxidants. SOD, CAT, and GSH levels were markedly reduced (12±1 pg/mL, 7±0.5 ng/L, and 70±5 ng/L, respectively) compared to control (26±2, 19±1, and 201±14). Lupeol treatment significantly restored antioxidant levels, with SOD (20±1), CAT (15±0.8), and GSH (120±9) showing marked improvement. Metformin treatment further normalized these levels (SOD: 25±1, CAT: 20±1.0, GSH: 180±10), indicating strong antioxidant restoration.

Parameters	Control	HFD Diabetes	HFD-T2DM + Lupeol	HFD-T2DM+ Metformin
SOD (pg/mL)	26±2	12±1[a]	20±1[b]	25±1[b]
CAT (ng/L)	19±1	7±0.5[a]	15±0.8[ab]	20±1.0[bc]
GSH (ng/L)	201±14	70±5[a]	120±9[ab]	180±10[abc]

Table 2: Effect of lupeol on enzymic and non-enzymic antioxidants in the gracilis muscle of diabetic adult male rat. Each bar represents mean ± SEM of 6 animals. Significance at $p< 0.05$, a-compared with control, b-compared with diabetic control.

Effect of Lupeol on insulin signaling-related gene expression in gracilis muscle of HFD-induced T2DM rats

In high-fat diet (HFD)-induced type 2 diabetic rats, the mRNA expression levels of key insulin signaling genes - AKT, insulin receptor (IR), IRS-1, and GLUT-4 were significantly reduced compared to the control group,

reflecting impaired insulin sensitivity and diminished glucose uptake in gracilis muscle. Treatment with lupeol markedly enhanced the expression of these genes relative to the diabetic group, indicating a restoration of insulin signaling, as evidenced by increased AKT, IR, and IRS-1 levels, along with improved glucose transport through upregulated GLUT-4 expression. Notably, metformin treatment produced a more substantial effect, with gene expression levels nearing those of the control group. Overall, these findings suggest that lupeol ameliorates insulin resistance and enhances glucose uptake by modulating the insulin signaling pathway.

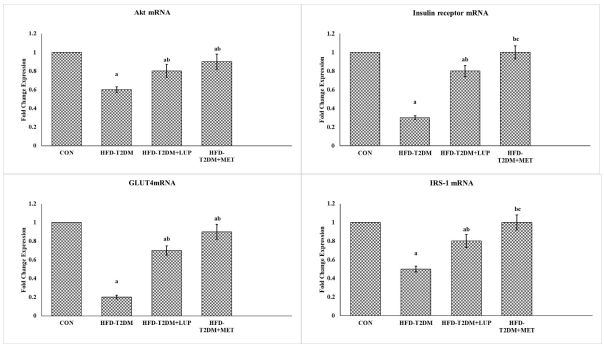

Figure 1: Relative mRNA expression levels of AKT, insulin receptor (IR), IRS-1, and GLUT-4 were analyzed. HFD-T2DM rats showed significant downregulation of these genes compared to control, indicating impaired insulin signaling. Lupeol treatment significantly restored gene expression, improving glucose uptake mechanisms. Metformin-treated rats showed near-normal expression levels. Values are expressed as mean ± SEM (n = 6). Statistical significance at $p < 0.05$. a: compared with control; b: compared with HFD-T2DM; c: compared with Lupeol-treated group.

Effect of Lupeol on AMPK-mediated metabolic gene expression in gracilis muscle of HFD-induced T2DM rats

In HFD-induced T2DM rats, the mRNA expression levels of CaMKKβ, CPT-1, PPAR-γ, and SIRT1 were significantly reduced compared to controls, indicating suppressed AMPK signaling, decreased fatty acid oxidation, disrupted lipid metabolism, and mitochondrial dysfunction. Lupeol treatment markedly upregulated these genes, suggesting restoration of metabolic homeostasis; increased CaMKKβ points to activation of upstream AMPK signaling, elevated CPT-1 reflects enhanced fatty acid β-oxidation, upregulated PPAR-γ indicates improved lipid metabolism, and higher SIRT1 expression suggests enhanced mitochondrial biogenesis and energy regulation. Metformin treatment exhibited a comparable but slightly more pronounced effect. Overall, these findings indicate that lupeol improves metabolic function by activating the CaMKKβ–AMPK signaling pathway and promoting mitochondrial efficiency.

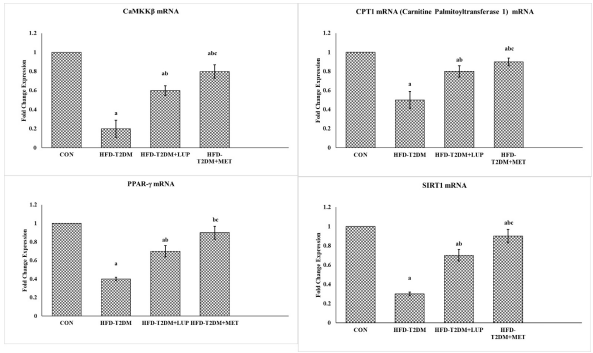

Figure 2: Relative mRNA expression levels of CaMKKβ, CPT-1, PPAR-γ, and SIRT1 were evaluated. HFD-T2DM rats showed significant downregulation, indicating impaired AMPK signaling, reduced fatty acid oxidation, and mitochondrial dysfunction. Lupeol treatment significantly upregulated these genes, suggesting activation of AMPK-mediated pathways. Metformin treatment showed comparable restoration. Values are expressed as mean ± SEM (n = 6). Statistical significance at $p < 0.05$. a: compared with control; b: compared with HFD-T2DM; c: compared with Lupeol-treated group.

Effect of Lupeol on lipogenic and inflammatory gene expression in gracilis muscle of HFD-induced T2DM rats

In HFD-induced diabetic rats, the mRNA expression of SREBP-1c and TNF-α was significantly elevated compared to controls, indicating increased lipogenesis and heightened inflammatory responses in gracilis muscle. Lupeol treatment markedly reduced the expression of both genes, suggesting suppression of lipid synthesis through downregulation of SREBP-1c and attenuation of inflammation via decreased TNF-α levels. Metformin treatment produced a more pronounced effect, with gene expression levels approaching those of the control group. Overall, these findings demonstrate that lupeol alleviates metabolic stress in diabetic muscle by exerting anti-lipogenic and anti-inflammatory effects.

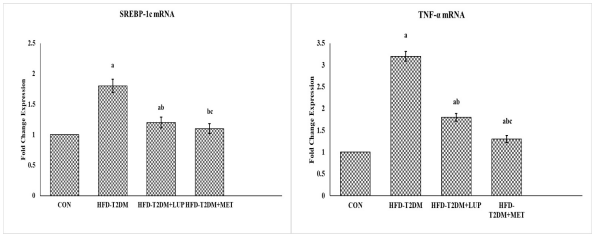

Figure 3: Relative mRNA expression levels of SREBP-1c and TNF-α were assessed. HFD-T2DM rats showed significant upregulation of both genes, indicating increased lipogenesis and inflammation. Lupeol treatment significantly reduced their expression, demonstrating anti-lipogenic and anti-inflammatory effects. Metformin treatment showed a similar trend. Values are expressed as mean ± SEM (n = 6). Statistical significance at $p < 0.05$. a: compared with control; b: compared with HFD-T2DM; c: compared with Lupeol-treated group.

DISCUSSION

The present study demonstrates that lupeol, a pentacyclic triterpenoid, effectively ameliorates skeletal muscle insulin resistance in HFD-induced T2DM rats through a multifaceted mechanism involving attenuation of oxidative stress, restoration of antioxidant defenses, enhancement of insulin signaling pathways, activation of the CaMKKβ–AMPK–SIRT1 axis, and suppression of lipogenesis and inflammation. These findings highlight lupeol as a promising multi-targeted phytotherapeutic agent for the management of T2DM, consistent with growing evidence supporting its diverse pharmacological properties. Skeletal muscle is responsible for nearly 70–80% of insulin-mediated glucose uptake and plays a central role in the pathogenesis of insulin resistance. In the present study, HFD-T2DM rats exhibited pronounced oxidative stress, as evidenced by elevated lipid peroxidation (LPO) and hydroxyl radical levels, along with diminished antioxidant enzyme activities (SOD, CAT, and GSH) in gracilis muscle. Lupeol treatment significantly mitigated oxidative damage and restored antioxidant capacity. These findings are in agreement with Özdemir et al. (2024), who reported that lupeol enhances endogenous antioxidant defenses while reducing oxidative stress markers (11). Similarly, Das et al. (2022) demonstrated that lupeol attenuates oxidative stress and inflammation via inhibition of NF-κB signaling in diabetic models (12).

Impairment of insulin signaling is a hallmark of T2DM, characterized by reduced expression of key components such as IR, IRS-1, AKT, and GLUT-4. In this study, lupeol significantly upregulated these genes, suggesting improved insulin sensitivity and glucose uptake. These results are consistent with Daniel (2023), who observed enhanced insulin signaling and reduced inflammatory cytokine expression following lupeol treatment in diabetic rats (13). The restoration of GLUT-4 is particularly critical, as it governs insulin-stimulated glucose transport in skeletal muscle. Supporting this, Che et al. (2026) and Yao et al. (2023) demonstrated that activation of the AMPK–GLUT4 pathway by natural compounds significantly improves glucose uptake and insulin sensitivity, reinforcing the importance of this signaling axis. A major mechanistic insight from this study is the activation of the CaMKKβ–AMPK–SIRT1 pathway. AMPK acts as a key metabolic regulator that enhances glucose uptake, fatty acid oxidation, and mitochondrial function while inhibiting lipogenesis. The observed upregulation of CaMKKβ, CPT-1, PPARγ, and SIRT1 indicates that lupeol activates this energy-sensing pathway (14,15). Kemelo and Moseki (2025) emphasized that AMPK–SIRT1 activation plays a crucial role in improving metabolic homeostasis and is a target of widely used antidiabetic drugs such as metformin (16). Furthermore, Montalvo et al. (2026) demonstrated the essential role of AMPK activation in maintaining skeletal muscle metabolic function, while Bengal and Aviram (2025) highlighted its therapeutic potential in combating insulin resistance (17,18).

SIRT1 activation further contributes to metabolic regulation by enhancing mitochondrial biogenesis and reducing oxidative stress. Taheripak et al. (2024) showed that SIRT1 protects skeletal muscle cells from lipotoxicity-induced apoptosis by promoting PGC-1α-mediated mitochondrial function (19). Additionally, Hoseini et al. (2025) reported that SIRT1 improves metabolic homeostasis through modulation of autophagy and oxidative stress pathways. The increased expression of CPT-1 observed in this study further suggests enhanced fatty acid β-oxidation, supporting improved lipid metabolism (20). In line with this, Peng et al. (2024) demonstrated that natural compounds can restore lipid homeostasis via AMPK-mediated pathways in diabetic conditions. Lupeol also significantly reduced the expression of lipogenic and inflammatory markers,

including SREBP-1c and TNF-α, indicating suppression of lipid accumulation and inflammation. Chronic inflammation is a major contributor to insulin resistance, primarily through TNF-α-mediated disruption of insulin signaling (21). Park et al. (2024) reported that lupeol inhibits NF-κB signaling and reduces pro-inflammatory mediators such as COX-2 and iNOS (22). Similarly, Gayathri et al. (2024) demonstrated that lupeol reduces inflammatory cytokine expression in diabetic models, highlighting its systemic anti-inflammatory effects (23). Selvaraju et al. (2025) further showed that lupeol mitigates adipocyte hypertrophy and lipotoxicity, reinforcing its role in improving metabolic dysfunction (24).

The systemic benefits of lupeol have been demonstrated across multiple organs affected by diabetes. Sundaram et al. (2024) reported that lupeol improves carbohydrate metabolism and alleviates hepatic, renal, and cardiac complications in diabetic rats (25). Malekinejad et al. (2023) also showed that lupeol reduces insulin resistance and oxidative stress in metabolic disorders such as NAFLD and PCOS (26). Furthermore, Qin et al. (2024) demonstrated that lupeol improves bile acid metabolism and reduces hepatic steatosis, suggesting its broader role in metabolic regulation (27). Overall, the present findings provide strong evidence that lupeol exerts protective effects against skeletal muscle insulin resistance through coordinated modulation of oxidative stress, insulin signaling, energy metabolism, and inflammatory pathways. By targeting the CaMKKβ–AMPK–SIRT1 axis and restoring metabolic balance, lupeol emerges as a promising candidate for the development of novel therapeutic strategies in T2DM management. Further clinical studies are warranted to validate its efficacy and translational potential in humans.

CONCLUSION

The present study demonstrates that lupeol exerts significant protective effects against skeletal muscle insulin resistance in HFD-induced type 2 diabetic rats by modulating multiple interconnected pathways. Lupeol effectively attenuated oxidative stress, restored antioxidant defenses, improved insulin signaling (IR/IRS-1/AKT/GLUT-4), and activated the CaMKKβ–AMPK–SIRT1 axis, leading to enhanced fatty acid oxidation and mitochondrial function, while simultaneously suppressing lipogenesis and inflammation through downregulation of SREBP-1c and TNF-α. These findings highlight lupeol as a promising multi-targeted phyto-therapeutic agent with efficacy comparable to metformin. However, future studies are warranted to validate these effects at the protein and phosphorylation levels, explore dose-dependent responses, and assess long-term efficacy and safety. Additionally, improving the bioavailability of lupeol through advanced drug delivery systems such as nanoparticle-based formulations, along with translational and clinical studies, will be essential to establish its therapeutic potential in the management of type 2 diabetes mellitus.

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