Original Article

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Shenling Guchang prescription ameliorates intestinal barrier inflammation in gestational diabetes rats *via* TLR4/NF-κB pathway

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SUMMARY Gestational diabetes mellitus (GDM) is linked to a greater risk of various maternal and fetal complications, including the possibility of long-term metabolic issues in offspring. Our initial research suggests that the Traditional Chinese Medicine formula, Shenling Guchang prescription (SLGP), may have an impact on the gut microbiota. However, the specific mechanisms through which it affects intestinal barrier inflammation in GDM are still not fully understood. This study explored SLGP's mechanisms in GDM. Firstly, network pharmacology predicted key bioactive constituents targeting toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF-κB), guiding experimental design. Subsequently, the pregnant female rats were induced with GDM through intraperitoneal streptozotocin injection and then divided into control, model, metformin, and SLGP treatment groups. Blood samples were collected for ELISA analysis to measure levels of inflammatory markers, intestinal tissues were examined histologically using hematoxylin-eosin (HE) staining, and western blot analysis was conducted to evaluate TLR4 and NF-κB expression. Relative to control rats, model group animals exhibited significant increases in the levels of inflammatory markers (IL-1β, IL-6, TNF-α, TGF-β, CRP), as well as enhanced TLR4 and p-NF-κB p65 expression, along with intestinal histopathological changes. Treatment with SLGP notably reduced inflammatory markers and protein expression in the colonic tissue of GDM rats, leading to a decrease in histopathological damage. Overall, SLGP was found to modulate the TLR4/NF-κB pathway, resulting in enhancements in insulin resistance and a reduction in inflammatory responses in GDM rats, thereby providing protection for the intestines. This study demonstrates the potential therapeutic effectiveness of SLGP in addressing intestinal inflammation linked to GDM.

Keywords Gestational diabetes mellitus, intestinal inflammation, inflammatory marker, toll-like receptor 4, nuclear factor-kappa B

1. Introduction

Obesity is a pressing global health issue that has fueled a rise in gestational diabetes mellitus (GDM) incidence, which now impacts approximately 4-16.5% of pregnant women globally (*1*), exposing both the mother and the fetus to health risks that can persist even after the perinatal period ends (*2-4*). The precise causes of GDM are incompletely understood, but key drivers of this condition include inflammation, oxidative stress, and insulin resistance (*5,6*). The gut microbiome, a complex assembly of diverse microorganisms in the gastrointestinal tract, plays a central role in shaping host immune activity and metabolic functionality (*7,8*). Recent studies suggest that gut dysbiosis are linked to GDM incidence (*9,10*). The disruption of normal microbial homeostasis within the gut can lead to the production of a variety of signaling molecules and metabolites that can modulate the function of the intestinal barrier (*11,12*). Such disturbances can be linked to an increase in gut permeability such that lipopolysaccharide (LPS) and other compounds can access the bloodstream and trigger

TLR4/MyD88/NF-κB pathway-mediated inflammation (*13*). The consequent oxidative stress and systemic inflammation can lead to the onset of insulin resistance associated with GDM (*14*).

At present, the outcomes associated with common treatments for GDM such as exercise, dietary changes, and pharmacological interventions remain highly variable (*15*). There is thus a clear need to devise new treatment options that are more efficacious. Traditional Chinese medicine (TCM) has been established as a promising approach to the treatment of GDM, although the mechanisms through which TCM prescriptions exert their effects are incompletely understood, particularly as they pertain to the TLR4/NF-κB pathway (*16*).

This study was developed to assess the impact of Shenling Guchang prescription (SLGP), a TCM formula, a traditional Chinese medicine formula has been used to treat various gastrointestinal diseases in clinical practice, and its potential benefits in controlling GDM. To deeply understand SLGP's therapeutic potential, network pharmacology was first utilized to predict its active components and relevant targets. Following this, a GDM rats model was implemented to explore the mechanisms of action. By merging network pharmacology with vivo experimentation, the study aimed to clarify SLGP's role in GDM's pathophysiological processes.

2. Materials and Methods

2.1. Network pharmacology

2.1.1. Collection of chemical composition and target prediction of SLGP

The chemical composition and target prediction of SLGP were conducted using Chinese traditional medicine. Active ingredients from Pseudostellaria heterophylla, Atractylodes Macrocephalae Koidz, Poria cocos, Coicis Semen, Dioscorea opposita Thunb, Crataegi Folium, Puerariae Lobatae Radix, Agrimoniae Herba, and Glycyrrhizae Radix et Rhizoma were retrieved based on criteria from the Traditional Chinese Medicine Database and Analysis Platform (TCMSP, *https://tcmsp-e.com/*), specifically targeting compounds with oral bioavailability (OB) ≥ 30% and medicinal likeness (DL) ≥ 0.18. After sorting, Uniprot (*https://www.uniprot.org/*) data were used to standardize gene names by removing non-human genes and eliminating duplicate targets.

2.1.2. Acquisition of GDM-related targets

Targets associated with Gestational Diabetes Mellitus (GDM) were obtained by que-rying GeneCards (*https://www.genecards.org/*) and OMIM (*https://www.omim.org/*) databases using the keyword "GDM". Integrated database entries were scrutinized in Excel to remove duplicate genes and validate gene information against the Uniprot database.

2.1.3. Drug-disease target prediction results

Drug component targets and disease targets were mapped, and a Venn diagram was created to identify intersecting genes. The Drug-Ingredient-Target network was sub-sequently constructed using Cytoscape 3.7.2 software.

2.1.4. Construction of target protein interaction network

To investigate the protein-protein interactions of SLGP in treating GDM, drug-intersecting genes were uploaded to the STRING database (*https://string-db.org/*) to construct a Protein-Protein Interaction (PPI) network. Species specificity was set to "Homo sapiens", and a minimum interaction score of 0.7 was applied to ensure study credibility. Results were exported in TSV format and analyzed in Cytoscape 3.7.2. Node size and color reflected Degree centrality, while edge thickness indicated CombineScore, highlighting core targets in the network diagram.

2.1.5. GO enrichment analysis and KEGG pathway analysis

Intersection genes were imported into the Drug_Disease.txt file, and their symbols were converted to EntrezIDs using the org.Hs.eg.db package in RStudio. GO enrichment and KEGG pathway analyses were performed using the clusterProfiler package, with human species specified and significance threshold set at $P < 0.05$. The top 10 results were visualized using the ggplot2 package, elucidating the role of SLGP target proteins in GDM treatment across Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF). Additionally, KEGG pathway enrichment analysis provided further insights into the therapeutic targets of SLGP in GDM treatment.

2.2. Animals

In total, 62 specific-pathogen-free (SPF) healthy female Sprague-Dawley (SD) rats (8 weeks old, 200 ± 20 g) and 36 SPF healthy male SD rats of a similar age and weight were purchased from Changsha Tianqin Biotechnology Co., Ltd. (experimental animal qualification certificate number SCXK (Xiang) 2021-0005). These rats were individually housed at the Animal Experiment Center of Guizhou University of Chinese Medicine with free food and water access under controlled conditions (22 ± 1°C, 12 h light/dark cycle). The Guizhou University of Chinese Medicine approved all animal studies, and efforts were made to minimize animal suffering wherever possible.

2.3. Pregnant rat preparation

After allowing 1 week for acclimatization, the 62 female rats were fasted overnight with free access to water, followed by analyses of their blood glucose levels, with levels < 6.1 mmol/L being sufficient for inclusion in this study. The 62 enrolled rats that met these criteria were allowed to acclimate for an additional week, after which they were co-housed for one week with male rats at a 1.5:1 ratio. In the morning, rats were examined for vaginal plugs, with the day that a plug was observed was set as day 0.5 of pregnancy. Blood glucose was measured on day 0.5 to exclude any potential for pre-gestational diabetes. After one week, any rats who did not conceive were excluded from this analysis. In total, 48 rats successfully became pregnant.

2.4. GDM modeling and treatment

The 48 pregnant female rats were further subdivided at random into 6 groups (n = 8/group), including a blank control group in which rats received a normal diet. The remaining 40 rats underwent GDM modeling by administering a high-fat/high-sugar diet (59% regular feed, 18% lard, 20% sugar, and 3% egg yolk; Ke Ao Xie Li, Beijing, China) for 5 days. After this 5-day period, all pregnant rats underwent overnight fasting with free access to water, followed by the measurement of their body weight and fasting blood glucose (*17*). Rats in the modeling groups were then administered freshly prepared STZ (35 mg/kg; Solarbio, Beijing, China, batch number is S8050-100mg) once per day for 3 days, whereas the rats in the blank control group were injected with a similar volume of vehicle control (0.1 mmol/L sodium citrate buffer). At 24, 48, and 72 h post-administration, fasting blood glucose levels in these rats were analyzed, with modeling being considered successful if the rats exhibited blood glucose levels above 11.1 mmol/L on three consecutive days. Body weights were again measured after successful modeling.

The 40 rats that had undergone GDM modeling were further divided with a random number table into model, metformin, and SLGP treatment (low-, medium-, or high-dose) groups. Per the "Laboratory Animals" dosage conversions, a dose equivalent to the daily dose for a 60 kg adult was calculated. SLGP was obtained from The Intelligent Granule Pharmacy of the Second Affiliated Hospital of Guizhou University of Chinese Medicine, and consisted of 15 g Pseudostellaria heterophylla, 15 g Atractylodes Macrocephalae Koidz, 15 g Poria cocos, 20 g Coicis Semen, 30 g Atractylodis Dioscorea opposita Thunb, 15 g Crataegi Folium, 15 g Puerariae Lobatae Radix, 15 g Agrimoniae Herba, and 6 g Glycyrrhizae Radix et Rhizoma (Table 1). Square granules from Sichuan New Green Medicine Industry (Batch numbers: 20110141, 21080031, 21080103, 21060067, 21050095, 21040030, 20080222, 21020023, 20080229, and 21070040) were administered *via* gavage to rats in the low-, medium-, and high-dose treatment groups at 4.5, 9, and 18 mg/kg. Metformin (500 mg/tablet; Tianfang Pharmaceutical, Xi'an, China) was obtained from the Western Medicine Pharmacy of the same hospital and was administered to rats in the appropriate group at 52.5 mg/kg *via* gavage. Rats in the model and blank groups instead received an equal volume of 0.9% saline. Treatment was repeated once per day for 2 weeks, after which all rats were euthanized following food and water deprivation for 12 h. Blood glucose and body weight values were measured for rats in each group, after which samples of blood and colon tissue were harvested for analysis.

2.5. ELISAs

After allowing blood samples to stand for 30 min at room temperature, they were centrifuged (15 min, 2,000 rpm; radius: 5 cm), and serum was then stored at -80°C. Serum levels of insulin (Elabscience, Wuhan, Hubei, China), C-reactive protein (CRP, eBioscience, Wuhan, Hubei, China), IL-1β, IL-6 (MultiSciences, Shanghai, China), lipopolysaccharide (LPS, MSKBIO, Wuhan, China), TGF-β, tumor necrosis factor-α (TNF-α), TLR-4 (Bioswamp, Wuhan, Hubei, China), and phospho-NF-κB p65 (MSKBIO, Wuhan, Hubei, China).

2.6. Histopathological staining

Samples of colon tissue from the same location in each rate were fixed for 48 h in 4% paraformaldehyde,

Table 1. Composition of SLGP

English name	Chinese name	Content (g)	Main components
Pseudostellaria heterophylla	Taizishen	15	Polysaccharides, amino acids, trace elements
Atractylodes Macrocephalae Koidz	Baizhu	15	Starch, triterpenoids, flavonoids
Poria cocos	Fulin	20	Lignans, polysaccharides, alkaloids
Coicis Semen	Yiyiren	20	Polysaccharides, proteins, amino acids
Dioscorea opposita Thunb	Shanyao	30	Starch, glycoproteins, saponins
Crataegi Folium	Shanzhaye	15	Flavonoids, triterpenoids, sterols
Puerariae Lobatae Radix	Gegeng	15	Saponins, flavonoids, polysaccharides
Agrimoniae Herba	Xianhecao	15	Alkaloids, flavonoids, tannins
Glycyrrhizae Radix et Rhizoma	Gancao	6	Glycyrrhizin, flavonoids, polysaccharides

paraffin-embedded, and cut into 4-6 μm sections. These sections were then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) solution (Servare,Wuhan,Hubei,China) as directed, followed by imaging with a microscope.

2.7. Western immunoblotting

Colon tissue samples were collected from the same location in rats from each group and stored at -80°C for analysis. RIPA buffer (Kangwei Century,Qingdao,China) was used to homogenize these colon tissue samples, which were then centrifuged (5 min, 15,000 rpm), and supernatants were collected. After measuring the total protein content in these supernatants, 30 μg per sample was separated *via* SDS-PAGE and transferred to a PVDF membrane (Millipore,Shanghai,China) with a semi-dry transfer technique. Blocked membranes were incubated overnight with primary antibodies specific for TLR4 (Bioswamp), p-NF-κB p65 (CST, Shanghai, China), and GAPDH (Abcam,Shanghai,China) at 4°C. After a 1 h incubation with secondary antibodies (Kangwei Century), a chemiluminescent substrate was used for membrane development. ImageJ was used for densitometric analyses of protein bands, with GAPDH as a loading control.

2.8. Statistical analysis

GraphPad Prism 8.0 and SPSS 19.0 were used to analyze all data, which are reported as means ± standard deviations (SD). Results were compared with one-way ANOVAs and multiple comparisons testing as appropriate. $P < 0.05$ was selected as the cut-off to define significance.

3. Results

3.1. Acquisition of active compounds and its targets in SLGP

Following removal of ineffective components and criteria of OB ≥ 30% and DL ≥ 0.18, the following components were collected: 6 from Pseudostellariae Radix, 5 from Radix Pseudostellariae, 4 from Radix Pseudostellariae, 6 from Hawthorn Leaf, 88 from Licorice, 6 from Poria Poria, 12 from Yam, 4 from Pueraria Root, and 9 from Coix Seed. In total, 122 ingredients were identified from all pharmaceutical ingredients. Collecting all disease targets of these 122 active compounds, a components-targets network was constructed. The top five components identified were MOL000098 (quercetin), MOL000422 (kaempferol), MOL000006 (luteolin), MOL000449 (stigmasterol), and MOL000392 (formononetin).

3.2. PPI network analysis of core targets

Using a Relevance Score > 5 as the criterion for inclusion from GeneCards, 2,636 targets were included, and an additional 522 targets were obtained from the OMIM database. After deduplication, 3,056 GDM-related targets were identified. Intersection of GDM target genes and drug target genes yielded 134 overlapping genes, representing potential interaction targets for GDM treatment (Figure 1A). After intersection of all drug and GDM targets, 134 overlapping genes were identified and analyzed using the String database (*https://string-db.org/*) for protein-protein interaction prediction in Homo sapiens, with a confidence threshold of 0.7. The resulting network file was saved in TSV format and imported into Cytoscape 3.7.2 to construct a protein interaction network. Nodes with Degree > 5 were selected for topology analysis, revealing 104 nodes and 2230 edges. The top 20 target genes including *IL6*, *TP53*, *STAT3*, *AKT1*, *IL1B*, *TNF*, *EGFR*, *JUN*, *CASP3*, *MMP9*, *PTGS2*, *MYC*, *BCL2*, *IL10*, *CXCL8*, *HIF1A*, *ESR1*, *MAPK3*, *FOS*, and *STAT1* were screened using the cytohuba plug-in (Table 2, Figure 1B).

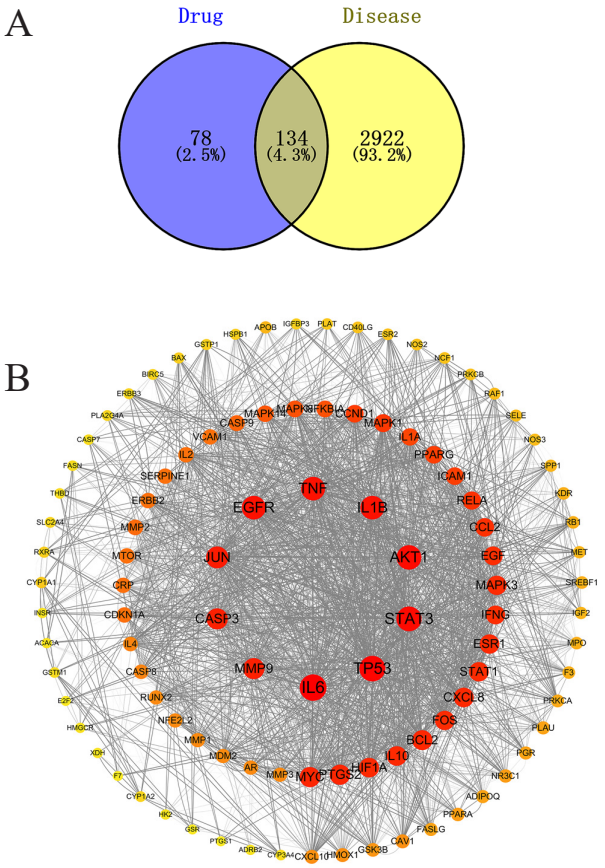

Figure 1. Components and targets analysis of SLGP in treating GDM. (A) The core regulatory genes of SLGP in treating GDM. (B) PPI network showed the protein relationship between SLGP and GDM. The network was re-edited by Cytoscape. The node's color is marked from red to yellow according to the degree value in descending order.

Table 2. Top 20 target genes of SLGP

Rank	Gene	Protein name	Score
1	*IL6*	Interleukin-6	63
2	*TP53*	Cellular tumor antigen p53	60
3	*STAT3*	Signal transducer and activator of transcription 3	57
4	*AKT1*	RAC-alpha serine/threonine-protein kinase	56
5	*IL1B*	Interleukin-1 beta	53
6	*TNF*	Tumor necrosis factor	53
7	*EGFR*	Epidermal growth factor receptor	53
8	*JUN*	Jun proto-oncogene	47
9	*CASP3*	Caspase-3	44
10	*MMP9*	Matrix metalloproteinase-9	44
11	*PTGS2*	Prostaglandin G/H synthase 2	41
12	*MYC*	Myelocytomatosis oncogene	41
13	*BCL2*	B-cell lymphoma-2	40
14	*IL10*	Interleukin-10	39
15	*CXCL8*	C-X-C motif chemokine ligand 8	39
16	*HIF1A*	Hypoxia-inducible factor 1-alpha	39
17	*ESR1*	Estrogen receptor	38
18	*MAPK3*	Mitogen-activated protein kinase 3	38
19	*FOS*	Proto-oncogene c-Fos	38
20	*STAT1*	Signal transducer and activator of transcription 1	38

3.3. Biological function enrichment analysis

3.3.1. GO enrichment analysis

ClusterProfiler package identified 2,742 enriched GO terms, including 2473 bio-logical processes (BP), 78 cellular components (CC), and 191 molecular functions (MF). Top 10 GO terms for BP, CC, and MF were plotted. BP involved responses to lipopolysaccharides, chemical stress, nutrient levels, reactive oxygen species metabolism, drug responses, oxidative stress, muscle cell proliferation, and cellular response to oxidative stress. CC encompassed membrane rafts, plasma membrane rafts, vesicular lumen, and transcriptional regulatory complexes. MF included nuclear receptor activity, ligand-activated transcription factor activity, DNA-bindintranscription factor binding, and cytokine receptor binding, *etc*. (Figure 2A).

3.3.2. KEGG pathway enrichment analysis

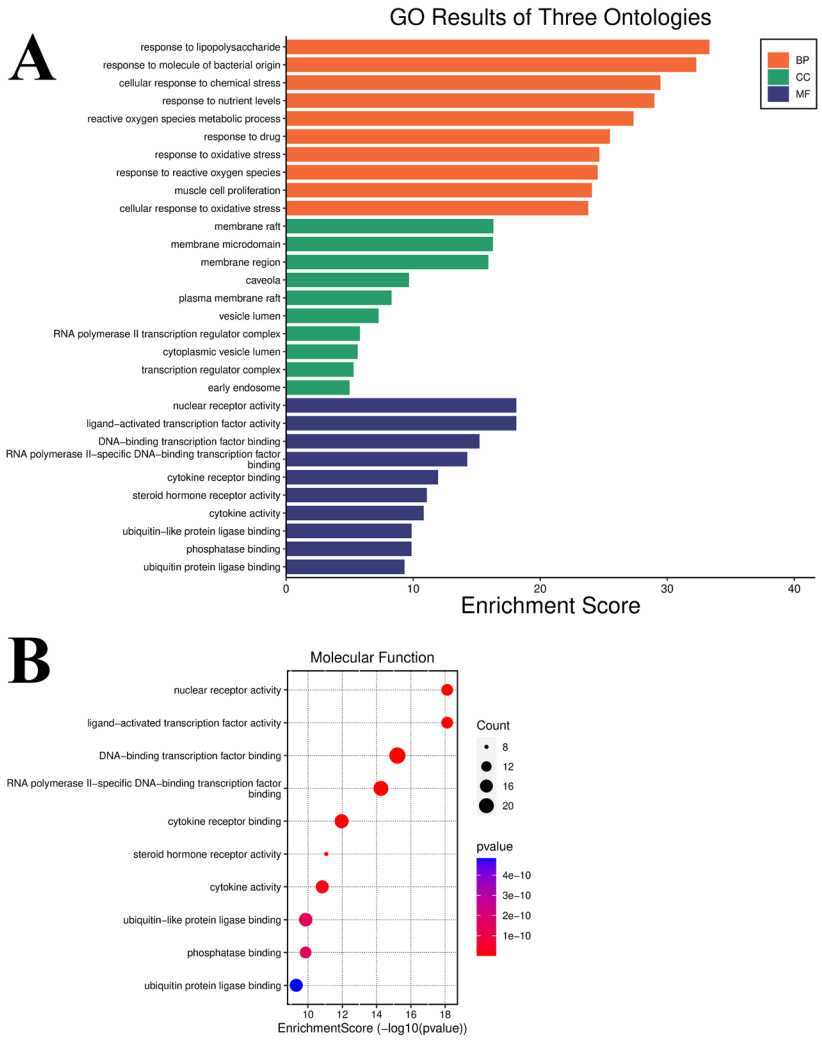

Figure 2. Enrichment analysis of SLGP on GDM. (A) The biological process, cellular component, and molecular function of GO analysis were shown. **(B)** KEGG pathway analysis showed the top 20 enrichment pathways.

KEGG Pathway analysis identified 179 pathways relevant to GDM treatment, with the top 20 pathways selected for mapping. Pathways included lipid and atherosclerosis, AGE-RAGE signaling pathway in complications of diabetes, hepatitis B, fluid shear stress and atherosclerosis, prostate cancer, and others, suggesting potential regulatory mechanisms of Chinese herbal compounds, *etc*. (Figure 2B).

3.4. SLGP treatment improves glucose metabolism in GDM model rats

GDM model rats were gavaged with SLGP (4.5, 9, or 18 mg/kg) or metformin (52.5 mg/kg), while blank and model group rats were instead gavaged with an equal volume of 0.9% saline once per day for two weeks. Relative to blank controls, GDM model groups exhibited significantly elevated insulin levels ($P < 0.01$). Compared with the model group, high-dose SLGP treatment significantly lowered insulin levels in these experimental rats (Table 3, $P < 0.05$).

3.5. SLGP suppresses intestinal barrier-related inflammatory factor production

Relative to blank controls, significant increases in CRP, IL-6, IL-1β, TGF-β, TNF-α, and LPS levels were detected in the model group ($P < 0.05$). SLGP treatment significantly reduced the levels of these inflammatory factors relative to the model group ($P < 0.05$), and similar reductions were observed in the metformin group, albeit without any significant differences in TGF-β and TNF-α levels (Table 4).

Table 3. The impact of SLGP on glycometabolic activity ($\bar{x} \pm s$, $n = 8$)

Groups	*n*	INS/pg·mL^{-1}
Normal	8	115.89 ± 48.66**
GDM	8	239.96 ± 47.97
Low-dose SLGP	8	193.65 ± 93.82
Medium-dose SLGP	8	224.47 ± 55.61
High-dose SLGP	8	137.30 ± 55.26*
Metformin group	8	205.16 ± 20.96

$^{*}P < 0.05$ *vs.* GDM; $^{**}P < 0.01$ *vs.* GDM.

3.6. SLGP modulates serum TLR4 and p-NF-κB p65 levels in rats

Significantly elevated TLR4 and p-NF-κB p65 levels were detected in the model group as compared to the blank group ($P < 0.05$), while these levels were significantly reduced relative to model rats in serum samples from the medium-dose SLGP group (Table 5, $P < 0.05$).

3.7. SLGP treatment alters intestinal barrier histopathology in GDM model rats

Control group rats exhibited a colonic structure that was intact without any apparent evidence of inflammatory cell infiltration or necrosis. Model group rats presented with degenerative changes in the mucosal layer, a congested and edematous submucosal layer, a thinner muscle layer, an enlarged intestinal lumen, and some degree of crypt loss. For rats in the low-, medium-, and high-dose SLGP treatment groups, mucosal degeneration and submucosal congestion and edema were still evident, but the degree of inflammatory cell infiltration was reduced as compared to that observed for GDM model rats. Mucosal and submucosal necrosis were evident in the metformin group, together with mild edema, congestion, and inflammatory cell infiltration (Figure 3).

3.8. SLGP affects colon TLR4, p-NF-κB p65 protein expression

Western immunoblotting revealed significantly elevated TLR4 and p-NF-κB p65 protein levels in the model group as compared to the blank group ($P < 0.05$), while these levels were significantly reduced relative to model rats in colon samples from the medium- and high-dose SLGP groups and the metformin group (Figure 4, $P < 0.05$).

4. Discussion

GDM is a metabolic disorder that frequently arises during pregnancy, exposing both the mother and fetus to substantial health risks. Women diagnosed with GDM also face an elevated risk of subsequently developing postpartum diabetes and cardiovascular

Table 4. The impact of SLGP on inflammatory factors related to the intestinal barrier in rats ($\bar{x} \pm s$, $n = 8$)

Groups	*n*	CRP/ng·mL^{-1}	IL-6/pg·mL^{-1}	IL-1β/pg·mL^{-1}	TGF-β/pg·L^{-1}	TNF-α/ng·L^{-1}	LPS/ng·L^{-1}
Normal	8	262.45 ± 17.87**	42.55 ± 16.71**	75.88 ± 37.77**	30.81 ± 11.46**	159.37 ± 43.08**	37.89 ± 5.97**
GDM	8	326.25 ± 31.81	88.57 ± 11.55	217.48 ± 41.73	55.24 ± 19.38	254.33 ± 84.96	58.26 ± 11.56
Low-dose SLGP	8	284.36 ± 45.45*	30.79 ± 22.69**	139.34 ± 62.27*	31.78 ± 13.44*	210.62 ± 59.41	42.41 ± 11.24**
Medium-dose SLGP	8	267.09 ± 24.45**	24.46 ± 14.25**	130.06 ± 72.32*	35.034 ± 14.09*	163.27 ± 23.93**	43.58 ± 6.33**
High-dose SLGP	8	274.50 ± 40.09**	39.26 ± 15.18**	114.63 ± 41.36**	32.03 ± 56.68*	168.20 ± 24.85*	43.99 ± 7.41**
Metformin	8	271.175 ± 20.96**	34.59 ± 23.36**	141.07 ± 58.72*	38.47 ± 19.60	211.25 ± 58.21	38.68 ± 4.49**

$^{*}P < 0.05$ *vs.* GDM; $^{**}P < 0.01$ *vs.* GDM.

Table 5. The impact of SLGP on signaling pathway molecules in GDM model rats ($\bar{x} \pm s$, $n = 8$)

Groups	*n*	TLR4/ng·mL^{-1}	p-NF-κBp65/pg·mL^{-1}
Normal	8	0.88 ± 0.10*	177.15 ± 70.85*
GDM	8	1.15 ± 0.08	267.23 ± 82.00
Low-dose SLGP	8	0.95 ± 0.26	227.50 ± 32.08
Medium-dose SLGP	8	0.84 ± 0.19**	183.39 ± 54.98*
High-dose SLGP	8	0.99 ± 0.19	223.90 ± 28.37
Metformin	8	1.27 ± 0.17	296.95 ± 77.15

$^{*}P < 0.05$ *vs.* GDM; $^{**}P < 0.01$ *vs.* GDM.

diseases, while their infants are also more likely to develop complications including macrosomia, neonatal hypoglycemia, and respiratory distress syndrome (*18,19*), and they may also develop long-term health issues including childhood obesity and cardiovascular diseases when they reach adulthood (*20,21*). Insulin resistance is a hallmark of GDM and a key driver of the pathogenesis of this condition (*22*). A variety of complex interactions between environmental, inflammatory, and genetic factors underlie GDM development (*23,24*). The gut

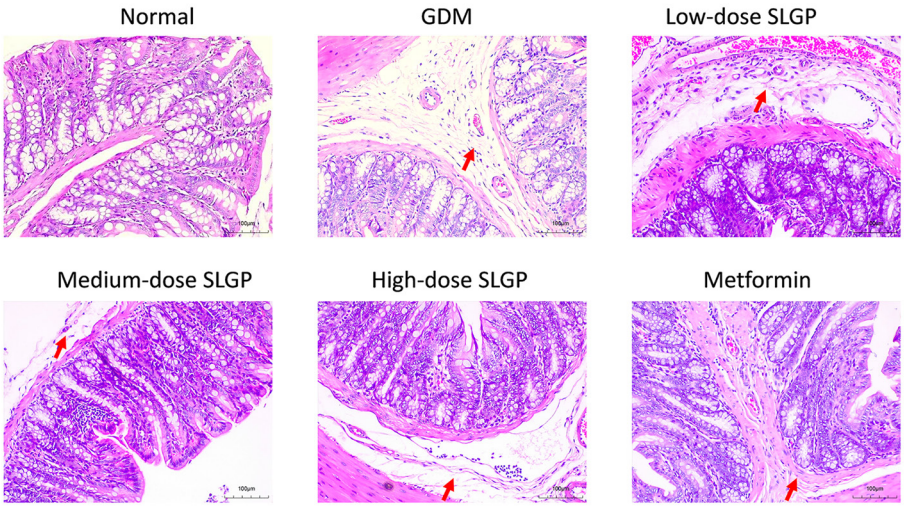

Figure 3. The impact of SLGP on colon tissue pathology in rats (H&E, 100×).

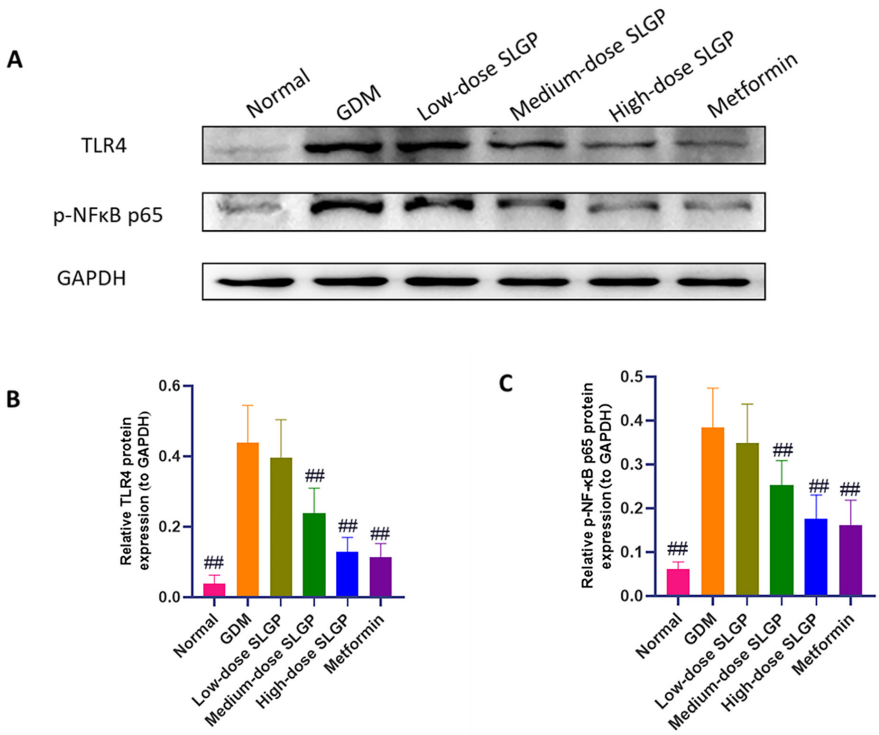

Figure 4. Colon tissue TLR4 and p-NF-κB p65 protein levels in different groups as detected by Western immunoblotting. Data are means ± SD and were compared with one-way ANOVAs and Tukey's post hoc test. $^{\#\#}P < 0.05$ *vs.* GDM group. **(A)** TLR4, p-NF-κB p65, and GAPDH were detected by Western immunoblotting. **(B, C)** Statistical analyses of TLR4/GAPDH **(B)** and p-NF-κB p65/GAPDH **(C)** levels.

microbiome and dysfunction of the intestinal barrier layer have both been demonstrated to be closely associated with GDM onset (*25,26*). Moreover, hyperglycemia has been shown to exert detrimental effects on the intestinal barrier function. The intestinal barrier is crucial for maintaining homeostasis and preventing the translocation of potentially harmful substances from the gut lumen into the circulation. Under high glucose conditions, the intestinal epithelial cells can undergo changes that compromise the integrity of tight junctions and adherens junctions, leading to increased intestinal permeability (*27*). Furthermore, hyperglycemia has been associated with an increase in the risk of enteric infection by compromising the intestinal barrier's ability to control microbial translocation. This was demonstrated in a study where hyperglycemia was shown to drive intestinal barrier dysfunction and risk for enteric infection, potentially leading to a vicious cycle of inflammation and further impairment of barrier function (*28*). Under physiological conditions, the intestinal barrier functions as a key boundary interface between the host and the lumen of the gut which is vital to the maintenance of systemic homeostasis (*29*).

Our network pharmacology analysis precisely identified a select group of target genes pivotal to inflammatory pathways in GDM, including *IL6*, *TNF*, and *IL1β*, represents the cornerstone of the inflammatory response, with well-established roles in immune regulation and pro-inflammatory cytokine production. Furthermore, GDM has been proposed to be related to intestinal mucosal damage that leads to LPS leakage into systemic circulation as a consequence of greater intestinal permeability (*30-32*). This, in turn, triggers inflammatory signaling mediated by TLR4 and other pattern recognition receptors, driving NF-κB pathway activation that culminates in pro-inflammatory cytokine production and the onset and/or exacerbation of insulin resistance (*33,34*).

The gut microflora comprises a complex microecological system that is integral to the control of host immunity and metabolic function. Intestinal dysbiosis has been linked to GDM in the past. Gut microbiota disruptions can affect metabolite and signaling molecule production, thereby compromising the integrity of the intestinal barrier and giving rise to systemic inflammatory activity (*35,36*). The restoration of gut microbiota homeostasis may thus be an effective and novel approach to GDM management (*37-40*).

In this study, a rat model of GDM was employed to study how SLGP affects intestinal barrier inflammation and TLR4/NF-κB pathway signaling. In prior studies, we have found that SLGP offers promise owing to its ability to modulate the composition of the gut microbiome and to improve glycometabolic activity in GDM model rats, possibly through its effects on TLR4/NF-κB signaling induced by LPS. In this study, the precise underlying mechanisms whereby SLGP can treat GDM were explored at length, focusing in particular on TLR4/NF-κB signaling. These analyses ultimately suggest that SLGP can inhibit the stimulatory effects of LPS, thereby helping to reduce inflammation and insulin resistance. Together, these findings suggest that SLGP is capable of enhancing the degree of glycemic control in GDM model rats through the alleviation of inflammation and insulin resistance.

These results suggest that SLGP treatment can strengthen the intestinal barrier in GDM model rats, as evidenced by observed histopathological improvements in the colon tissues from these SLGP-treated rats. These improvements in barrier integrity may help mitigate LPS translocation across the compromised barrier interface. Consistent with such a model, TLR4 and p-NF-κB p65 protein levels were reduced by SLGP treatment with a concomitant reduction in inflammatory factor expression consistent with the suppression of the inflammatory response.

While this study underscores the promising therapeutic utility of SLGP as an approach to GDM management, there are certain limitations to this therapeutic strategy. Firstly, there is a lack of research on the effect of STZ on intestinal barrier, so it is not clear whether STZ has an effect on intestinal barrier. Secondly, the GDM modeling, which induced by STZ, involves the destruction of pancreatic β-cells, results in a form of diabetes that is more akin to type 1 diabetes rather than GDM, which is primarily characterized by insulin resistance during pregnancy. Thirdly, most studies of the efficacy of SLGP-based management of GDM to date have been conducted in animal model systems, while clinical data availability remains limited. Further clinical trials are thus warranted to inform patient treatment. Accordingly, future studies will center on the implementation of large-scale, multi-center, randomized controlled clinical trials based on the present results in order to help bridge the gap between basic preclinical research and clinical utility. This strategy will ultimately help provide an evidence-based foundation for the management of GDM using traditional Chinese medicine.

In summary, these results suggest that SLGP may offer value as a treatment for GDM through its ability to suppress TLR4/NF-κB pathway activity and to restore the integrity and function of the intestinal barrier.

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