ORIGINAL RESEARCH

Effects of Moringa Oleifera Leaf Extract Plus Rosiglitazone on Serum Leptin and Glucose and Lipid Metabolism in Type 2 Diabetic Rats

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ABSTRACT

Objective • To investigate the effects of Moringa Oleifera Leaf Extract (MOLE) plus rosiglitazone (RSG) on glucose and lipid metabolism, serum leptin, and the Akt/GSK3β/β-Catenin signaling pathway in type 2 diabetic (T2D) rats.

Methods • Sixty male Sprague-Dawley (SD) rats were randomly divided into six groups: the normal group, the model group, the RSG group, the low- and high-dose MOLE group, and the MOLE+RSG group. The normal group was fed a standard rat diet, while the other groups were given a single intraperitoneal injection of low-dose streptozomycin (STZ) (35 mg/kg) and fed a high-sugar and high-fat diet. After 8 weeks, the treatment outcomes were evaluated by measuring key parameters of blood glucose and lipid metabolism and the protein kinase B (AKT) / Glycogen synthase kinase 3beta (GSK3β) /β-Catenin signaling pathway in the T2D rats.

Results • Compared with the normal group, the model group showed significantly increased levels of blood glucose, blood lipids, serum leptin, free fatty acid (FFA), and tumor necrosis factor-α (TNF-α). Compared with the model group, the RSG, low-dose MOLE, and high-dose MOLE groups displayed effective control of blood glucose, blood lipids, serum leptin, FFA, and TNF-α. The MOLE+RSG group surpassed the RSG group in regulating glucose, lipid metabolism, and serum leptin levels in T2D rats. In addition, the MOLE+RSG group also had superiority over the RSG group in activating the AKT/GSK3β/β-Catenin pathway.

Conclusion • MOLE plus RSG can effectively reduce blood glucose and blood lipids in T2DM rats. (*Altern Ther Health Med.* 2023;29(8):650-655).

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INTRODUCTION

Diabetes mellitus is a common endocrine disease attributable to the interaction of genetic and environmental factors, and type 2 diabetes mellitus (T2DM) accounts for approximately 90% of all cases. Existing evidence shows that abnormal blood lipid metabolism, usually concomitant with vascular lesions, is the most frequently occurring pathophysiological change in T2DM and can ultimately induce multi-organ damage and failure.[1,2] Considering the large number of patients with T2DM and the serious associated hazards, it is a major public health concern how to better prevent and control T2DM and treat related abnormalities of glucose and lipid metabolism.[3]

Moringa oleifera leaves exert an anti-diabetic effect by improving pancreatic function and regulating intestinal function and glycogen metabolism. Studies have demonstrated that Moringa oleifera leaf extract (MOLE) has diverse pharmacological properties that offer significant lipid- and glucose-lowering, anticancer, hepatoprotective, and antioxidant benefits, especially in the prevention and control of diabetes mellitus.[4-7]

Rosiglitazone (RSG) belongs to a class of novel, potent oral thiazolidinediones (TZDs) and has become a first-line drug for T2DM treatment, particularly in overweight and obese patients.[8] RSG can effectively activate peroxisome proliferator-activated receptor gamma (PPARγ) of insulin-activating tissues, regulate the transcription of insulin-responsive genes, and control the production, transportation, and utilization of blood glucose in the body, thereby gaining proper control of blood sugar. RSG can enhance the glucose uptake-facilitating ability of glucose transporter type 4 (GLUT4), which can reduce and ultimately improve blood

glucose levels in the body. Patients receiving TZDs may experience side effects such as edema, increased plasma volume, liver damage, and heart failure.[9-13] Therefore, it is particularly important to research drugs that can be combined with RSG as potential solutions to RSG-induced side effects.[8-13]

Aiming to explore the therapeutic effect of MOLE plus RSG on diabetes mellitus, this study investigated the effects of the combination therapy on related parameters of glucose and lipid metabolism and the AKT/GSK3β/β-Catenin signaling pathway in T2D rats.

MATERIALS AND METHODS

Laboratory animals

Sixty healthy male clean-grade Sprague-Dawley (SD) rats weighing (200 ± 20) g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All rats were kept in a room at 20-22°C under 12-h:12-h light-dark (LD) cycles, with free access to food and water.

Drugs and reagents

Moringa oleifera leaf powder was obtained from Shaanxi Taike Biotech Co., Ltd. Moringa oleifera leaf aqueous extract is prepared as described in a previous study[14]: Add 2 L of ultrapure water to 200 g of Moringa oleifera leaf powder, mix thoroughly and boil for 5 min, cool down to room temperature, remove undissolved impurities with a 2 μm filter membrane, obtain brown powder by vacuum freeze-drying, and store in a freezer. Rosiglitazone (Sigma, USA, Cat No. R2408), streptozotocin (Sigma, USA, Cat No. V900890), the rat tumor necrosis factor-α (TNF-α) ELISA kit (Cat No. SEA133Ra), and the leptin ELISA kit (Cat No. SEA084Ra) were purchased from Cloud-Clone Corp., Wuhan. The glucose (GLU) assay kit (Cat No. BC8323), total cholesterol (TC) assay kit (Cat No. BC1985), triglyceride (TG) assay kit (Cat No. BC0625), low-density lipoprotein cholesterol (LDL-C) assay kit (Cat No. BC5335) and high-density lipoprotein cholesterol (HDL-C) assay kit (Cat No. BC5325) were obtained from Beijing Solarbio Science & Technology Co., Ltd. The free fatty acid (FFA) assay kit (Cat No. YX-W-B400) was purchased from Hefei Laier Biotechnology Co., Ltd. Akt antibody (Cat No. #9272), p-Akt (Ser473) antibody (Cat No. #9271), GSK3β antibody (Cat No. #9315), p-GSK3β antibody (Cat No. #9336), β-Catenin antibody (Cat No. #8814), p-β-Catenin antibody (Cat No. #9561), and β-Tubulin antibody (Cat No. #2128) were purchased from CST Inc.

Animal group modeling and dosing

Sixty male SD rats were purchased and subject to adaptive feeding with a standard diet composed of 16% protein, 8% fat, and 50% carbohydrate. Seven days later, 10 male SD rats were randomly selected out of 60 as a normal group and continued to receive the standard diet. The remaining rats were assigned to an experimental group fed a high-fat diet consisting of 15% protein, 25% fat, 50% carbohydrates, and 5% cholesterol. Following 4 weeks of the high-fat diet, these rats were intraperitoneally injected with 35 mg/kg STZ (freshly prepared with 0.1M citric acid-citrate buffer at pH 4.4 before use). Tail vein blood was collected 120 hours after injection for random blood glucose testing, with a result of or over 16.7 $mmol \cdot L^{-1}$ indicative of successful modeling of diabetes. Subsequently, the diabetic rats were randomized into 5 groups: the model, RSG, low-dose MOLE (L-MOLE), high-dose MOLE (H-MOLE), and MOLE+RSG groups. The RSG group was given 0.35 mg/kg RSG suspension via gastric lavage. The L-MOLE and H-MOLE groups were administered daily with 100 mg/kg and 200 mg/kg MOLE via gastric lavage, respectively. The MOLE+RSG group received 0.35 mg/kg RSG suspension and 100 mg/kg MOLE via gastric lavage. The blank control received an equal quantity of solvent every day using the identical method. All medications were freshly prepared and administered via gastric lavage once daily for 8 consecutive weeks. Random blood glucose tests were arranged every two weeks and conducted by tail vein blood collection.

Sample collection and parameter measurement

At the end of the 8-week treatment, animals were anesthetized with 10% chloral hydrate by intraperitoneal injection after fasting for 12 hours. Blood samples were collected from the abdominal aorta, and serum was isolated and stored at -20°C. Blood glucose and blood lipids were measured with biochemical methods. Serum insulin, leptin, and FFA were measured according to the protocols provided with the assay kits.

Western blotting

After drug intervention, liver tissue was harvested and chopped into small pieces. Then, 200 μL of histone lysate was added for homogenization on ice for 20 min, followed by centrifugation at 12000× g at 4°C for 15 min. The total protein in the supernatant was measured using the bicinchoninic acid (BCA) assay kit to determine the sample load. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and polyvinylidene fluoride (PVDF) membrane transfer were performed using 30 μg of the obtained protein separately. After mounting with 5% BSA, the primary antibody was added to a shaking table for overnight incubation at 4°C. The primary antibody was diluted as follows: Akt antibody (1:1000; Cat No: 60203-2-Ig; Proteintech, China), p-AKT antibody (1:1000; Cat No. 80455-1-RR; Proteintech, China), GSK3β antibody (1:1000; Cat No. 22104-1-AP; Proteintech, China), p-GSK3β antibody (1:1000; Cat No. 9322S; CST, USA), β-Catenin antibody (1:1000; Cat No. 51067-2-AP; Proteintech, China), p-β-Catenin antibody (1:1000; Cat No. 28776-1-AP; Proteintech, China), and β-Tubulin antibody (1:1000; Cat No. 10094-1-AP; Proteintech, China). The next day, the HRP-conjugated secondary antibody was added for further incubation at room temperature for 1 h, followed by electrochemical luminescence. Finally, grayscale analysis was performed using ImageJ.

Statistical analysis

Data are expressed as $\bar{x} \pm s$ and processed using SPSS version 16.0. Comparisons between the two groups were analyzed by independent sample *t* tests. Comparisons between three or more groups were examined by uni-variance analysis (UNOVA). The significant level was set at $P < .05$.

RESULTS

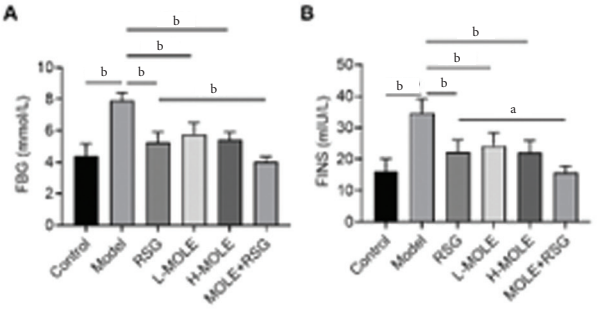
Effects of MOLE plus RSG on glucose metabolism in T2D rats

MOLE and RSG were used simultaneously to observe whether the combination therapy would affect glucose metabolism in T2D rats. As shown in Figure 1A, the fasting blood glucose (FBG) level in the model group (7.91 ± 0.52 mmol/L) is significantly higher than in the control group (4.38 ± 0.78 mmol/L) ($P < .0001$); the FBG level is significantly lower in the RSG (5.26 ± 0.65 mmol/L), L-MOLE (5.78 ± 0.74 mmol/L), and H-MOLE (5.43 ± 0.48 mmol/L) groups than in the model group ($P < .0001$); the FBG level is significantly improved in the MOLE+RSG group (4.03 ± 0.31 mmol/L) than in the RSG group ($P < .0001$). Additionally, there were significant changes in fasting serum insulin (FINS) levels. As shown in Figure 1B, the FINS level is significantly higher in the model group (34.72 ± 4.36 mIU/L) than in the control group (16.07 ± 3.98 mIU/L); the FINS level is significantly lower in the RSG (22.31 ± 3.82 mIU/L), L-MOLE (24.27 ± 4.07 mIU/L), and H-MOLE (22.12 ± 3.85 mIU/L) groups than in the model group ($P < .0001$); the FINS level is significantly lower in the MOLE+RSG group (15.79 ± 2.02 mIU/L) than in the RSG group ($P < .001$). These findings demonstrated that the combined use of MOLE and RSG has favorable glucose-lowering activity.

Effects of MOLE plus RSG on blood lipids in T2D rats

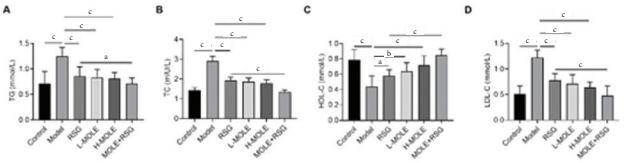
MOLE plus RSG was administered to analyze its effect on blood lipids in T2D rats. As shown in Figures 2A and 2B, serum levels of TG and TC are significantly higher in the model (1.25 ± 0.17 mmol/L for TG; 2.91 ± 0.24 mIU/L for TC) group than in the control group (1.71±0.24 mmol/L for TG; 1.44 ± 0.13 mIU/L for TC) ($P < .0001$ for TG; $P < .0001$ for TC); TG and TC levels are significantly reduced in the RSG (1.86 ± 0.18 mmol/L for TG; 1.93 ± 0.16 mIU/L for TC), L-MOLE (0.83 ± 0.16 mmol/L for TG; 1.86 ± 0.19 mIU/L for TC), and H-MOLE (0.81 ± 0.12 mmol/L for TG; 1.79 ± 0.17 mIU/L for TC) groups as compared with the model group ($P < .0001$ for TG; $P < .0001$ for TC). Notably, compared with RSG group, decreases in TG and TC are more evident in the MOLE+RSG group (0.71 ± 0.10 mmol/L for TG; 1.33 ± 0.11 mIU/L for TC) ($P = .033$ for TG; $P < .0001$ for TC). The potential effects of MOLE plus RSG on HDL-C and LDL-C levels were also analyzed in the present study (Figures 2C and 2D). Compared with the model group (0.44 ± 0.14 mmol/L for HDL-C; 1.22 ± 0.15 mmol/L for LDL-C), the results showed that HDL-C was significantly increased while LDL-C was significantly decreased in the T2D rats treated with RSG (0.58 ± 0.08 mmol/L for HDL-C; 0.78 ± 0.13 mmol/L for LDL-C) ($P = .013$ for HDL-C; $P < .0001$ for LDL-C), L-MOLE (0.64 ± 0.11 mmol/L for HDL-C; 0.71 ± 0.18 mmol/L for LDL-C) ($P = .002$ for HDL-C; $P < .0001$ for LDL-C) and H-MOLE (0.72 ± 0.12 mmol/L for HDL-C; 0.64 ± 0.10 mmol/L for LDL-C) ($P < .0001$ for HDL-C; $P < .0001$ for LDL-C) as sole. Further, the combined use of MOLE and RSG (0.85 ± 0.08 mmol/L for HDL-C; 0.48 ± 0.19 mmol/L for LDL-C) ($P < .0001$ for HDL-C; $P < .0001$ for LDL-C) exceeded RSG in regulating HDL-C and LDL-C levels. These findings suggest that MOLE plus RSG can significantly reduce blood lipid levels in T2D rats.

Figure 1. Effects of MOLE plus RSG on glucose metabolism in T2D rats. (A) Effects on FBG levels; (B) effects on FINS levels.



[a]$P < .001$
[b]$P < .0001$

Figure 2. Effects of MOLE plus RSG on blood lipids in T2D rats. (A) Effects on TG levels; (B) effects on TC levels; (C) effects on HDL-C levels; and (D) effects on LDL-C levels.

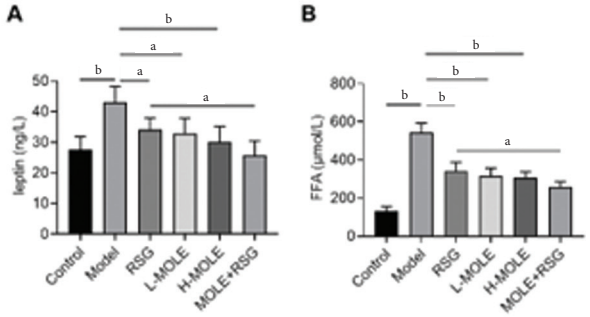


[a]$P < .05$
[b]$P < .01$
[c]$P < .0001$

Effects of MOLE plus RSG on serum leptin and FFA levels in T2D rats

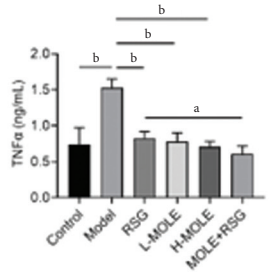
Serum leptin and FFA concentrations are associated with lipid and glucose metabolism, and endocrine function.[15] The results showed that serum leptin and FFA levels were significantly higher in the model group (43.09 ± 5.12 ng/L for leptin; 543.98 ± 49.23 μmol/L for FFA) than in the control group (27.61 ± 4.22 ng/L for leptin; 133.25 ± 20.16 μmol/L for FFA) ($P < .0001$ for leptin; $P < .0001$ for FFA) (Figures 3A and 3B). Compared with the model group, the RSG (34.12 ± 3.78 ng/L for leptin; 341.27 ± 46.35 μmol/L for FFA) ($P < .001$ for leptin; $P < .0001$ for FFA), L-MOLE (32.93 ± 4.82 ng/L for leptin; 316.30 ± 38.72 μmol/L for FFA) ($P < .001$ for leptin; $P < .0001$ for FFA), and H-MOLE (30.17 ± 5.02 ng/L for leptin; 304.71 ± 31.63 μmol/L for FFA) ($P < .0001$ for leptin; $P < .0001$ for FFA) groups showed significant reductions in serum leptin and FFA levels. In addition, MOLE plus RSG

Figure 3. Effects of MOLE plus RSG on serum leptin and FFA levels in T2D rats. (A) Effects on serum leptin levels; (B) effects on FFA levels.



[a]$P < .001$
[b]$P < .0001$

Figure 4. Effects of MOLE plus RSG on TNF-α in T2D rats.



[a]$P < .001$
[b]$P < .0001$

Figure 5. Effects of MOLE on the protein levels of the Akt/GSK3β signaling pathway in T2D rats

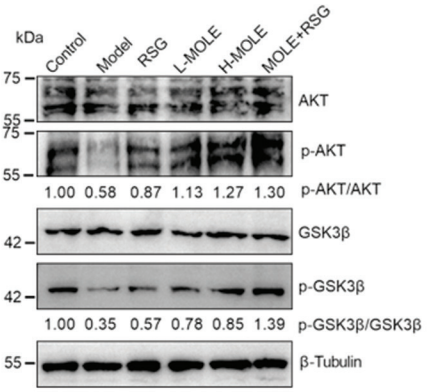
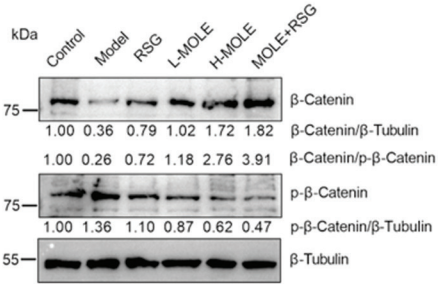


Figure 6. Effects of MOLE on the protein levels of the Wnt/β-Catenin signaling pathway in T2D rats



outperformed RSG in improving serum leptin and FFA in T2D rats, and the differences were significant (25.81 ± 4.72 ng/L for leptin; 258.26 ± 26.37 μmol/L for FFA) ($P < .001$ for leptin; $P < .001$ for FFA) (Figures 3A and 3B).

Effects of MOLE plus RSG on TNF-α levels in T2D rats

TNF-α is known to be in close association with plasma insulin, glucose metabolism, and lipid metabolism.[16] High levels of TNF-α can inhibit insulin function, induce hyperinsulinemia, and strengthen insulin resistance.[17] Therefore, this study also investigated the effects of MOLE plus RSG on TNF-α levels in T2D rats. As shown in Figure 4, the TNF-α level is significantly higher in the model group (1.53 ± 0.12 ng/mL) than in the control group (0.74 ± 0.23 ng/mL) ($P < .0001$). The TNF-α level is significantly reduced in the RSG (0.83 ± 0.09 ng/mL) ($P < .0001$), L-MOLE (0.71 ± 0.07 ng/mL) ($P < .0001$), and H-MOLE (1.53 ± 0.12 ng/mL) ($P < .0001$) groups as compared with the model group. In addition, compared with the RSG group, the TNF-α level is significantly reduced in the MOLE plus RSG group (0.61 ± 0.11 ng/mL) ($P < .001$).

Effects of MOLE plus RSG on the Akt/GSK3β signaling pathway in T2D rats

Numerous studies have proven that the Akt/GSK3β signaling pathway in the liver plays a role in glycogen synthesis and storage, while decreased GSK3β phosphorylation will impede glycogen synthesis and storage.[18] First of all, the p-Akt/Akt level in the liver was found to be decreased in the model group and significantly increased in the RSG, L-MOLE, and H-MOLE groups. Further, the increase in p-Akt/Akt was more significant in the MOLE+RSG group than in the RSG group (Figure 5). In the Akt/GSK3β signal pathway, GSK3β is regulated by Akt as a direct downstream molecule of Akt. In the model group, the p-GSK3β/GSK3β level was reduced with the p-Akt level. In the RSG, L-MOLE, and H-MOLE groups, p-GSK3β/GSK3β was increased in the T2D rat liver tissues. Compared with the RSG group, the MOLE+RSG group had a higher level of p-GSK3β/GSK3β (Figure 5). These findings suggest that MOLE plus RSG can significantly activate the Akt/GSK3β signaling pathway in T2D rats.

Effects of MOLE plus RSG on the Wnt/β-Catenin signaling pathway in T2D rats

This study also analyzed the effects of MOLE on β-Catenin and phosphorylated β-Catenin levels. The results showed that β-Catenin was reduced in the model group as compared with the control group; in contrast, β-Catenin in rat liver tissues was gradually increased in the RSG, L-MOLE, and H-MOLE groups as compared with the model group (Figure 6). In addition, p-β-Catenin was increased in the model group, while its level was gradually decreased in the rat liver tissues treated with RSG, L-MOLE, and H-MOLE. β-Catenin/p-β-Catenin was reduced in the model group as

compared with the control group. Contrarily, β-Catenin/p-β-Catenin was increased gradually in the RSG, L-MOLE, and H-MOLE groups as compared with the model group. However, β-Catenin and β-Catenin/p-β-Catenin were both increased significantly while p-β-Catenin was reduced significantly in the MOLE+RSG group as compared with the RSG group. These findings suggest that MOLE plus RSG can significantly activate the Wnt/β-Catenin signaling pathway in T2D rats.

DISCUSSION

Diabetes is a chronic metabolic disorder that can cause chronic complications of the heart and great vessels. Cardiovascular disease has become one of the most serious complications and leading causes of death in patients with diabetes mellitus.[19-21] An abnormal increase in blood glucose is the primary symptom of the disease. Moreover, lipid disorders often occur concomitantly with glucose metabolism disorders, remarkably increasing the risk of cardiovascular and cerebrovascular diseases.[19-23]

Among all available western medicines for T2DM, RSG is one of the most frequently used drugs in the clinical treatment of T2DM. It can increase receptor gamma through peroxisome proliferator activation and improve insulin sensitivity in tissues, thereby enhancing insulin resistance in the body.[24] However, a multitude of cases have shown that RSG produces a therapeutic effect less than ideal; further, prolonged use may lead to adverse drug reactions.[25-28] MOLE has extensive pharmacological effects and demonstrates significant lipid- and glucose-lowering, anticancer, hepato-protective, and antioxidant activities, especially in the prevention and control of diabetes mellitus.[29] The active components of Moringa oleifolia leaf (flavonoids, proteins, polysaccharides, etc.) show their anti-diabetic effects through three ways of action: (1) Acts on the pancreas by regulating the Akt signaling pathway, cAMP/PKA and PI3K/Akt signaling pathways and up-regulating the insulin receptor substrate 2 (IRS2) in the FoxO1 signaling pathway[30]; (2) To improve liver function and glucose metabolism by up-regulating PPAR-α and GLUT2 gene expression and down-regulating SREBP-1, PEPCK, and G6Pase gene expression[31]; (3) It acts on the intestine by inhibiting the activities of salivary α-amylase, pancreatic α-amylase, intestinal α-glucosidase, and sucrase, thereby inhibiting the decomposition of starch and the absorption of sugar by the intestine, and improving the intestinal function by increasing the diversity of flora and regulating the disturbance of flora.[32] Finally, through the above three ways of action to achieve the purpose of anti-diabetes.

The therapeutic effect of MOLE plus RSG in the treatment of T2DM is still unclear.[24-29] This study focused on the effects of MOLE plus RSG on glucose metabolism, blood lipids, serum leptin, FFA, and TNF-α in T2DM. Experiments in this study provide supportive evidence for the combined use of MOLE and RSG superior to the sole use of RSG. Many experiments have shown that MOLE can regulate glucose metabolism, repair damaged liver and pancreatic tissues, and modulate liver and kidney function in diabetic rats, thereby improving the blood levels of insulin, triglycerides, cholesterol, and malondialdehyde and ultimately achieving the goal of lowering blood glucose.[33-35] Some researchers argue that the anti-diabetic effect of Moringa oleifera is attributed to its active ingredients such as flavones, proteins, and polysaccharides, which can significantly reduce blood glucose levels in diabetic mice.[36,37] Therefore, future studies may investigate the key ingredients of MOLE and different hypoglycemic drugs to identify an ideal combination therapy. Reportedly, the AKT/GSK3β signaling pathway is involved in liver glycogen synthesis and storage, and decreased GSK3β phosphorylation can hinder glycogen synthesis and storage.[38,39] GSK3β serves as a key regulator in the Wnt/β-Catenin signaling pathway.[18,40] Given that MOLE is found to regulate the AKT/GSK3β signaling pathway, it is necessary to clarify its effect on the Wnt/β-Catenin signaling pathway. Moreover, our results showed that the combined use of MOLE and RSG has an advantage over RSG alone in activating the AKT/GSK3β/β-Catenin pathway.

CONCLUSION

MOLE plus RSG can reduce blood glucose and blood lipids in T2D rats and offer a reference for the clinical treatment of T2DM in the developing framework of integrated traditional Chinese and Western medicine.

AUTHOR DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

DATA AVAILABILITY

Please contact the corresponding author for a data request.

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AUTHOR CONTRIBUTIONS

CZ and JZ conceived the study. CZ performed the study. CS, HR, JL, and YS analyzed the data. YL performed animal feeding, sampling, and data recording. All authors drafted the article and approved the final version.

ETHICS STATEMENT

The animal experiment was approved by the Animal Care and Use Committee of Cangzhou Medical College (Approval No. [2022] 04).

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