RESEARCH PAPER

Assessment Of Renin–Angiotensin System (Ras) Axis Modulation In Response To Lupeol Treatment In High-Fat Diet-Induced Type 2 Diabetic Rats

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Abstract

Background: Type 2 diabetes mellitus (t2dm) involves an imbalance in the renin–angiotensin system, with overactivation of the ace/ang ii/at1r axis and suppression of the ace2/masr axis, leading to oxidative stress, inflammation, and insulin resistance. Lupeol, a bioactive pentacyclic triterpenoid known for its antioxidant and antidiabetic effects, remains unexplored for its potential role in regulating ras dysfunction in diabetes.

Aim: To investigate the effect of lupeol on serum ras components, including renin, aldosterone, classical axis markers (ace, ang ii, at1r), and protective axis markers (ace2, masr), in a high-fat diet (hfd)/streptozotocin (stz)-induced t2dm rat model.

Materials and Methods: Adult male wistar albino rats (180–220 g) were randomly divided into four groups (n = 6 per group): group i – normal control; group ii – hfd/stz-induced t2dm (hfd for 4 weeks followed by a single intraperitoneal injection of stz, 35 mg/kg); group iii – hfd-t2dm treated with lupeol; and group iv – hfd-t2dm treated with metformin. Diabetes was confirmed by fasting blood glucose levels ≥250 mg/dl. Serum levels of renin, aldosterone, ace, ang ii, at1r, ace2, and masr were quantified using elisa kits. Statistical analysis was performed using one-way anova followed by duncan's multiple range test, with $p < 0.05$ considered significant.

Results: Hfd-t2dm rats exhibited a significant ($p < 0.05$) reduction in serum aldosterone (100 ± 7 vs. 189 ± 10 pg/ml) and renin (46 ± 2.5 vs. 101 ± 9 pg/ml) compared to controls. This was accompanied by a marked elevation in classical ras components (ace, ang ii, at1r) and a concomitant reduction in protective ras markers (ace2, masr). Treatment with lupeol significantly restored aldosterone (160 ± 9 pg/ml) and renin (79 ± 3.4 pg/ml) levels toward normal, attenuated the classical ras axis, and enhanced the protective axis. Metformin treatment produced comparable effects (aldosterone: 172 ± 11 pg/ml; renin: 86 ± 3.4 pg/ml). Both treatment groups showed significant improvement ($p < 0.05$) compared to untreated diabetic rats.

Conclusion: Lupeol effectively restores ras homeostasis in hfd/stz-induced t2dm by suppressing the classical ace/ang ii/at1r axis and enhancing the ace2/masr protective axis, along with normalization of renin and aldosterone levels. Its effects are comparable to metformin, highlighting its potential as a novel phytotherapeutic agent for the management of t2dm and prevention of ras-mediated complications.

Keywords: Lupeol, Renin–Angiotensin System, Type 2 Diabetes Mellitus, Ace2, Mas Receptor, At1r, High-Fat Diet, Streptozotocin.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder marked by sustained hyperglycemia, insulin resistance, and progressive dysfunction of pancreatic β-cells. It represents a significant and growing global health challenge due to its rapidly increasing prevalence (1). The chronic complications associated with T2DM such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases are largely driven by disturbances in various neurohumoral systems, among which the renin–angiotensin system (RAS) plays a central role (2). The RAS is a complex hormonal network essential for regulating blood pressure, fluid homeostasis, and electrolyte balance. It consists of two opposing functional arms. The classical or deleterious axis includes renin, angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and the angiotensin II type 1 receptor (AT1R). Activation of this pathway leads to vasoconstriction, sodium retention, oxidative stress, inflammation, and fibrosis (3,4). In contrast, the protective axis is composed of angiotensin-converting enzyme 2 (ACE2), angiotensin-(1–7), and the Mas receptor (MasR), which counteract the effects of the classical pathway by promoting vasodilation and exerting anti-inflammatory, antioxidant, and anti-fibrotic actions (5,6).

Under diabetic conditions, chronic hyperglycemia induces overactivation of the classical RAS axis, resulting in elevated levels of Ang II. This exacerbates insulin resistance, impairs β-cell function, and accelerates both microvascular and macrovascular complications (7). Concurrently, the protective ACE2/Ang-(1–7)/MasR axis is suppressed in diabetic tissues, further shifting the balance toward pathological damage and metabolic dysfunction (8). Thus, therapeutic strategies aimed at restoring the balance between these two RAS axes have gained considerable attention in the management of T2DM and its associated complications (4). The high-fat diet (HFD) combined with low-dose streptozotocin (STZ) model is widely recognized as a reliable experimental model for T2DM, as it closely replicates the progression of human disease from insulin resistance to partial β-cell failure (9-11). Importantly, this model is also associated with activation of both systemic and tissue-specific RAS components, making it suitable for investigating interventions targeting RAS modulation (12,13).

Lupeol (20(29)-Lupen-3β-ol) is a naturally occurring pentacyclic triterpenoid present in various fruits, vegetables, and medicinal plants (14,15). It has been extensively studied for its pharmacological properties, including anti-inflammatory, antioxidant, antihyperglycemic, and antihyperlipidemic effects. These effects are mediated through the modulation of key signaling pathways such as NF-κB, PI3K/Akt, and MAPK (16). Previous studies have demonstrated that lupeol can protect pancreatic β-cells by reducing oxidative stress and inflammation in diabetic models (17), enhance insulin signaling, and suppress pro-inflammatory cytokines such as TNF-α, IL-6, and NF-κB in adipose tissues of HFD-induced diabetic rats (18). Additionally, lupeol has been reported to attenuate neuroinflammation in the brain and mitigate metabolic complications associated with non-alcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS) (19,20). Despite substantial evidence supporting the metabolic and anti-inflammatory benefits of lupeol, its role in modulating the renin–angiotensin system particularly its effects on both the classical (ACE/Ang II/AT1R) and protective (ACE2/Ang-(1–7)/MasR) axes remains insufficiently explored. Given the critical involvement of RAS imbalance in diabetic pathophysiology and the multi-targeted pharmacological actions of lupeol, it is hypothesized that lupeol may exert therapeutic effects by restoring RAS homeostasis. Therefore, the present study aims to evaluate the impact of lupeol on key RAS components, including renin, aldosterone, ACE, Ang II, AT1R, ACE2, and MasR, in HFD/STZ-induced T2DM rats.

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 renin, angiotensin II (Ang II), aldosterone, ACE, ACE2, AT1R, and Mas receptor (MasR) 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.

Sample Collection

At the end of the experimental period, animals were fasted overnight and anesthetized. Blood samples were collected via cardiac puncture and centrifuged at 3000 rpm for 15 minutes to separate serum. Serum samples were stored at −80°C until further biochemical analysis.

Estimation of Serum RAS Components

Serum levels of renin, angiotensin II (Ang II), and aldosterone were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Assessment of Classical RAS Axis Markers

The classical RAS axis components, including angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and angiotensin II type 1 receptor (AT1R), were evaluated using ELISA kits. The expression levels were calculated and compared among experimental groups.

Assessment of Protective RAS Axis Markers

Protective RAS axis markers such as angiotensin-converting enzyme 2 (ACE2) and Mas receptor (MasR) were analyzed using ELISA kits. These markers were used to assess the counter-regulatory arm of the RAS pathway.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunccan's multiple range test using GraphPad Prism software. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Serum parameters

Induction of T2DM using HFD/STZ significantly reduced circulating aldosterone and renin levels compared to the control group, indicating suppression of systemic RAS activity. Treatment with lupeol markedly restored both aldosterone (160±9 pg/mL) and renin (79±3.4 pg/mL) levels toward normal, demonstrating partial recovery of endocrine RAS function. Metformin showed a slightly greater restoration (aldosterone: 172±11 pg/mL; renin: 86±3.4 pg/mL), though the effect of lupeol was comparable, suggesting its efficacy in normalizing circulating RAS components.

PARAMETERS	CONTROL	HFD-T2DM	HFD-T2DM+LUP	HFD-T2DM+MET
Aldosterone (pg/mL)	189±10	100±7^{p}	160±9^{qr}	172±11^{pqr}
Renin (pg/mL)	101±9	46±2.5^{p}	79±3.4^{pq}	86±3.4^{pq}

Table 1: Effect of lupeol on serum renin and aldosterone levels in control and experimental groups.

Data are presented as mean ± SEM. HFD-T2DM significantly reduced renin and aldosterone levels compared to control, whereas treatment with lupeol and metformin significantly restored these parameters toward normal levels.

Effect of Lupeol on Classical RAS Axis Markers (ACE, AT1R, Ang II)

Diabetic rats exhibited a significant upregulation of classical RAS markers, with increased ACE, AT1R, and Ang II mRNA expression compared to controls, confirming activation of the deleterious RAS arm under hyperglycemic conditions. Lupeol treatment significantly downregulated these markers, reducing ACE and Ang II expression and attenuating AT1R levels, though not completely to control values. Metformin treatment produced a more pronounced reduction, approaching near-normal expression levels. These findings indicate that lupeol effectively suppresses the hyperactivated classical RAS axis in T2DM.

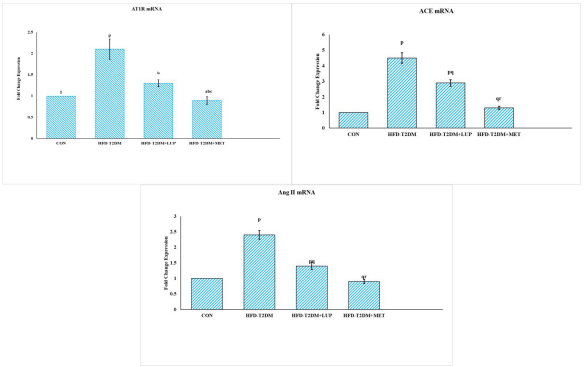

Figure 1: Effect of lupeol on classical renin–angiotensin system (RAS) markers (ACE, AT1R, and Ang II) mRNA expression in control and experimental groups. HFD-T2DM rats showed significant upregulation of ACE, AT1R, and Ang II expression compared to control, indicating activation of the classical RAS axis. Treatment with lupeol significantly downregulated these markers, while metformin produced a more pronounced normalization toward control levels. Data are expressed as fold change (mean ± SEM). Statistical significance: $p < 0.05$ vs control; $q < 0.05$ vs HFD-T2DM; $r < 0.05$ vs HFD-T2DM+LUP.

Effect of Lupeol on Protective RAS Axis (ACE2, MasR)

A significant downregulation of ACE2 and MasR mRNA expression was observed in the diabetic group, reflecting impairment of the protective RAS pathway. Administration of lupeol significantly upregulated both ACE2 and MasR expression compared to untreated diabetic rats, indicating restoration of the counter-regulatory axis. Metformin exhibited a slightly stronger effect, bringing expression levels closer to those of the control group. Overall, lupeol demonstrated a substantial to rebalance RAS by enhancing the protective axis.

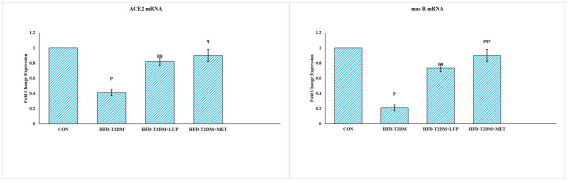

Figure 2: Effect of lupeol on protective renin–angiotensin system (RAS) markers (ACE2 and Mas receptor) mRNA expression in control and experimental groups. HFD-T2DM rats exhibited significant downregulation of ACE2 and MasR expression compared to control, indicating impairment of the protective RAS axis. Treatment with lupeol significantly upregulated both ACE2 and MasR levels, while metformin showed a more pronounced restoration toward normal expression. Data are presented as fold change (mean ± SEM). Statistical significance: $p < 0.05$ vs control; $q < 0.05$ vs HFD-T2DM; $r < 0.05$ vs HFD-T2DM+LUP.

DISCUSSION

The present study elucidates the modulatory role of lupeol on the renin–angiotensin system (RAS) in high-fat diet (HFD)/streptozotocin (STZ)-induced type 2 diabetic rats. The findings demonstrate a pronounced imbalance between the classical and counter-regulatory arms of the RAS in diabetic conditions, which was effectively normalized following lupeol administration, with effects comparable to those observed with metformin. In the current study, HFD/STZ-induced diabetic rats exhibited significantly reduced circulating levels of renin and aldosterone compared to the control group. This observation aligns with emerging evidence indicating that systemic RAS activity may be suppressed during chronic hyperglycemia, whereas tissue-specific RAS particularly intrarenal components remain paradoxically activated. Such a dissociation between circulating and local RAS activity has been increasingly reported in diabetic nephropathy and related complications (2,4). Recent quantitative analyses further suggest that alterations in renin and aldosterone levels correlate with insulin resistance indices and renal dysfunction markers across different stages of T2DM (21).

Concomitantly, diabetic rats displayed marked upregulation of key components of the classical RAS

axis, including angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and angiotensin II type 1 receptor (AT1R), indicating hyperactivation of the deleterious arm of the system. This is consistent with substantial evidence demonstrating that Ang II, via AT1R signaling, promotes oxidative stress, inflammation, fibrosis, and insulin resistance under hyperglycemic conditions (22). Mechanistically, Ang II has been shown to impair insulin receptor signaling and inhibit Akt phosphorylation through PKC-dependent pathways, thereby contributing to metabolic dysfunction (23). Additionally, activation of the AT1R–NADPH oxidase axis exacerbates oxidative stress in pancreatic β-cells and renal tissues, further aggravating diabetic complications (24,25).

In contrast, the protective arm of the RAS comprising ACE2 and the Mas receptor (MasR) was significantly downregulated in diabetic animals. This suppression is widely recognized as a critical factor that amplifies the pathological effects of the classical RAS axis in diabetes (26). The ACE2/Ang-(1–7)/Mas axis plays a pivotal role in maintaining metabolic homeostasis by enhancing insulin sensitivity, promoting glucose uptake, preserving pancreatic β-cell function, and suppressing hepatic gluconeogenesis (27). Recent findings further indicate that Ang-(1–7) enhances insulin secretion and improves peripheral glucose utilization via MasR-mediated signaling (28). Moreover, ACE2-mediated conversion of Ang II to Ang-(1–7) confers renoprotective effects by attenuating inflammation and fibrosis, with clinical evidence demonstrating that higher circulating ACE2 levels are associated with reduced progression of diabetic kidney disease (29,30).

Importantly, lupeol treatment significantly restored renin and aldosterone levels toward normal values while simultaneously suppressing the overactivation of ACE, Ang II, and AT1R, and enhancing ACE2 and MasR expression. These findings suggest that lupeol exerts a dual regulatory effect on the RAS by inhibiting the classical axis and activating the protective axis. To the best of current knowledge, this represents one of the first demonstrations of lupeol-mediated RAS modulation in a T2DM model. The underlying mechanisms of this dual modulation may be attributed to the well-documented anti-inflammatory and antioxidant properties of lupeol. Previous studies have shown that lupeol suppresses key inflammatory signaling pathways, including NF-κB, MAPK, and PI3K/Akt, thereby reducing pro-inflammatory cytokine production and improving insulin sensitivity (14,16). Experimental evidence indicates that lupeol attenuates NF-κB activation and cytokine expression in pancreatic and adipose tissues, leading to improved metabolic outcomes in diabetic models (17). Given that chronic inflammation and oxidative stress are major drivers of RAS overactivation, these effects likely contribute to the observed suppression of the ACE/Ang II/AT1R axis. In addition, recent studies highlight the role of lupeol in activating the NRF2/HO-1 antioxidant pathway, which provides cytoprotective effects against oxidative stress (31). This is particularly relevant in the context of RAS regulation, as excessive AT1R activation has been shown to inhibit NRF2 signaling and promote oxidative damage. Restoration of NRF2 activity has been associated with reduced inflammation and fibrosis, suggesting that lupeol-mediated NRF2 activation may indirectly counteract classical RAS overactivation (32). Furthermore, lupeol has been reported to improve hepatorenal and cardiac function by modulating carbohydrate metabolism and oxidative stress in diabetic conditions (33), supporting its multifaceted therapeutic potential.

Notably, the efficacy of lupeol was comparable to that of metformin in restoring RAS components, despite being administered at a lower dose. Metformin is well known for its pleiotropic actions, including anti-inflammatory and antioxidant effects. The comparable outcomes observed in the present study suggest that lupeol may serve as a promising natural alternative or adjunct therapy for RAS modulation in T2DM. Similar findings have been reported in other metabolic disorder models, where lupeol improved insulin sensitivity and reduced oxidative stress to an extent comparable with metformin (20). From a translational perspective, targeting both arms of the RAS is gaining increasing attention as a therapeutic strategy for diabetes and its complications. While conventional RAAS inhibitors primarily suppress the classical axis, emerging approaches aim to enhance the ACE2/Ang-(1–7)/Mas pathway for broader protective effects (34,35). Recent experimental studies have demonstrated that pharmacological activation of the Mas receptor improves cognitive and metabolic dysfunction in diabetic models, highlighting the systemic benefits of this pathway (36). In this regard, lupeol offers a unique advantage as a dual modulator of RAS balance. Support for the role of phytochemicals in RAS modulation is further reinforced by studies on structurally related compounds. For instance, luteolin has been shown to regulate both classical and

protective RAS axes and exhibit strong ACE inhibitory potential (37). Additionally, pentacyclic triterpenoids such as ursolic acid and oleanolic acid have demonstrated significant ACE inhibitory and antidiabetic activities, suggesting that compounds like lupeol may share similar mechanisms of action (38).

The study primarily evaluated circulating RAS components, without assessing tissue-specific expression in key organs such as the kidney, pancreas, and vasculature. Given the known divergence between systemic and local RAS activity, future studies should incorporate molecular analyses including gene and protein expression profiling (RT-PCR, Western blotting, immunohistochemistry). Furthermore, the precise molecular mechanisms underlying lupeol-mediated RAS modulation whether through direct enzyme inhibition, receptor interaction, or transcriptional regulation remain to be elucidated. Advanced approaches such as molecular docking and pathway-specific investigations will be essential to clarify these mechanisms. Finally, the present study provides compelling evidence that lupeol effectively restores RAS homeostasis in HFD/STZ-induced T2DM by suppressing the classical ACE/Ang II/AT1R axis and enhancing the ACE2/Ang-(1–7)/Mas protective axis, alongside normalization of renin and aldosterone levels. These findings highlight the potential of lupeol as a novel phyto-therapeutic agent for the management of T2DM and its complications through comprehensive RAS modulation.

CONCLUSION

The present study demonstrates for the first time that lupeol (40 mg/kg/day) effectively modulates the renin–angiotensin system in HFD/STZ-induced type 2 diabetic rats by suppressing the deleterious ACE/Ang II/AT1R axis and enhancing the protective ACE2/MasR axis, while restoring serum renin and aldosterone levels toward normal; its efficacy was comparable to metformin, highlighting its potential as a multitarget phytotherapeutic agent. These effects may be attributed to its anti-inflammatory and antioxidant properties, particularly inhibition of NF-κB signaling and reduction of oxidative stress, which help rebalance RAS activity in diabetes. Future research should focus on tissue-specific expression of RAS components using molecular techniques, in silico studies to evaluate direct binding interactions, and advanced nanoformulations to improve bioavailability, along with long-term safety, dose optimization, and combination therapy studies to establish its clinical relevance.

REFERENCES

1. Nabi AN, Ebihara A. Diabetes and renin-angiotensin-aldosterone system: pathophysiology and genetics. InRenin-angiotensin aldosterone system 2021; IntechOpen.
2. Karimi F, Maleki M, Movahedpour A, Alizadeh M, Kharazinejad E, Sabaghan M. Overview of the renin-angiotensin system in diabetic nephropathy. Journal of the Renin-Angiotensin-Aldosterone System. 2024; 25:14703203241302966.
3. Batista JP, Faria AO, Ribeiro TF, Simões e Silva AC. The role of renin–angiotensin system in diabetic cardiomyopathy: a narrative review. Life. 2023 Jul 21;13(7):1598.
4. Sanglard A, Miranda BC, Vieira AL, Macedo MV, Santos RL, Campos AS, Piva AC, Simões e Silva AC. The role of renin-angiotensin system in diabetic nephropathy: an update. Mini-Reviews in Medicinal Chemistry. 2025;25(8):591-600.
5. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clinical research in cardiology. 2020;109(5):531-8.
6. Sarkar B, Singh J, Verma D, Mutsuddi M, Mukherjee A. Regulation of Notch signaling by multiple Ankyrin repeat containing protein Mask. Cell Communication and Signaling. 2025;23(1):358.
7. Luo H, Wang X, Chen C, Wang J, Zou X, Li C, Xu Z, Yang X, Shi W, Zeng C. Oxidative stress causes imbalance of renal renin angiotensin system (RAS) components and hypertension in obese Zucker rats. Journal of the American heart association. 2015;4(2):e001559.
8. Liao W, Wu J. The ACE2/Ang (1–7)/MasR axis as an emerging target for antihypertensive peptides. Critical Reviews in Food Science and Nutrition. 2021;61(15):2572-86.
9. Kaydash OA, Ivanov VV, Vengerovsky AI, Buyko EE, Schepetkin IA. The experimental model of type 2 diabetes mellitus caused by a high-fat diet with low-dose streptozotocin in rats. Bulletin of Siberian Medicine. 2020;19(2):41-7.
10. Wickramasinghe AS, Attanayake AP, Wijesiri TW, Peiris HH, Mudduwa LK. Pancreatic and hepatic histopathology of high-fat diet fed streptozotocin-induced Wistar rat model of type 2

diabetes mellitus. Ceylon Journal of Science. 2024;53(2).

11. 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, 431.
12. Mosili P, Mkhize BC, Ngubane P, Sibiya N, Khathi A. The dysregulation of the hypothalamic–pituitary–adrenal axis in diet-induced prediabetic male Sprague Dawley rats. Nutrition & Metabolism. 2020;17(1):104.
13. Chen CY, Lin MW, Xie XY, Lin CH, Yang CW, Wu PC, Liu DH, Wu CJ, Lin CS. Studying the roles of the renin–angiotensin system in accelerating the disease of high-fat-diet-induced diabetic nephropathy in a db/db and ACE2 double-gene-knockout mouse model. International journal of molecular sciences. 2023;25(1):329.
14. Singha A, Chakraborty A, Sil SK. Lupeol: ethnopharmacological insights and therapeutic potential in human health. 3 Biotech. 2025;15(7):199.
15. Rehman S, Siddiqui A, Rehman S. Importance of Lupeol Containing Plants and Plant Products in Traditional Medicine with an Emphasis on the Unani System of Medicine. InLupeol 2025 (pp. 234-269). CRC Press.
16. Parvez A, Rahman MA, Rahman MM, Shimki AI, Ahmmed S, Supti FA, Hasan MH, Bristi MS, Ansari SA, Islam MT. Broad-spectrum therapeutic potentials of the multifaceted triterpene lupeol and its derivatives. Chemistry & Biodiversity. 2025;22(7):e202402286.
17. Das AK, Hossain U, Ghosh S, Biswas S, Mandal M, Mandal B, Brahmachari G, Bagchi A, Sil PC. Amelioration of oxidative stress mediated inflammation and apoptosis in pancreatic islets by Lupeol in STZ-induced hyperglycaemic mice. Life sciences. 2022; 305:120769.
18. Razi FB, Ashraf H. The Benefcial Effect of Lupeol 8 against Diabetes and Obesity. Key Features.:93.
19. Esha Gayathri K, Veeraraghavan VP, Jayaraman S, Gayathri R. Effect of lupeol on the expression of neuro inflammatory signalling molecules in brain tissues of high fat diet and sucrose fed type-2 diabetic rats. management. 2024;1(5).
20. Malekinejad F, Kheradmand F, Khadem-Ansari MH, Malekinejad H. Lupeol synergizes with doxorubicin to induce anti-proliferative and apoptotic effects on breast cancer cells. DARU Journal of Pharmaceutical Sciences. 2022;30(1):103-15.
21. Wang N, Li J, Tian E, Li S, Liu S, Cao F, Kong J, Yue B. Renin-angiotensin-aldosterone system variations in type 2 diabetes mellitus patients with different complications and treatments: Implications for glucose metabolism. Plos one. 2025;20(3): e0316049.
22. Li X, Fu YH, Tong XW, Zhang YT, Shan YY, Xu YX, Pu SD, Gao XY. RAAS in diabetic retinopathy: mechanisms and therapies. Archives of endocrinology and metabolism. 2024;68: e230292.
23. Gutierrez-Rodelo C, Arellano-Plancarte A, Hernandez-Aranda J, Landa-Galvan HV, Parra-Mercado GK, Moreno-Licona NJ, Hernandez-Gonzalez KD, Catt KJ, Villalobos-Molina R, Olivares-Reyes JA. Angiotensin II inhibits insulin receptor signaling in adipose cells. International Journal of Molecular Sciences. 2022;23(11):6048.
24. Lin X, Wang X, Feng W, Wan Y, Chai J, Li F, Xu M. The Counteracting Effects of Ang II and Ang-(1-7) on the Function and Growth of Insulin-secreting NIT-1 Cells. Current Diabetes Reviews. 2024;20(10):80-92.
25. Ha TS, Seong SB, Ha DS, Kim SJ. Upregulation of NADH/NADPH oxidase 4 by angiotensin II induces podocyte apoptosis. Kidney research and clinical practice. 2023;42(2):202.
26. Li X, Qu S. Novel insights into the central protective role of ACE2 in diabetic cardiomyopathy: from underlying signaling pathways to therapeutic perspectives. Molecular and Cellular Biochemistry. 2025;480(6):3535-51.
27. Zhao S, Sun W, Jiang P. Role of the ACE2/Ang-(1-7)/Mas axis in glucose metabolism. Reviews in Cardiovascular Medicine. 2021; 22(3):769-77.
28. Rodriguez-Blanco J, Salvador AD, Suter RK, Swiderska-Syn M, Palomo-Caturla I, Kliebe V, Shahani P, Peterson K, Turos-Cabal M, Vieira ME, Wynn DT, Howell AJ, Yang F, Ban Y, McCrea HJ, Zindy F, Danis E, Vibhakar R, Jermakowicz A, Martin V, Coss CC, Harris BT, de Cubas A, Chen XS, Barnoud T, Roussel MF, Ayad NG, Robbins DJ. Triptolide and its prodrug Minnelide target high-risk MYC-amplified medulloblastoma in preclinical models. J Clin Invest. 2024;134(15):e171136.
29. Nomura H, Kuruppu S, Rajapakse NW. Stimulation of angiotensin converting enzyme 2: a

novel treatment strategy for diabetic nephropathy. Frontiers in Physiology. 2022; 12:813012.

30. Ueno A, Onishi Y, Mise K, Yamaguchi S, Kanno A, Nojima I, Higuchi C, Uchida HA, Shikata K, Miyamoto S, Nakatsuka A. Plasma angiotensin-converting enzyme 2 (ACE2) is a marker for renal outcome of diabetic kidney disease (DKD)(U-CARE study 3). BMJ Open Diabetes Research & Care. 2024;12(3).
31. Saha S, Capozzi A, Profumo E, Alessandri C, Sorice M, Saso L, Buttari B. Lupeol Attenuates Oxysterol-Induced Dendritic Cell Activation Through NRF2-Mediated Antioxidant and Anti-Inflammatory Effects. International Journal of Molecular Sciences. 2025;26(15):7179.
32. Godoy-Lugo JA, Thorwald MA, Mendez DA, Rodriguez R, Nakano D, Nishiyama A, Ortiz RM. Glucose increases hepatic mitochondrial antioxidant enzyme activities in insulin resistant rats following chronic angiotensin receptor blockade. International Journal of Molecular Sciences. 2022;23(18):10897.
33. Sundaram R, Muthu K. Chrysin attenuates hyperglycemia associated with hepatorenal and glycoprotein abnormalities via regulation of glucose metabolism in HFD/STZ-induced diabetic rats. Phytomedicine plus. 2024;4(4):100632.
34. Skwiersky S, Iwuala S, Chillumuntala S, Osafehinti D, Karam J. Prevention of Type 2 Diabetes: Mechanistic Insights and Evidence. Renin-Angiotensin Aldosterone System. 2021; 103.
35. Kale A, Shelke V, Sankrityayan H, Dagar N, Gaikwad AB. Klotho restoration via ACE2 activation: A potential therapeutic strategy against acute kidney injury-diabetes comorbidity. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2022;1868(12):166532.
36. Tian S, Wu T, Zhang Z, Lv S, Ji X, Zhao Z, Ma X, Wang J, Bi Y. Activation of central Angiotensin-(1-7)/Mas receptor alleviates synaptic damage in diabetes-associated cognitive impairment via modulating AKT/FOXO1/PACAP axis. International Journal of Biological Sciences. 2025; 21(6):2824.
37. Rongpan S, Pocasap P, Iampanichakul M, Tong-Un T, Maneesai P, Kittipreeya P, Prachaney P, Pakdeechote P. Luteolin restores the renin-angiotensin system to alleviate blood pressure and cardiac changes in secondary hypertensive rats. Journal of Functional Foods. 2025; 134:107075.
38. Chakraborty R, Roy S. Angiotensin-converting enzyme inhibitors from plants: A review of their diversity, modes of action, prospects, and concerns in the management of diabetes-centric complications. Journal of Integrative Medicine. 2021;19(6):478-92.