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Original Research Article

Mogroside V attenuates gestational diabetes mellitus via SIRT1 pathway in a rat model

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Abstract

Purpose: *To investigate the function of mogroside V in gestational diabetes mellitus in a rat model.*
Methods: *Rats were divided into three groups: control rats (N = 6), rats with gestational diabetes mellitus (N = 6), and mogroside V-treated rats with gestational diabetes mellitus (GDM, N = 6). Rats in the gestational diabetes mellitus group were injected intraperitoneally with 35 mg/kg streptozotocin while rats in mogroside V group were orally gavaged with 100 mg/kg mogroside V for 20 days. Blood sugar, insulin, and leptin levels were measured using commercial kits. Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) levels were also assessed using commercial kits. Hematoxylin-eosin staining was used to determine morphological changes in placenta and pancreas.*
Results: *Streptozotocin injection significantly increased blood sugar, insulin, and leptin levels and elevated the body weight of both fetal and gestational diabetes mellitus rats ($p < 0.01$). The serum levels of TG, TC and HDL also increased in rats with gestational diabetes mellitus. However, mogroside V treatment reduced blood sugar, insulin, and leptin levels, and lowered the serum levels of TG, TC and HDL. Streptozotocin injection induced morphological damage in placenta and pancreas, but mogroside V administration ameliorated the pathologic damage. Additionally, mogroside V administration attenuated the streptozotocin injection-induced decrease in SIRT1 levels in the rats.*
Conclusion: *Mogroside V alleviates pathological damage in the placenta and pancreas of rats with gestational diabetes mellitus by down-regulating SIRT1, indicating the potential of mogroside V for managing gestational diabetes mellitus.*

Keywords: *Mogroside V, Placenta, Pancreas, Gestational diabetes mellitus, SIRT1*

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INTRODUCTION

Pregnant women often develop hyperinsulinemia and insulin resistance, and 10% of pregnant women with pancreatic dysfunction develop gestational diabetes mellitus [1]. Gestational diabetes mellitus is characterized by glucose intolerance in pregnant women, leading to gestational hypertension, pre-eclampsia, and liver or kidney damage [2]. Additionally, the

adverse outcomes of gestational diabetes mellitus include fetal macrosomia, stillbirth, and children at a higher risk of developing type 2 diabetes [3]. Elucidating the underlying mechanism in gestational diabetes mellitus may facilitate the development of therapeutic drugs for its effective prevention.

The hypoglycemic pharmacology of *Siraitia grosvenorii*, a traditional Chinese medicine, has attracted extensive attention in type 2 diabetes mellitus [4]. Increasing evidence has uncovered the pharmacological characteristics of mogroside V, the primary bioactive component of *S. grosvenorii*, such as anti-tussive, antioxidant, and anti-inflammatory effects [5]. In high-fat diet-treated mice and free fatty acid-treated LO2 cells, mogroside V regulated lipolysis and fatty acid oxidation to reduce hepatic steatosis [6]. Mogroside V restored insulin resistance and glucose metabolism in high glucose-induced HepG2 cells and attenuated the fasting blood glucose level, insulin sensitivity, and liver damage in type 2 diabetes mellitus rats [7]. However, the effect of mogroside V on gestational diabetes mellitus has not been reported to date.

In this study, a rat model of gestational diabetes mellitus was established, and then the effects of mogroside V on gestational diabetes mellitus symptoms, including hyperglycemia and tissue damage, were investigated.

EXPERIMENTAL

Animal model establishment

Experiments were approved by The Affiliated Hospital of Southwest Medical University (approval no. 2020831) and were performed in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [8]. Eighteen female Wistar rats were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The rats were divided into two groups: 6 in the control group and 12 in the gestational diabetes mellitus group. The rats in the control group were fed a basic diet, and those in the gestational diabetes mellitus group were fed a high-fat diet (10% sugar, 24.15% corn starch, 20% casein, 13.2% dextrin, 0.1% cholesterol, 5% cellulose, 22% lard, 3.5% minerals, 0.3% L-cysteine, 1% vitamins, 0.5% bile salts, and 0.25% choline chloride) for 8 weeks. The female rats were paired with male rats at a ratio of 2:1. Female rats were subjected to vaginal smears. Microscopic examination was used to detect sperm, and rats with sperm were labeled as pregnant rats. The pregnant rats in the gestational diabetes mellitus group were then injected intraperitoneally with 35 mg/kg streptozotocin (Sigma-Aldrich, St. Louis, MO, USA). The pregnant rats in the control group were subjected to buffer injection. Three days later, the fasting blood glucose level was measured, and rats with a blood glucose level greater than 13.8 mmol/L were included in the gestational diabetes mellitus group.

Mogroside V administration

Rats with gestational diabetes mellitus were divided into two groups: six in the gestational diabetes mellitus (GDM) with control group and six in the GDM with mogroside V group. Rats in the GDM group were gavaged with sterile saline once a day. Rats in the GDM with mogroside V group were orally gavaged with 100 mg/kg of mogroside V (Nanjing Plant Origin Biological Technology Co., Ltd; Nanjing, China) daily for 20 days.

Biochemical analysis

A top-loading balance (Thermo Fisher Scientific, Rockford, IL, USA) was used to detect the body weight. Rats were euthanized by carbon dioxide inhalation. Blood was collected and subjected to centrifugation to separate plasma for biochemical analysis. Blood sugar levels were measured using a glucometer (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Serum levels of insulin, leptin, TG, TC, and HDL were measured using commercial ELISA kits (Thermo Fisher Scientific).

Hematoxylin-eosin staining

The placenta and pancreas were isolated from the rats, fixed in 4% paraformaldehyde and embedded in paraffin. Next, the tissues were sliced into sections, which were then stained with hematoxylin and eosin (Sigma-Aldrich) before observation under a microscope (Olympus, Tokyo, Japan).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the islet tissues using the RNAeasy Mini Kit (QIAGEN, Gaithersburg, MD, USA) and then was reverse transcribed into cDNAs using the QuantiTect Reverse Transcription Kit (QIAGEN). The QuantiTect SYBR Green PCR Kit (QIAGEN) was used to detect the expression level of SIRT1 using the following conditions: 95 °C for 10 min, 40 cycles of 95 °C for 10 s and 60 °C for 60 s. The primers used are shown in Table 1.

Table 1: Primers used in PCR

Gene	Forward	Reverse
SIRT1	5'-TCACCACCAGATTCTTCAGTG-3'	5'-CCTCTTGATCATCTCCATCAGTC-3'
GAPDH	5'-AGCCACATCGCTCAGACAC-3'	5'-GCCCAATACGACCAAATCC-3'

Western blot assay

Islet tissues were homogenized in RIPA buffer (Shanghai Biocolor BioScience & Technology Co., Shanghai, China), and the bicinchoninic acid protein quantitative kit (Pierce, Rockford, IL, USA) was used to determine the protein concentration in the supernatant of islet tissues. Each sample (40 μg) was separated by SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane. The membrane was blocked in Tris-buffered saline-0.1% Tween-20 buffer containing 5% non-fat dry milk. Specific antibodies, anti-SIRT1 (1:3000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-GAPDH (1:4000; Santa Cruz Biotechnology), were used to probe the membrane. Horseradish peroxidase-conjugated secondary antibodies (1:5000; Kangcheng Inc., Shanghai, China) were incubated with the membrane, and samples were visualized using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA) with a bio-image analysis system (Bio-Rad, Baltimore, MD, USA).

Statistical analysis

The data from at least triplicate experiments are presented as mean ± standard deviation. Statistical analysis between two groups was analyzed using Student' s t test under GraphPad Prism 7 (GraphPad Inc., San Diego, CA, USA), and one-way analysis of variance was used to analyzed comparisons among multiple groups. A p value <0.05 was considered statistically significant.

RESULTS

Mogroside V attenuated diabetes mellitus symptoms in rats with gestational diabetes mellitus

The effects of mogroside V on gestational diabetes mellitus were investigated by evaluating diabetes mellitus symptoms. Streptozotocin injection significantly increased the blood sugar (Figure 1 A), insulin (Figure 1 B), and leptin (Figure 1C) levels in rats compared with those in the control. However, mogroside V administration restored the levels of blood sugar (Figure 1 A), insulin (Figure 1B), and leptin (Figure 1 C) to those of the control. Additionally, the streptozotocin-induced increase in the maternal (Figure 1 D) and fetal (Figure 1 E) body weight was reduced by mogroside V administration, suggesting that mogroside V attenuated diabetes mellitus symptoms in rats with gestational diabetes mellitus.

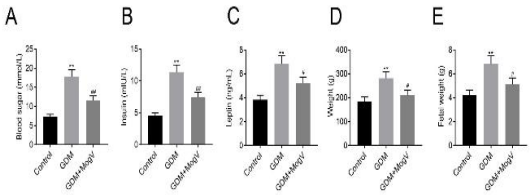

Figure 1: Effect of mogroside V on diabetes mellitus symptoms in rats with gestational diabetes mellitus. (A) Mogroside V administration attenuated the streptozotocin-induced increase in blood sugar levels. (B) Mogroside V administration attenuated the streptozotocin-induced increase in insulin. (C) Mogroside V administration attenuated the streptozotocin-induced increase in leptin. (D) Mogroside V administration attenuated the streptozotocin-induced increase in maternal body weight. (E) Mogroside V administration attenuated the streptozotocin-induced increase in fetal body weight. ##, ** $P < 0.01$. #, $p < 0.05$

Mogroside V suppressed lipid metabolism-related factors in rats with gestational diabetes mellitus

In addition to the diabetes mellitus symptoms, the effect of mogroside on lipid metabolism was also investigated. Levels of the lipid metabolism-related factors TG and TC were increased, while HDL levels were decreased in rats with gestational diabetes mellitus (Figure 2). Mogroside administration elevated HDL levels and reduced the levels of TG and TC (Figure 2), suggesting a protective effect against lipid metabolism in gestational diabetes mellitus.

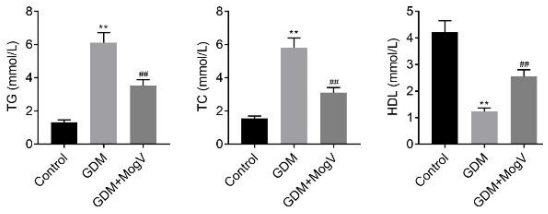

Figure 2: Mogroside V suppresses lipid metabolism-related factors in rats with gestational diabetes mellitus. Mogroside V administration attenuated the streptozotocin-induced decrease in HDL and increase in TG and TC. **, ##, $P < 0.01$

Mogroside V relieved placental and pancreatic tissue damage in rats with gestational diabetes mellitus

Hematoxylin-eosin staining was performed to evaluate tissue damage. The pathologic damage in streptozotocin-induced rats is shown in Figure 3. The placentas of rats with gestational diabetes mellitus were not clearly stratified, the cell distribution was loose and disordered, the intercellular space was enlarged, and capillary distribution was reduced (Figure 3). Additionally, in the pancreatic tissue of rats with gestational diabetes mellitus, the cells were disordered and lysed, and inflammatory cell infiltration, telangiectasia, hyperemia, and bleeding were visible (Figure 3). However, compared with the GDM group, the cell structure and morphology of the placental and pancreatic tissue of mogroside V-administered rats gradually became prominent, and the cell arrangement was improved (Figure 3). These results show that mogroside V ameliorated pathologic damage in placental and pancreatic tissues.

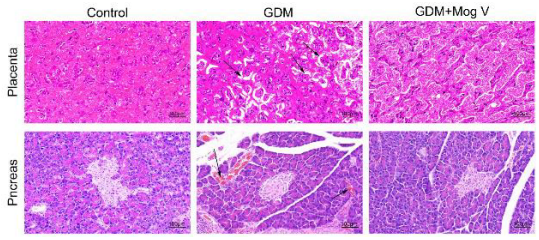

Figure 3: Mogroside V mitigates placental and pancreatic tissue damage in rats with gestational diabetes mellitus. Mogroside V administration attenuated streptozotocin-induced pathologic damage in placental and pancreatic tissues. Scale bars: 100 μm

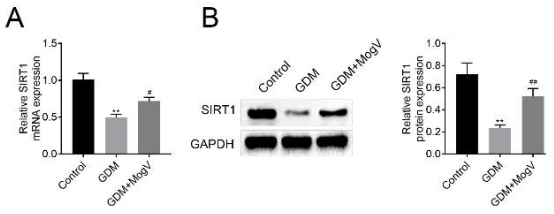

Figure 4: Mogroside V promotes SIRT1 in rats with gestational diabetes mellitus. (A) Mogroside V administration attenuates the streptozotocin-induced decrease in SIRT1 mRNA. (B) Mogroside V administration attenuated the streptozotocin-induced decrease in SIRT1 protein. **, ## $p < 0.01$

Mogroside V promoted SIRT1 in rats with gestational diabetes mellitus

The mRNA (Figure 4 A) and protein (Figure 4 B) expression of SIRT1 were down-regulated in the islet tissues of rats with gestational diabetes mellitus. Mogroside V administration attenuated the streptozotocin injection-induced decrease in SIRT1 (Figure 4 A and B), indicating that mogroside V promoted SIRT1 expression to alleviate gestational diabetes mellitus.

DISCUSSION

A previous study showed that mogroside derivatives from *S. grosvenorii* reduced blood glucose levels and exerted antidiabetic effects against type 2 diabetes mellitus rats by suppressing insulin resistance [7]. Additionally, metabolites of mogroside V are widely distributed in the plasma, bile, urine, and feces of rats, suggesting beneficial effects to treat type 2 diabetes mellitus [9]. The antidiabetic effect of mogroside V against gestational diabetes mellitus was investigated in this study.

Streptozotocin, an antibiotic that leads to pancreatic β-cell destruction, is widely used to establish gestational diabetic model rats in combination with a high-fat diet [10]. We showed that streptozotocin injection induced diabetes mellitus symptoms in rats, as demonstrated by enhanced blood sugar, insulin, and leptin levels and increased maternal and fetal body weight. Additionally, lipid metabolism disorders with reduced HDL levels and enhanced TC and TG levels were identified in streptozotocin-induced gestational diabetic rats, findings that were consistent with previous study findings [10]. The hypolipidemic effect of mogroside V was reported in high-fat diet-induced mice with down-regulation of lipogenesis and upregulation of lipolysis and fatty acid oxidation [6].

The present study showed that mogroside V administration attenuated the streptozotocin-induced decrease in HDL levels and increase in the TC and TG levels in rats. Therefore, mogroside V exerted hypoglycemic and hypolipidemic effects to ameliorate gestational diabetes mellitus. Additionally, the systematic pathologies in the placenta and pancreas of the maternal diabetes mellitus rats were ameliorated by mogroside V. These results indicate the protective effect of mogroside V against gestational diabetes mellitus. Inflammation is closely associated with a high risk of gestational diabetes mellitus, and suppression of the inflammatory response can ameliorate gestational diabetes mellitus symptoms [11]. Because mogroside V possesses anti-inflammatory activity in a murine ear edema model [12], the effect of mogroside V on gestational diabetes mellitus-associated

inflammation should be investigated in future research.

Activation of the PI3K/Akt pathway was implicated in the protective effect of mogroside V against type 2 diabetes mellitus [7]. The present study showed that mogroside V attenuated the streptozotocin-induced decrease in SIRT1, suggesting that mogroside V regulates SIRT1 to participate in the pathogenesis of gestational diabetes mellitus. SIRT1 expression is related to lipid metabolism, and SIRT1, a nutrient sensor, is considered a powerful regulator of cell metabolism [13]. Alterations in SIRT1 are likely associated with many pathological conditions, such as gestational diabetes mellitus [14].

Sirtuin 1 (SIRT1) enhances insulin sensitivity in insulin-sensitive tissues (liver, skeletal muscle, and adipose tissue) and induces insulin secretion from pancreatic beta cells [15]. Additionally, several studies have shown that SIRT1 downregulation is associated with impaired glucose tolerance, metabolic syndrome, and type 2 diabetes [16,17]. Mogroside V increases SIRT1 expression to reduce oxidative stress and alleviate the deterioration of oocyte quality [18]. The present study revealed that mogroside V administration enhanced SIRT1 expression in the islet tissues of rats with gestational diabetes mellitus. Therefore, mogroside V may ameliorate gestational diabetes mellitus by upregulating SIRT1.

CONCLUSION

Mogroside V administration improves glucose metabolism and ameliorates gestational diabetes mellitus symptoms by up-regulating SIRT1. Thus, mogroside V could be developed as an effective agent to treat gestational diabetes mellitus.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Teng Zhou and Xiaodong Fu designed the study and supervised the data collection. Hongyi Li analyzed and interpreted the data. Yan Yin prepared the manuscript for publication and reviewed the draft of the manuscript. All the authors have read and approved the manuscript.

REFERENCES

1. *Qiu M, Shangguan Z, Zhang H. Downregulation of miR-451a in plasma may promote prothrombin expression and contribute to high blood coagulation state in gestational diabetes mellitus. Trop J Pharm Res 2020; 19(10): 2079-2084.*
2. *Ankumah N-A, Tita ATN, Biggio JR, Harper LM. Pregnancy Outcomes in Women with 1-Hour Glucose Challenge Test ≥ 200 mg/dL. Am J Perinat 2016; 33(5): 490-494.*
3. *Muche AA, Olayemi OO, Gete YK. Effects of gestational diabetes mellitus on risk of adverse maternal outcomes: a prospective cohort study in Northwest Ethiopia. BMC Pregnancy Childbirth 2020; 20(1): 73.*
4. *Zhang Y, Zhou G, Peng Y, Wang M, Li X. Anti-hyperglycemic and anti-hyperlipidemic effects of a special fraction of Luohanguo extract on obese T2DM rats. J Ethnopharmacol 2020; 247: 112273.*
5. *Yang L, Chen Y, Liu Y, Xing Y, Miao C, Zhao Y, Chang X, Zhang Q. The Role of Oxidative Stress and Natural Antioxidants in Ovarian Aging. Front Pharmacol 2021; 11: 617843.*
6. *Lu Z-b, Liu S-h, Ou J-y, Cao H-h, Shi L-z, Liu D-y, Tian C-y, Zheng Y-r, Zhou H-l, Liu J-s. Forsythoside A inhibits adhesion and migration of monocytes to type II alveolar epithelial cells in lipopolysaccharide-induced acute lung injury through upregulating miR-124. Toxicol Appl Pharmacol 2020; 407: 115252.*
7. *Liu X, Zhang J, Li Y, Sun L, Xiao Y, Gao W, Zhang Z. Mogroside derivatives exert hypoglycemics effects by decreasing blood glucose level in HepG2 cells and alleviates insulin resistance in T2DM rats. J Funct Foods 2019; 63: 103566.*
8. *Health NIo. Guide for the care and use of laboratory animals: National Academies; 1985.*
9. *Zhou G, Zhang Y, Li Y, Wang M, Li X. The metabolism of a natural product mogroside V, in healthy and type 2 diabetic rats. J Chromatogr B Analyt Technol Biomed Life Sci 2018; 1079: 25-33.*
10. *Xu W, Tang M, Wang J, Wang L. Anti-inflammatory activities of puerarin in high-fat diet-fed rats with*

streptozotocin-induced gestational diabetes mellitus. Mol Biol Rep 2020; 47(10): 7537-7546.

11. *Zou C, Zhang Q, Zhang S. Mogroside IIIE attenuates gestational diabetes mellitus through activating of AMPK signaling pathway in mice. J Pharmacol Sci 2018; 138(3): 161-166.*
12. *Di R, Huang M-T, Ho C-T. Anti-inflammatory Activities of Mogrosides from Momordica grosvenori in Murine Macrophages and a Murine Ear Edema Model. J Agric Food Chem 2011; 59(13): 7474-7481.*
13. *Majeed Y, Halabi N, Madani AY, Engelke R, Bhagwat AM, Abdesselem H, Agha MV, Vakayil M, Courjaret R, Goswami N, et al. SIRT1 promotes lipid metabolism and mitochondrial biogenesis in adipocytes and coordinates adipogenesis by targeting key enzymatic pathways. Sci Rep 2021; 11(1): 8177.*
14. *Gui J, Potthast A, Rohrbach A, Borns K, Das AM, von Versen-Höynck F. Gestational diabetes induces alterations of sirtuins in fetal endothelial cells. Pediatr Res 2016; 79(5): 788-798.*
15. *Liang F, Kume S, Koya D. SIRT1 and insulin resistance. Nat Rev Endocrinol 2009; 5(7): 367-373.*
16. *Mac-Marcjanek K, Zieleniak A, Zurawska-Klis M, Cypryk K, Wozniak L, Wojcik M. Expression Profile of Diabetes-Related Genes Associated with Leukocyte Sirtuin 1 Overexpression in Gestational Diabetes. Int J Mol Sci 2018; 19(12): 3826.*
17. *Mishra JS, Zhao H, Hattis S, Kumar S. Elevated Glucose and Insulin Levels Decrease DHA Transfer across Human Trophoblasts via SIRT1-Dependent Mechanism. Nutrients 2020; 12(5): 1271.*
18. *Nie J, Sui L, Zhang H, Zhang H, Yan K, Yang X, Lu S, Lu K, Liang X. Mogroside V protects porcine oocytes from in vitro ageing by reducing oxidative stress through SIRT1 upregulation. Aging 2019; 11(19): 8362-8373.*