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Protective effect of saikosaponin D against gestational diabetes mellitus *via* regulating TLR4/MyD88/NF-κB and MAPK signaling pathway

Lu-Lu Wang[#], Chong Yang[#], Ye-Qing Su[✉], Fang Wang[✉]

Department of Gynecology and Obstetrics, Affiliated Hospital of Hebei University, No.212 Yuhua East Road, Baoding City, Hebei Province, 071000, China

ABSTRACT

Objective: To examine the protective effect of saikosaponin D against streptozotocin (STZ)-induced gestational diabetes mellitus in female rats.

Methods: Intraperitoneal administration of STZ (40 mg/kg) was used for the induction of diabetes in pregnant rats, and rats orally received sikosaponin D (10, 20, and 40 mg/kg). The body weight, placental weight, fetal weight, fetal index, and various biochemical parameters, including antioxidant, glucose level, cytokines, and apoptosis parameters, were estimated. The expression levels of various mRNAs were also analyzed.

Results: Saikosaponin D increased body weight and fetal weight while decreasing placental weight and placental index. Saikosaponin D significantly altered various biochemical parameters such as fasting blood glucose, glycated hemoglobin (HbA1c), hemoglobin, hepatic glycogen, advanced glycation end products, lipid parameters (total cholesterol, triglyceride, low density lipoprotein, high density lipoprotein, very low density lipoprotein), antioxidant parameters (superoxide dismutase, glutathione, glutathione peroxidase, malonaldehyde, catalase), inflammatory cytokines (tumor necrosis factor-α, interleukin-6, interleukin-1β, interleukin-10), apoptosis parameters (Bcl-2, Bax, caspase-3), resistin, adiponectin, leptin, intercellular adhesion molecule 1, vascular cell adhesion molecule-1, and monocyte chemotactic protein-1. Furthermore, saikosaponin D modulated the mRNA expression of *TLR4*, *MyD88*, *NF-κB*, *NLRP3*, *TNF-α*, *IL-6*, *CRP*, *SIRT1*, and *MAPK*.

Conclusions: Saikosaponin D exhibits a protective effect against STZ-induced gestational diabetes mellitus in rats *via* regulation of TLR4/MyD88/NF-κB and MAPK signaling pathways.

KEYWORDS: Saikosaponin D; Inflammation; Antioxidant; TLR4/MyD88/NF-κB; MAPK signaling pathway

1. Introduction

Gestational diabetes mellitus (GDM) is a complex metabolic disorder that affects a significant number of pregnancies worldwide. It is characterized by glucose intolerance that first appears or is diagnosed during pregnancy, most commonly in the second or third trimester[1,2]. Hyperglycemia during pregnancy, as seen in GDM, poses significant risks for both the mother and the developing fetus, potentially leading to numerous complications during pregnancy

Summary

Question: Does saikosaponin D effectively ameliorate streptozotocin-induced gestational diabetes mellitus disorder?

Findings: Saikosaponin D showed a protective effect against gestational diabetic mellitus by downregulation of the blood glucose level and body weight, along with modulating antioxidant status, inflammatory cytokines, inflammatory parameters, vascular protective, and apoptotic parameters. Additionally, it altered the mRNA expressions, suggesting the protective effect *via* modulation of TLR4/MyD88/NF-κB and MAPK signaling pathways.

Meaning: Saikosaponin D mitigates streptozotocin-induced gestational diabetes mellitus disorder *via* modulation of TLR4/MyD88/NF-κB and MAPK signaling pathways.

[✉]To whom correspondence may be addressed. E-mail: doctorsu810@outlook.com (YQ. Su); wf2014837@outlook.com (F. Wang).
[#]Both authors equally contributed to the article.

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and delivery. GDM is a multifactorial condition involving both increased insulin resistance and impaired insulin secretion. As the placenta develops and grows, it releases hormones that reduce the body's sensitivity to insulin, thereby increasing insulin resistance and controlling to enhance blood glucose levels[3,4]. In most cases, the pancreas compensates for enhanced insulin resistance during pregnancy by producing higher levels of insulin. However, in women who develop GDM, this compensatory response is inadequate, leading to persistent hyperglycemia. Inflammation and oxidative stress are recognized as the key contributors to the pathogenesis of GDM. These factors are closely associated with pancreatic β-cell dysfunction and can further exacerbate insulin resistance, disrupting glucose homeostasis during pregnancy. Consequently, both inflammation and oxidative stress play critical roles in the onset and progression of GDM, significantly impacting maternal health[5]. Disruption of metabolic homeostasis mediated by lipid peroxidation and an imbalance between prooxidant and antioxidants may initiate these pathological processes by dysregulated protein expression. The placenta, a vital organ for sustaining fetal life, is particularly susceptible to inflammation in the context of GDM. In a normal pregnancy, the inflammatory response is tightly regulated to maintain conditions favorable for fetal development. However, in GDM, this delicate balance is impaired, leading to an exaggerated inflammatory state that may compromise both fetal health and placental function[6]. GDM has been linked with the aberrant expression and function of placental nutrition transport proteins. The altered inflammatory conditions in the placenta, mother, and fetus during GDM can disrupt the activity of these proteins, potentially contributing to comorbidities linked with GDM pregnancies. Since inflammation reaction and oxidative stress significantly induce the onset of GDM and affect fetal development, a lot of therapeutic approaches aim to alleviate these underlying factors. By targeting oxidative stress and inflammation, researchers and healthcare professionals aim to suppress the detrimental effects of GDM on both maternal and fetal health[7].

The MAPK signalling pathway is the primary regulator of various cellular processes such as gene expression, differentiation, stress response, and proliferation. This pathway functions as a cascade of protein kinases that transmit signals from the cell surface to the nucleus, thereby influencing cellular behavior. In the context of GDM, the activation of specific MAPK subfamilies [including p38 MAPK and c-Jun *N*-terminal kinase (JNK)] has been implicated in the expansion of insulin resistance and impaired glucose regulation. These kinases can be activated by several stimuli, such as inflammatory cytokines, oxidative stress, and hyperglycemia, all of which are commonly observed in GDM[8]. GDM is linked with dysregulation of the MAPK signaling cascade. Overactivation of p38 MAPK and JNK can suppress insulin signaling *via* phosphorylation of insulin receptor substrate responses, thereby reducing peripheral insulin sensitivity. Prolonged activation of these kinases is also linked to pancreatic β-cell dysfunction and apoptosis, which can severely affect the glucose-regulating processes. Furthermore, MAPK signaling plays a crucial role in placentation and overall placental function. Its dysregulation has been associated with various pregnancy complications such as fetal macrosomia and preeclampsia, both of which are more common in GDM. Therefore, elucidation of the complex interactions between MAPK signaling pathways and GDM pathophysiology may offer valuable insights for the development of targeted therapies, ultimately improving maternal and neonatal health outcomes[9–11].

Bioactive compound saikosaponin D is derived from *Bupleuri radix*, also called Chai Hu in traditional Chinese medicine[12,13]. The herb is a key ingredient in shosaiko-to, an herbal formula commonly used in East Asian traditional medicine. *Bupleuri radix* is derived from the root of species of *Bupleurum*, its pharmacological profile has been extensively documented, with anti-inflammation, hepatoprotection, and immunomodulation[14,15]. Research indicates that saikosaponin D is associated with many biological activities, including anti-cancer, anti-viral, and anti-fibrotic effects[16,17]. It functions through cellular signaling pathway modulation, inflammatory mediator regulation, and gene expression[12,14,15,17]. The current study investigates the protective effect of saikosaponin D against streptozotocin (STZ)-induced GDM and explores the underlying mechanism.

2. Materials and methods

2.1. Main reagents and chemicals

STZ and saikosaponin D (95%) were purchased from Sigma Aldrich, USA. Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), NADH oxidase-2 (NOX-2), and monocyte chemotactic protein-1 (MCP-1) were estimated using the ELISA kits following the manufacturer's instructions (R&D system, USA). Advanced glycation end products (AGEs), glycated hemoglobin (HbA1c), Bax, Bcl-2, and caspase-3 were estimated using the ELISA kits following the manufacturer's instructions (Elabscicne, USA). The antioxidant parameters like catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), malonaldehyde (MDA); total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), high density lipoprotein (HDL) and very low density lipoprotein (VLDL); inflammatory cytokines like interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor-α (TNF-α) were estimated using the ELISA kit following the manufacturer's instructions (Abcam, USA).

2.2. Ethical approval

The study was approved by the Affiliated Hospital of Hebei University (HBU0711_MT1018).

2.3. Experimental rodent

Swiss Albino Wistar rats [weight (200±50) g, sex: both, aged 10-12 weeks] were used for this experimental study. The rats were kept in the single polyethylene cage in standard laboratory conditions [temperature (22±5)℃, relative humidity 60%-80%, and a 12/12 h dark/light cycle]. The rats received the standard pellet and water *ad libitum*.

2.4. Induction of diabetes

Intraperitoneal administration of STZ (40 mg/kg) was used for the induction of DM in the rats. STZ was prepared by dissolving it in the phosphate buffer (pH=4.5)[18,19].

2.5. Experimental protocol

The rats were randomly divided into the following groups:
Group Ⅰ: normal control (received 0.9% saline); Group Ⅱ: GDM; Group Ⅲ-Ⅴ: GDM+ saikosaponin D (10, 20, and 40 mg/kg), respectively[20,21].

The rats received the oral administration of saikosaponin D for the next 14 d[19]. Fasting blood glucose (FBG) levels and body weight were estimated at regular time intervals. At the end of the experimental study, the rats were sacrificed, and samples were collected to prepare the serum for further biochemical parameter estimation. The placental weight, placental index, and fetal weight of the experimental rats were scrutinized. The pancreas tissue was separated and employed for further analysis.

2.6. Biochemical parameters

The levels of AGEs and HbA1c, and apoptosis parameters, including Bax, Bcl-2, and caspase-3, were estimated using the ELISA kits following the manufacturer's instructions (Elabscicne, USA).

The antioxidant parameters like CAT, GSH, GPx, SOD, and MDA; lipid parameters like TC, LDL, TG, HDL, and VLDL; inflammatory cytokines like IL-1β, IL-6, IL-10, TNF-α were estimated using the ELISA kits following the manufacturer's instructions (Abcam, USA).

The levels of VCAM-1, ICAM-1, NOX-2, MCP-1, receptor for AGE (RAGE), and epidermal growth factor receptor (EGFR) were estimated using the ELISA kits following the manufacturer's instruction (R&D system, USA).

Resistin, adiponectin, leptin and hemoglobin (Hb) were estimated using the ELISA kits following the manufacturer's instructions (R&D system, USA).

2.7. Real-time polymerase chain reaction (RT-PCR)

For the determination of mRNA expression in hepatic tissue, total RNA was isolated using a commercial reagent, following the manufacturer's instructions (Invitrogen, CA, USA). One μg of total RNA was reverse transcribed into cDNA using the First Strand cDNA Synthesis Kit (Thermo, USA). The qRT-PCR was performed using the SYBR Green qPCR Master Mix Kit (Thermo, USA). Each reaction was performed in duplicate under the following conditions: 45 cycles at 95℃ for 10 s, 60℃ for 30 s, and 72℃ for 30 s, using the primer sequences listed in Supplementary Table 1. The expression levels of target genes were normalized to the reference gene *GAPDH*, and the results of the study were expressed as fold change relative to the control group.

2.8. Statistical analysis

The statistical analysis was performed using the GraphPad Prism software (Version 7). The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test. The results were shown as mean±standard deviation (SD). *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Body weight, fetal weight, placental weight, and placental index

GDM rats exhibited decreases in body weight (Figure 1A) and fetal weight (Figure 1B), along with an increase in placental weight (Figure 1C) and placental index (Figure 1D). Saikosaponin D significantly ($P<0.05$) improved body weight, and fetal weight while decreasing the placental weight and placental index, showing its potential protective effect.

3.2. FBG, HbA1c, Hb, hepatic glycogen, and AGEs

GDM rats exhibited significant increases in FBG (Figure 2A), HbA1c (Figure 2B), and AGEs (Figure 2E), as well as significant decreases in hepatic glycogen (Figure 2D) and Hb (Figure 2C). Saikosaponin D treatment significantly ($P<0.05$) modulated these levels.

3.3. Lipid parameters

The GDM group exhibited significant increases in levels of TC (Supplementary Figure 1A), LDL (Supplementary Figure 1B), VLDL (Supplementary Figure 1D), and TG (Supplementary Figure 1E), as well as significant decreases in HDL (Supplementary Figure 1C). Saikosaponin D treatment significantly (P<0.05) restored the level of lipid parameters.

3.4. Resistin, adiponectin, and leptin

GDM rats exhibited significant increases in resistin (Supplementary Figure 2A) and leptin (Supplementary Figure 2C), as well as significant decreases in adiponectin (Supplementary Figure 2B). Saikosaponin D treatment significantly (P<0.05) modulated the levels of these parameters.

3.5. Antioxidant parameters

GDM rats showed altered levels of SOD (Supplementary Figure 3A), GSH (Supplementary Figure 3B), GPx (Supplementary Figure 3C), MDA (supplementary Figure 3D), and CAT (Supplementary Figure 3E). Saikosaponin D treatment significantly (P<0.05) modulated the level of antioxidant parameters.

3.6. MCP–1, ICAM–1, and VCAM–1

GDM rats demonstrated elevated levels of MCP-1 (Figure 3A), ICAM-1 (Figure 3B), and VCAM-1 (Figure 3C). Saikosaponin D treatment significantly (P<0.05) suppressed these levels.

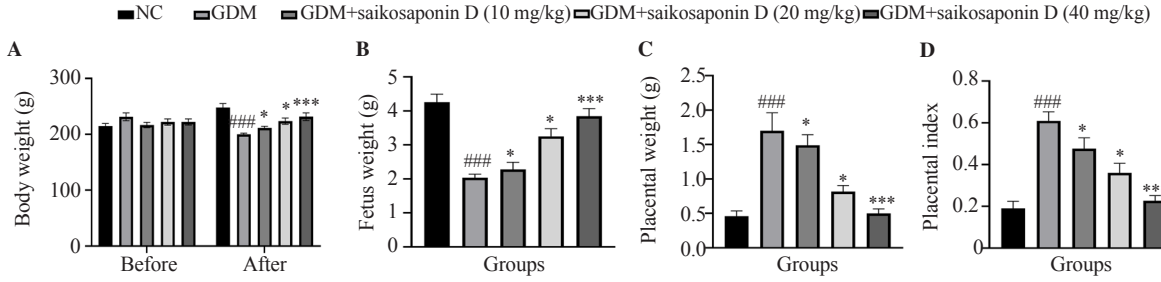

Figure 1. Effect of saikosaponin D on body weight, fetus weight, placental weight and placental index. A: body weight, B: fetus weight, C: placental weight, and D: placental index. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the gestational diabetes mellitus (GDM) group.

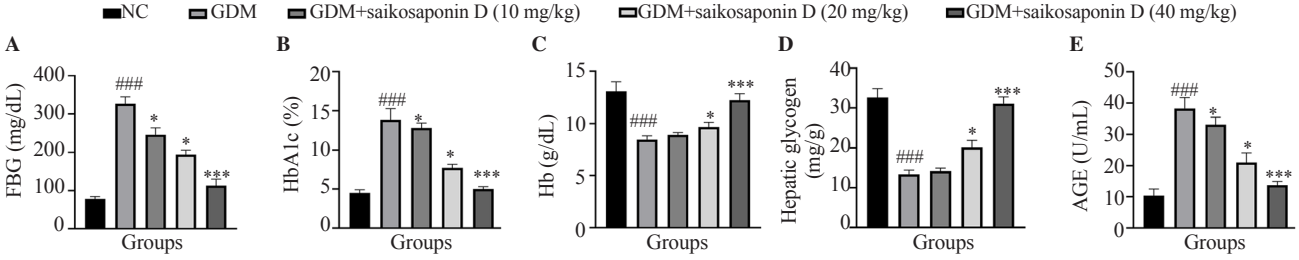

Figure 2. Effect of saikosaponin D on fasting blood glucose (FBG), glycated hemoglobin (HbA1c), hemoglobin (Hb), hepatic glycogen, and advanced glycation end products (AGEs). A: FBG, B: HbA1c, C: Hb, D: hepatic glycogen, and E: AGEs. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the GDM group.

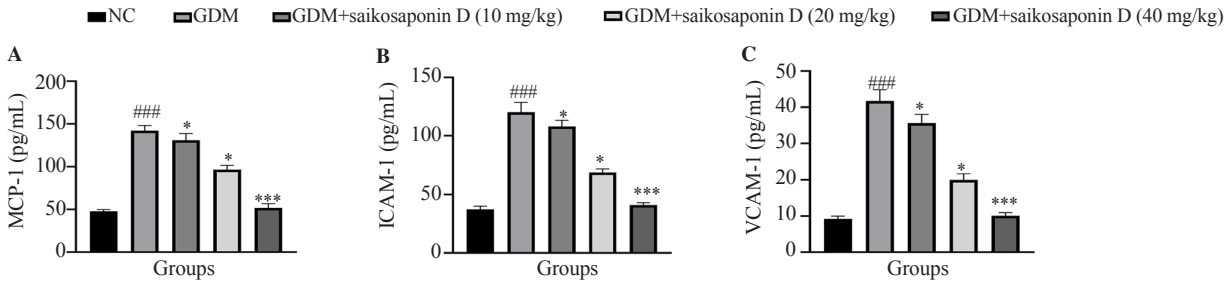

Figure 3. Effect of saikosaponin D on monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). A: MCP-1, B: ICAM-1, C: VCAM-1. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the GDM group.

3.7. RAGE, NOX-2, and EGFR

GDM rats demonstrated boosted levels of RAGE (Figure 4A), NOX-2 (Figure 4B), and EGFR (Figure 4C). Saikosaponin D treatment significantly (P<0.05) suppressed these levels.

3.8. Cytokines

GDM rats demonstrated elevated levels of TNF-α (Figure 5A), IL-1β (Figure 5B), and IL-6 (Figure 5C), and decreased IL-10 (Figure 5D). Saikosaponin D treatment significantly (P<0.05) restored these cytokine levels.

3.9. Apoptosis parameters

GDM rats showed elevated levels of Bax (Figure 6A) and caspase-3 (Figure 6B), and decreased Bcl-2 (Figure 6C). Saikosaponin D treatment significantly (P<0.05) restored these apoptosis-related parameters.

3.10. mRNA expression

GDM group rats demonstrated elevated mRNA expression levels of *TLR4* (Figure 7A), *MyD88* (Figure 7B), *NF-κB* (Figure 7C), *NLRP3* (Figure 7D), *TNF-α* (Figure 7E), *IL-6* (Figure 7F), *CRP* (Figure

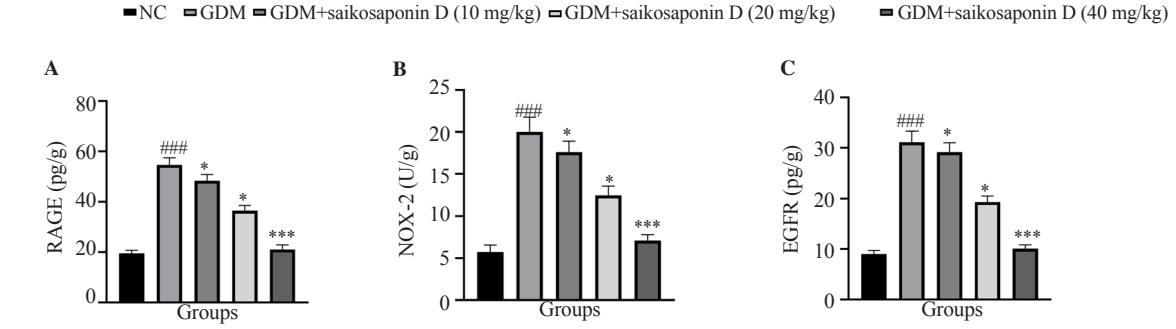

Figure 4. Effect of saikosaponin D on receptor for AGE (RAGE), NADH oxidase-2 (NOX-2), and epidermal growth factor receptor (EGFR). A: RAGE, B: NOX-2, C: EGFR. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the GDM group.

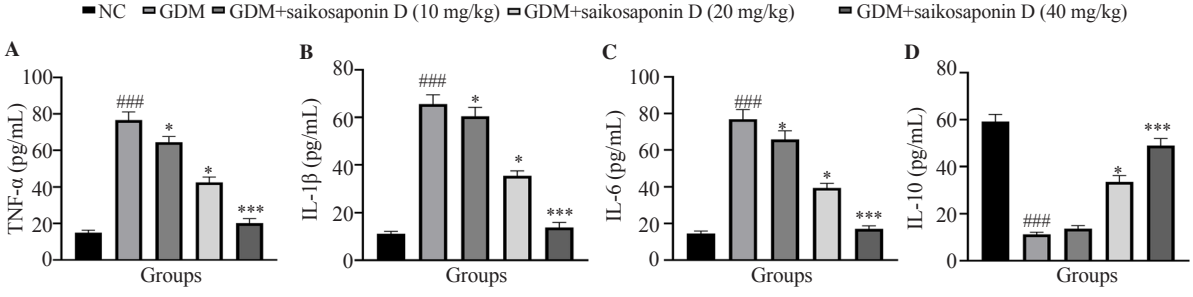

Figure 5. Effect of saikosaponin D on inflammatory cytokines. A: tumor necrosis factor-α (TNF-α), B: interleukin (IL)-1β, C: IL-6, and D: IL-10. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the GDM group.

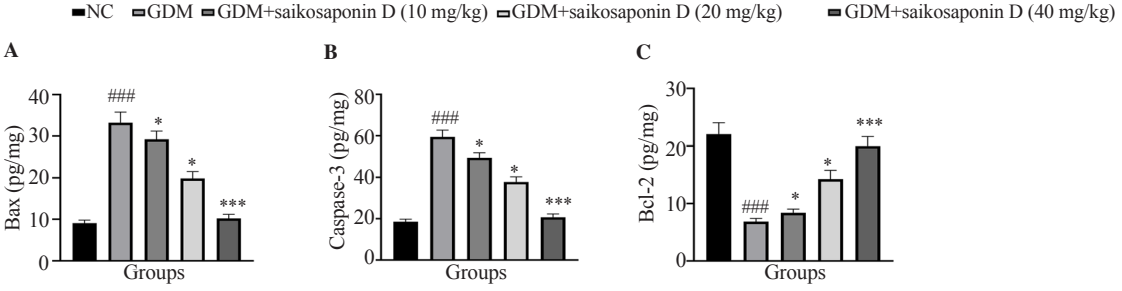

Figure 6. Effect of saikosaponin D on apoptosis parameters. A: Bax, B: Caspase-3, and C: Bcl-2. $^{###}P$<0.001 compared with the normal control; $^{*}P$<0.05, $^{***}P$<0.001 compared with the GDM group.

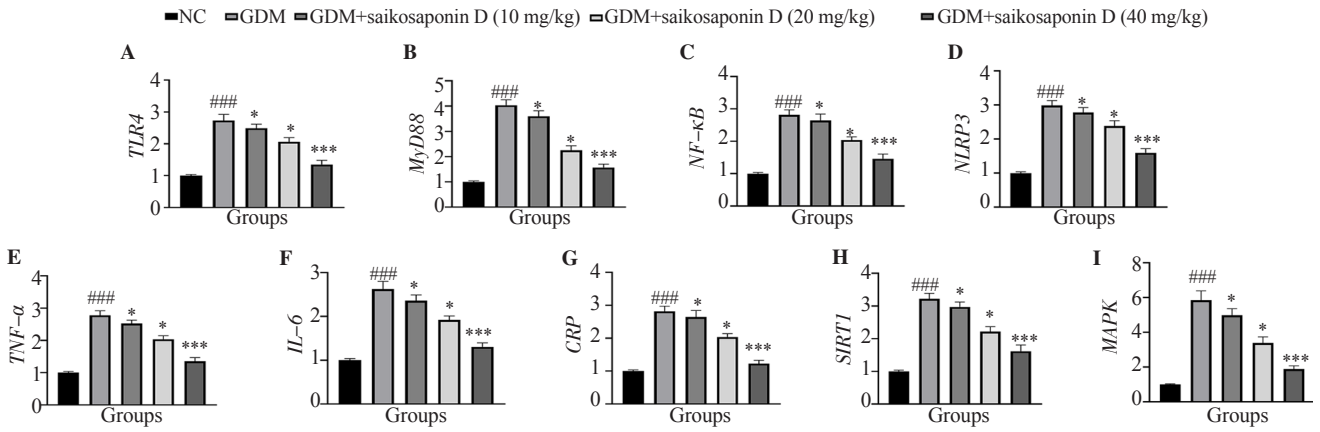

Figure 7. Effect of saikosaponin D on mRNA expression of relative genes. A: *TLR4*, B: *MyD88*, C: *NF–κB*, D: *NLRP3*, E: *TNF–α*, F: *IL–6*, G: *CRP*, H: *SIRT1*, I: *MAPK*. $^{###}P<0.001$ compared with the normal control; $^{*}P<0.05$, $^{***}P<0.001$ compared with the GDM Group.

7G), *SIRT1* (Figure 7H), and *MAPK* (Figure 7I). Saikosaponin D treatment significantly ($P<0.001$) decreased these mRNA expression levels.

4. Discussion

GDM is a complex metabolic disorder characterized by glucose intolerance that arises during pregnancy. It results from a disruption in the normal adaptations in insulin sensitivity, leading to decreased cellular responsiveness to insulin[22,23]. The pathophysiology of GDM is particularly complex, as it can develop without any preceding symptoms, making early detection and prevention challenging. The increasing incidence of GDM is strongly associated with two factors: the obesity epidemic and the increasing average maternal age at delivery. Both factors significantly contribute to an increased risk of insulin resistance during pregnancy[24,25]. This complexity necessitates further research to better understand the various risk factors contributing to the onset of GDM. Abnormal insulin secretion, coupled with progressive insulin resistance, develops a disrupted metabolic environment in affected patients, potentially leading to significant maternal and fetal health complications. These risks highlight the critical need for careful monitoring and effective treatment throughout pregnancy[3,26,27].

STZ is a potent diabetogenic agent that induces pancreatic β-cell damage *via* multiple mechanisms. The main mechanism involves DNA alkylation, wherein STZ modifies DNA bases, which further leads to breaks of the strand and genomic instability[28,29]. Moreover, STZ generates reactive oxygen species (ROS) and NO to exacerbate cellular damage. These cumulative effects activate apoptotic signaling pathways in pancreatic β-cells, ultimately resulting in their destruction and the progression of diabetic conditions[3].

The research results provide strong evidence for the role of AGEs in inducing oxidative stress in GDM. The increased level of MDA in the GDM rats shows the presence of oxidative stress. Moreover, the reduction in antioxidant enzymes involved in cellular redox homeostasis further corroborates this observation. AGEs inactivate GSH-related enzymes, leading to depleted circulating GSH levels, highlighting AGE accumulation as a cascade phenomenon that disrupts the antioxidant defense system[3,4,27]. The current study also investigates the complex interplay between GDM-related inflammatory reactions, AGEs, oxidative stress, and antioxidant mechanisms. Previous reports suggest that AGEs exert their biological effects *via* activation of NADP(H) oxidase, which may serve as a key trigger for the generation of ROS and reactive nitrogen species. Consequently, this leads to enhanced oxidative stress, as evidenced by elevated MDA levels. The concurrent reduction in antioxidant enzyme activities (GSH, SOD, and CAT) exacerbates oxidative imbalance, leading to a vicious cycle that could promote the onset and progression of GDM[3,5,30]. The significant positive association between urine AGEs and GDM suggests that targeting AGE formation or enhancing antioxidant defenses could represent novel approaches for the treatment of GDM and its complications.

AGEs interact with their receptor RAGE, triggering intracellular signaling pathways that lead to the secretion of inflammatory cytokines, NF-κB-dependent factors, and pro-atherogenic mediators[31,32]. In the GDM rat models, the upregulation of key proteins such as RAGE, p65, VCAM-1, MCP-1, EGFR, and NOX-2 has been observed, indicating an intensified inflammatory and oxidative stress response. The activation of the AGE-RAGE signaling pathway amplifies NF-κB activity, further enhancing the production and secretion of cytokines[27,33]. Furthermore, improved glycemic control has been associated with reduced AGE levels, suggesting a direct link between AGE formation and hyperglycemia. High levels of AGEs hurt fetal development in GDM, underscoring

the long-term implications[3,5,27,30].

The interaction between insulin resistance and inflammatory cytokines plays a crucial role in the expansion of GDM. This state of low-grade inflammation during pregnancy can have widespread effects, not only increasing the risk of complications like preeclampsia (high blood pressure in pregnancy), premature labor, and polyhydramnios (too much amniotic fluid), but it also affects fetal development[27,34]. Although the precise mechanisms linking inflammation to GDM are still unclear, pathological inflammatory responses are believed to contribute to insulin resistance, impaired glucose tolerance, and other metabolic abnormalities associated with GDM[3,27,35]. The complex relationship between inflammation and metabolic dysfunction underscores the importance of early diagnosis and intervention. Prompt identification and treatment of GDM in its early stages enable healthcare professionals to conduct interventions that mitigate inflammatory responses and enhance insulin sensitivity. This proactive approach has been shown to greatly decrease the risk of complications for both mother and fetus, highlighting the critical role of comprehensive prenatal care and continuous monitoring in GDM treatment[3,4,30].

NF-κB is an essential transcription factor that regulates the inflammatory response in various physiological and pathological processes. It regulates the expression of multiple genes associated with immune response, inflammation, proliferation, and cell survival. Upon activation, NF-κB translocates to the nucleus, where it interacts with specific DNA sequences, driving the transcription of target genes. This activation can be triggered by a broad spectrum of stimuli, including cytokines, pathogens, and environmental stressors[4,5,27,30]. NF-κB also mediates the production and secretion of key pro-inflammatory cytokines. Consequently, these cytokines can further activate NF-κB, inducing the inflammatory response. Additionally, NF-κB regulates the expression of adhesion molecules, chemokines, and enzymes involved in inflammatory processes, reinforcing its central role in the complex interplay of inflammatory mediators. It is an important step toward targeted therapy to modulate inflammatory responses in diseases by understanding in depth the mechanisms involving NF-κB activation and regulation[3,4,31].

Numerous studies have shown a strong relationship between the inflammatory response and insulin resistance. Inflammatory cytokines can suppress the insulin signal transduction by preventing the tyrosine phosphorylation of IRS-1. TLR4 plays a crucial role in initiating an inflammatory response by activating the NF-κB pathway, which subsequently promotes the release of pro-inflammatory mediators. In trophoblast cultures, TLR4 activation perpetuates inflammatory signaling through the adaptor protein MyD88, which leads to the MyD88-dependent activation of NF-κB and the expression of the inflammatory cytokines such as TNF-α[34]. However, recent well-designed studies have highlighted the therapeutic potential of traditional Chinese medicines against inflammatory response and GDM[3,26,27]. Inhibition of TLR4/MyD88/NF-κB signaling may contribute to the anti-inflammatory effects of saikosaponin D. Thus, saikosaponin D may represent a promising candidate for reducing inflammation and improving metabolic outcomes in GDM.

MAPKs are a family of protein kinases that form intricate signaling networks activated by different physiological and pathological stimuli, including cytokines, environmental stressors, and growth factors. During the GDM, MAPK pathways play a crucial role in altering insulin signaling, inflammatory responses, and glucose homeostasis[36]. The 3 major subfamilies of MAPKs, such as JNK, p38 MAPKs, and extracellular signal-regulated kinases, each have distinct functions in the pathophysiology of GDM. Dysregulated of these MAPK pathways in GDM has been shown to contribute to insulin resistance in peripheral tissue and to impair pancreatic β-cell function. Chronic activation of JNK and p38 MAPKs has been associated with exacerbated insulin resistance, particularly in skeletal muscle and adipose tissue. Moreover, these pathways can also amplify the inflammatory response by enhancing the secretion of pro-inflammatory cytokines, thereby worsening the metabolic abnormalities characteristic of GDM. Activation of MAPK pathways can also be mediated by oxidative stress, with excessive ROS activating MAPK pathways and generating a vicious cycle leading to cellular damage and dysfunction[37,38]. Despite the promising findings regarding the protective effects of saikosaponin D, especially over a short treatment duration, further validation in clinical settings is essential to assess its translational relevance. In this study, we explore the protective effect of saikosaponin D in short duration, long-term safety, efficacy, and potential side effects during pregnancy remain unexplored. Therefore, this study investigates the key inflammatory and metabolic pathways, which still need to explore the molecular mechanisms, such as interactions with placental function, insulin signaling, and more.

Saikosaponin D treatment reduced blood glucose levels and improved body weight. It also altered the level of lipid parameters, hepatic glycogen, oxidative stress, inflammatory cytokines, inflammatory parameters, and apoptosis parameters. Saikosaponin D modulated the mRNA expression of *TLR4*, *MyD88*, *NF-κB*, *NLRP3*, *TNF-α*, *IL-6*, *CRP*, *SIRT1*, and *MAPK*, suggesting the protective effect against GDM *via* alteration of TLR4/MyD88/NF-κB and MAPK signaling pathways.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

This study received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

LLW and CY performed the experimental study. YQS and FW designed the experimental study. All the authors interpreted and drafted the manuscript.

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Publisher's note

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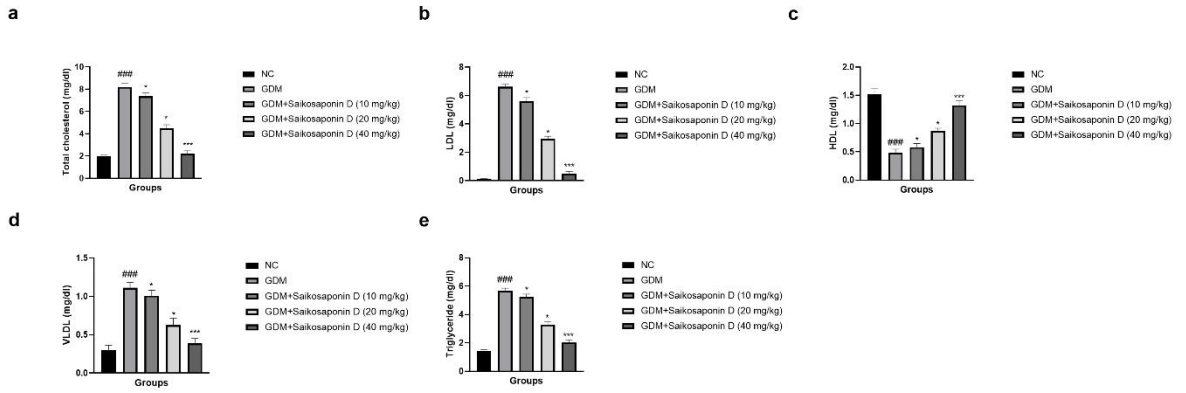
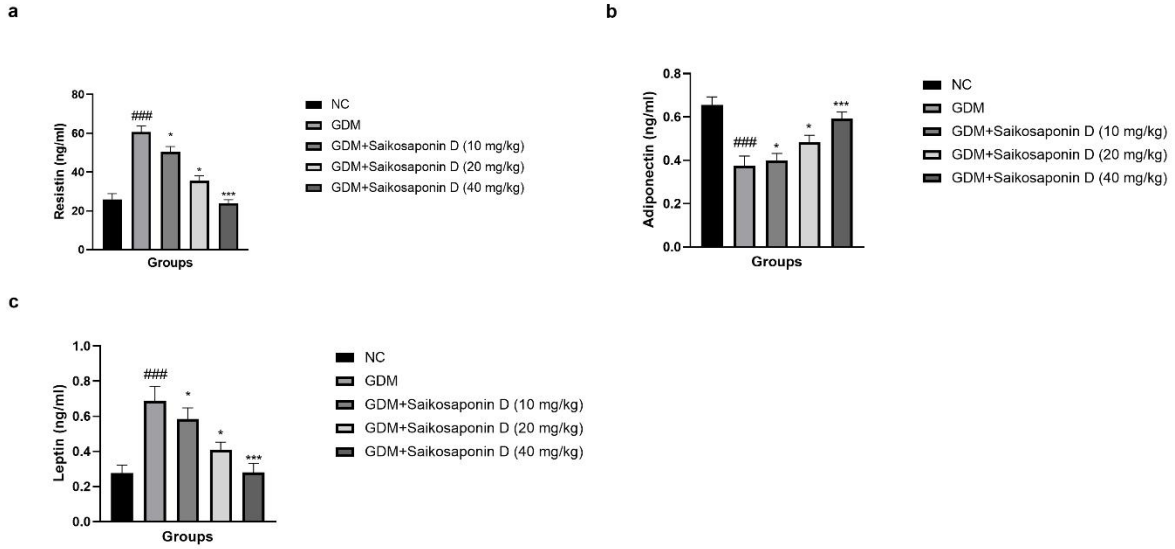
Supplementary Table 1. List of primers.

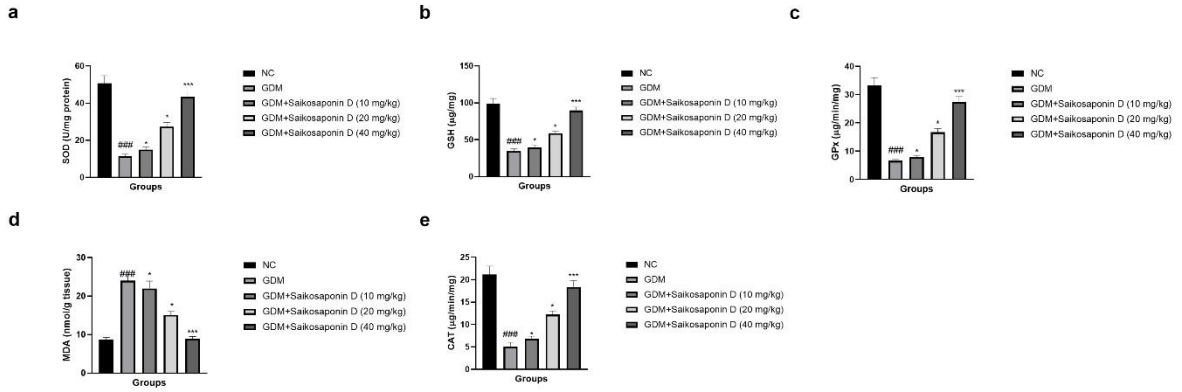
S. No	Gene	Reverse	Forward
1	*SIRT1*	CCTCTTGATCATCTCCATCAGTC	TCACCACCAGATTCTTCAGTG
2	*NF-κB*	CTGGTCCCGTGAAATACACC	CCCATCTTTGACAATCGTGC
3	*NLRP3*	CGCAGATCACACTCCTCAAA	TACGGCCGTCTACGTCTTCT
4	*TLR4*	GGTGGCTTAGGCTCTGATATGC	CTGCAGGTGCTGGATTTATCC
5	*MyD88*	CTCCTGCTGCTGCTTCAAGAT	ACTGCTCGAGCTGCTTACCAA
6	*TNF-α*	ATGATCTGAGTGTGAGGGTCTG G	GCTCTTCTGTCTACTGAACTTCG G
7	*IL-6*	AGCTTATCTGTTAGGAGACCAT TG	GTCCTTCAGAGAGATACAGAAA CT
8	*CRP*	TCAGAGCAGTGTAGAAATGGA GA	TTCCCAAGGAGTCAGATACTTCC
9	*p38 MAPK*	GTCAGATGGCAAGGGTTC	TTCCCAGCAGTCCTATCC
10	*GAPDH*	GATGGTGATGGGTTTCCCGT	AGTGCCAGCCTCGTCTCATA



Supplementary Figure 1. Effect of saikosaponin D on the total cholesterol, LDL, HDL, VLDL and triglyceride of normal and gestational diabetes mellitus (GDM) group of rats. **a:** total cholesterol, **b:** LDL, **c:** HDL, **d:** VLDL and **e:** triglyceride. $^{###}P<0.001$ compared with the normal control; $*P<0.05$, $***P<0.001$ compared with the GDM group.



Supplementary Figure 2. Effect of saikosaponin D on the resistin, adiponectin and leptin of normal and GDM group of rats. **a:** resistin, **b:** adiponectin and **c:** leptin. $^{###}P<0.001$ compared with the normal control; $^{*}P<0.05$, $^{***}P<0.001$ compared with the GDM group.



Supplementary Figure 3. Effect of saikosaponin D on the antioxidant parameters of normal and GDM group of rats. **a:** SOD, **b:** GSH, **c:** GPx, **d:** MDA and **e:** CAT. $^{###}P<0.001$ compared with the normal control; $^{*}P<0.05$, $^{***}P<0.001$ compared with the GDM group.