ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In Press.

Oxymatrine Attenuates High Glucose-induced NLRP3 Inflammasome-dependent Pyroptosis and Injury in Podocytes by Regulating SIRT1/NF-κB Pathway

Haiyan Ouyang[1], Dandan Chen[2], and Wei Liu[2]

[1] Department of Brain Disease Intensive Care Medicine, The Second People's Hospital of Hunan Province (Hunan Provincial Brain Hospital, Changsha, China
[2] Department of Endocrine, The Second People's Hospital of Hunan Province, (Hunan Provincial Brain Hospital), Changsha, China

Received: 2 September 2024; Received in revised form: 25 October 2024; Accepted: 5 November 2024

ABSTRACT

Diabetic nephropathy is a microvascular complication that leads to renal injury. Oxymatrine (OMT) is a matrine alkaloid and has been shown to ameliorate diabetic nephropathy. However, it is still unknown whether its mechanism involves podocytes, which play a critical role in diabetic nephropathy.

High glucose-induced podocytes (MPC5) were treated with OMT, the NOD-like receptor protein 3 (NLRP3) inhibitor MCC950, and the sirtuin 1 (SIRT1) inhibitor EX527. The effects on podocyte proliferation and apoptosis were assessed using cell counting kit-8 and flow cytometry. Immunofluorescence staining was performed to detect the expression of podocyte-associated proteins, NLRP3 inflammasome, and SIRT1. The levels of interleukin (IL)-1β and IL-18 were measured by enzyme-linked immunosorbent assay. Additionally, Western blot analysis was conducted to evaluate podocyte-related proteins, NLRP3 inflammasome-dependent pyroptosis-related proteins, and SIRT1/nuclear factor kappa B (NF-κB) pathway proteins, aiming to elucidate the mechanisms by which OMT improves podocyte injury.

OMT significantly promoted the proliferation of podocytes exposed to high glucose, inhibited their apoptosis, increased the levels of nephrin, Wilms tumor 1, podocin, and zonula occludens-1, and reduced pyroptosis-related proteins, IL-1β, and IL-18 ($p<0.05$). It also increased SIRT1 and decreased the acetylation of NF-κB p65 ($p<0.05$). The NLRP3 inhibitor MCC950 reduced podocyte pyroptosis under high glucose conditions, while the SIRT1 inhibitor EX527 reversed the protective effects of OMT on NLRP3 inflammasome-dependent pyroptosis and podocyte injury.

OMT ameliorates high glucose-induced podocyte injury by regulating the SIRT1/NF-κB pathway and inhibiting NLRP3 inflammasome-dependent pyroptosis.

Keywords: NOD-like receptor protein 3 inflammasome; Oxymatrine; Podocytes; Pyroptosis; SIRT1/NF-κB pathway

Corresponding Author: Wei Liu, MD;
Department of Endocrine, Hunan Provincial Brain Hospital (The Second People's Hospital of Hunan Province), Changsha, China.

Tel: (+86 158) 7410 1318, Fax: (+98 731) 8522 6120, Email: danzhe5@163.com

*The first and second authors contributed equally to this study.

Copyright©, Iran J Allergy Asthma Immunol. All rights reserved.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

IN PRESS

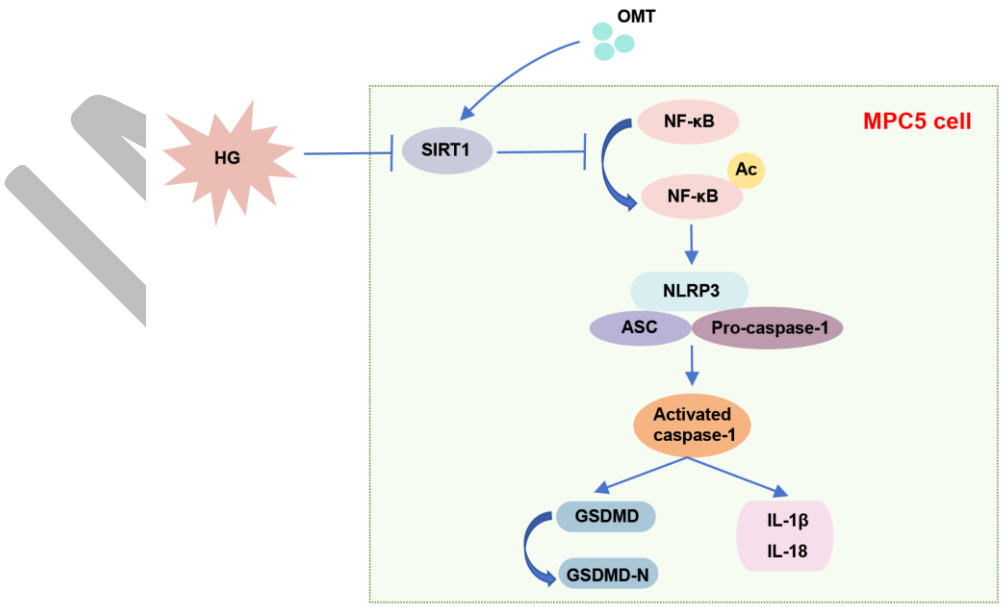
INTRODUCTION

Diabetic nephropathy (DN) is caused by diabetic microangiopathy. The clinical features are characterized by impaired glomerular filtration function, podocyte injury.[1] At present, the clinical treatment of DN includes blood pressure and blood glucose control, as well as therapies aimed at reducing albuminuria. However, these strategies will only slow the progression. As the core barrier of the glomerular filtration membrane, podocyte injury can lead to the occurrence of proteinuria, which is the key link to cause and aggravate DN. Hyperglycemia can cause an inflammatory response in podocytes and further induce podocyte apoptosis.[2] The decrease in the number of podocytes causes glomerular filtration dysfunction and reduces renal function.[3] Therefore, for the treatment of DN, alleviating hyperglycemia-triggered podocyte injury is considered essential.

Oxymatrine (OMT) is a kind of matrine alkaloid from Sophora flavescens, which has antifibrosis, antioxidative, metabolic regulation, and other pharmacological effects.[4-7] OMT can protect blood vessels through the sirtuin 1 (SIRT1)/Nrf2 pathway to inhibit NOD-like receptor protein 3 (NLRP3).[8] It can also improve endothelial cell injury and ulcerative colitis by inhibiting NLRP3-mediated pyroptosis.[8,9] At the same time, studies have found that OMT is helpful for the treatment of DN, which can improve DN by reducing tubulointerstitial fibrosis.[10-12] However, little is known about whether it can improve podocyte injury.

Inflammatory response is a hot topic in DN.[13] Pyroptosis is a kind of programmed cell death accompanied by a severe inflammatory response, which is characterized by the formation of the NLRP3 inflammasome, and the release of interleukin (IL)-1β and IL-18.[14] High glucose activates the NLRP3 inflammasome, causing pyroptosis damage to kidney tissue cells.[15] As the final barrier of the glomerular filtration membrane, podocyte injury is the core event of DN. Exploring the mechanism of podocyte injury, the key node of DN injury is essential for early intervention and treatment of DN. Therefore, regulating podocyte pyroptosis can be used as an important target to improve DN renal injury.

Based on the above, we speculate that OMT may improve podocyte injury by inhibiting NLRP3 inflammasome-mediated pyroptosis, thereby improving DN. By establishing an MPC5 cell injury model inhibited by NLRP3 and SIRT1, podocyte-related proteins, pyroptosis-related proteins, and pathways were investigated to provide an experimental basis for OMT for the clinical treatment of DN.



Graphical Abstract. Oxymatrine (OMT) attenuates high glucose-induced NOD-like receptor protein 3 (NLRP3) inflammasome-dependent pyroptosis and injury in podocytes by regulating the sirtuin 1 (SIRT1)/nuclear factor kappa B (NF-κB) pathway.

MATERIALS AND METHODS

Cell Culture and Treatment

The immortal mouse podocyte cell line MPC5 was purchased from Wuhan Qisai Biotechnology Co., Ltd. (QS-M014, Wuhan, China). 10% fetal bovine serum (S9020, Solarbio, Beijing, China), 1% penicillin-streptomycin solution (P7630, Solarbio, China), and 10 IU/mL interferon (IFN)-γ (300-02, PeproTech, Cranbury, New Jersey, USA) were added to Dulbecco's Modified Eagle Medium (DMEM) low-glucose medium (31600, Solarbio, China). MPC5 cells were placed in this medium and subcultured at 37℃ and 5% CO_2. The cells were cultured in an IFN-γ–free medium for 14 days (37℃, 5% CO_2) to promote cell differentiation. When the density was higher than 80%, it was used for subsequent experiments.

MPC5 cells were processed with 30 mM glucose (G8150, Solarbio, China, high glucose, HG) to establish a DN model, with 5.6 mM glucose (normal glucose, NG) as the normal group. To test the influences of OMT (B21470, source leaf, Shanghai, China) and NLRP3 inhibitor MCC950 (IM1340, Solarbio, China) on MPC5 cell injury, cells were processed with 2 μM, 4 μM, 8 μM OMT, and 10 μM MCC950 for 1 hour, and then induced by HG for 48 hours. To test the effect of SIRT1 inhibitor EX527 (10009798, cayman, Ann Arbor, Michigan, USA) on MPC5 cell injury, cells were processed with 8 μM OMT + 1 μM EX527 for 1 hour, and then induced by high glucose for 48 hours.

Cell Counting Kit-8 (CCK-8)

MPC5 cells were seeded in 96-well plates at 1×10^4 cells/well. Each well was marked, and the corresponding culture medium and drug of 200 μL were added to each well. Each group was set up with 3 duplicate wells, and the cell incubator was incubated for 24 hours. 20 μL CCK-8 working fluid (CA1210, Solarbio, China) was added to each well and gently shaken and mixed. The fully mixed cells were incubated for 2 h. The absorbance of the microplate reader (1410101, Thermo Fisher Scientific, Waltham, MA, USA) was set to 450 nm to detect the OD value.

Flow Cytometry

Podocytes were digested with a trypsin digestion solution (T1350, Solarbio, China). The supernatant was discarded. About 1×10^6 cells were collected from each group, and 500 μL Binding Buffer was added. After repeated blowing and beating, 5 μL Annexin V-FITC and PI were added and mixed. The cells were placed in the darkroom for 10 min, and then the apoptosis was detected by flow cytometry (2010284AA, Agilent, Santa Clara, CA, USA) and the apoptosis rate was calculated.

Enzyme-Linked Immunosorbent Assay

The cells were collected after 48 h of intervention, and the supernatant was obtained by low-temperature centrifugation (12000 r/min). IL-1β and IL-18 were tested according to the operation steps of ELISA kits (SEKH-0002, SEKH-0028, Solarbio, China).

Immunofluorescence

MPC5 cells were fixed in 4% paraformaldehyde (P1110, Solarbio, China), permeabilized with TBS (T1080, Solarbio, China) solution containing 0.25% Triton X-100 (IR9073, Solarbio, China), blocked with 5% BSA (SW3015, Solarbio, China) for 1 hour, and then incubated with primary antibodies SIRT1 (1:100, Abcam, Cambridge, UK), NLRP3 (1:50, Abcam, UK), Nephrin (1:200, Abcam, UK) and podocin (1:200, Abcam, UK) overnight. The cells were incubated with a secondary antibody (1:5000, GB21303, Servicebio, Wuhan, China) for 1 hour. After adding the fluorescence quencher dropwise, the coverslips were covered and photographed under a fluorescence microscope (MF52-N, Guangzhou Ming-Mei Technology Co., Ltd, Guangdong, China).

Western Blot

After the cell samples were collected, the supernatant was obtained by full lysis. The BCA kit (PC0020, Solarbio, China) was used to determine protein concentration. gel preparation, electrophoresis, and protein transfer to the polyvinylidene fluoride (PVDF) membrane (YA1700, Solarbio, China). The cells were incubated with 5% skimmed milk powder (LP0033B, Solarbio, China) for 2 h. Nephrin (ab235903, 1:1000, Abcam, UK), zonula occludens 1 (ZO-1, ab307799, 1:1000, Abcam, UK), Wilms tumor 1 (WT1, ab267377, 1:1000, Abcam, UK), NLRP3 (ab270449, 1:1000, Abcam, UK), podocin (ab181143, 1:2000, Abcam, UK), nuclear factor kappa B (NF-κB) p65 (ab288751, 1:1000, Abcam, UK), apoptosis-associated speck-like protein (ASC, ab283684, 1:1000, Abcam, UK), p-NF-κB p65 (ab76302, 1:1000, Abcam, UK),

IN PRESS

cysteinyl aspartate specific proteinase (Caspase)-1 p20 (ab207802, 1:1000, Abcam, UK), SIRT1 (ab189494, 1:1000, Abcam, UK), gasdermin D(GSDMD)-N (ab215203, 1:1000, Abcam, UK), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (TA-08, 1:1000, ZSGB-BIO, Beijing, China) were added and incubated in a refrigerator. On the second day, Tris-Buffered Saline with Tween-20 (TBST) buffer (T1082, Solarbio, China) was washed and a secondary antibody (1:20000, Abcam, UK) was added and incubated for 1 hour. TBST buffer was washed 5 times, 5 minutes each time. Enhanced chemiluminescence (ECL, PE0010, Solarbio, China) reagent was used to react for 2 to 3 minutes, and then the Tanon 5200 Multi automatic chemiluminescence imaging system (Tanon 5200 Multi, Shanghai Tianneng Technology Co., Ltd., Shanghai, China) was used for imaging.

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analysis and image drawing were performed using GraphPad 9.0. Student's *t* test was used to analyze the difference between the 2 groups, and One-way analysis of variance (ANOVA) was used to compare multiple groups. $p<0.05$ was considered statistically significant.

RESULTS

OMT Attenuates High Glucose-induced MPC5 Cell Injury

We first analyzed the survival rate of podocytes, the cell viability of the HG group declined markedly. OMT at concentrations of 2, 4, and 8 μM could significantly reverse the decrease of cell viability induced by HG. At the same time, compared with the NG group, OMT alone did not affect cell viability ($p<0.05$; Figure 1A), suggesting that OMT had no toxic effect on podocytes and improved podocyte injury. At the same time, the apoptosis rate of podocytes increased, and the apoptosis rate declined after 2, 4, and 8 μM OMT treatment ($p<0.05$; Figure 1B-C). Then we analyzed podocyte-related proteins, nephrin, podocin, WT1, and ZO-1 proteins in the HG group were markedly decreased, and the protein expression levels increased after 2, 4, and 8 μM OMT treatment ($p<0.05$; Figure 1D–E). At the same time, the immunofluorescence experiments of nephrin and podocin also illustrated this result ($p<0.05$; Figures 1F–H). In conclusion, OMT alleviates high glucose-induced MPC5 cell damage.

NLRP3 Inflammasome-dependent Pyroptosis Mediates High Glucose-induced MPC5 Cell Injury

In order to confirm whether NLRP3 inflammasome-dependent pyroptosis is involved in podocyte injury, we cocultured MPC5 cells with the NLRP3 inhibitor MCC950. NLRP3 was significantly upregulated, while NLRP3 in the HG+MCC950 group was reduced ($p<0.05$; Figure 2A-B), indicating that NLRP3 was significantly activated under HG conditions. Then we used Western blot to detect pyroptosis-related proteins. The experimental results showed that NLRP3, ASC, Caspase-1 p20, GSDMD-N, IL-1β, and IL-18 were markedly elevated, while in the HG+MCC950 group, they were significantly reduced ($p<0.05$; Figure 2C–I), suggesting that the inflammatory environment was enhanced under HG conditions. Combined with the results of Figures 2J–L, the cell viability was significantly raised, and the apoptosis rate of podocytes was reversed in the HG+MCC950 group ($p<0.05$), indicating that podocytes under the action of HG were accompanied by programmed cell death of severe inflammatory response. In summary, NLRP3 inflammasome-dependent pyroptosis mediates HG-induced MPC5 cell damage.

OMT Attenuates HG-induced MPC5 Cell Injury by Inhibiting NLRP3 Inflammasome-dependent Pyroptosis

In order to verify that OMT can reduce podocyte injury through NLRP3 inflammasome, we detected pyroptosis-related proteins, the NLRP3, ASC, Caspase-1 p20, and GSDMD-N were elevated, and reduced after 2, 4, and 8 μM OMT treatment ($p<0.05$; Figure 3A-E). The contents of IL-1β and IL-18 were significantly higher ($p<0.05$; Figure 3F-G) and reduced significantly after OMT treatment. Taken together, OMT alleviates HG-induced MPC5 cell damage by inhibiting NLRP3 inflammasome-dependent pyroptosis.

IN PRESS

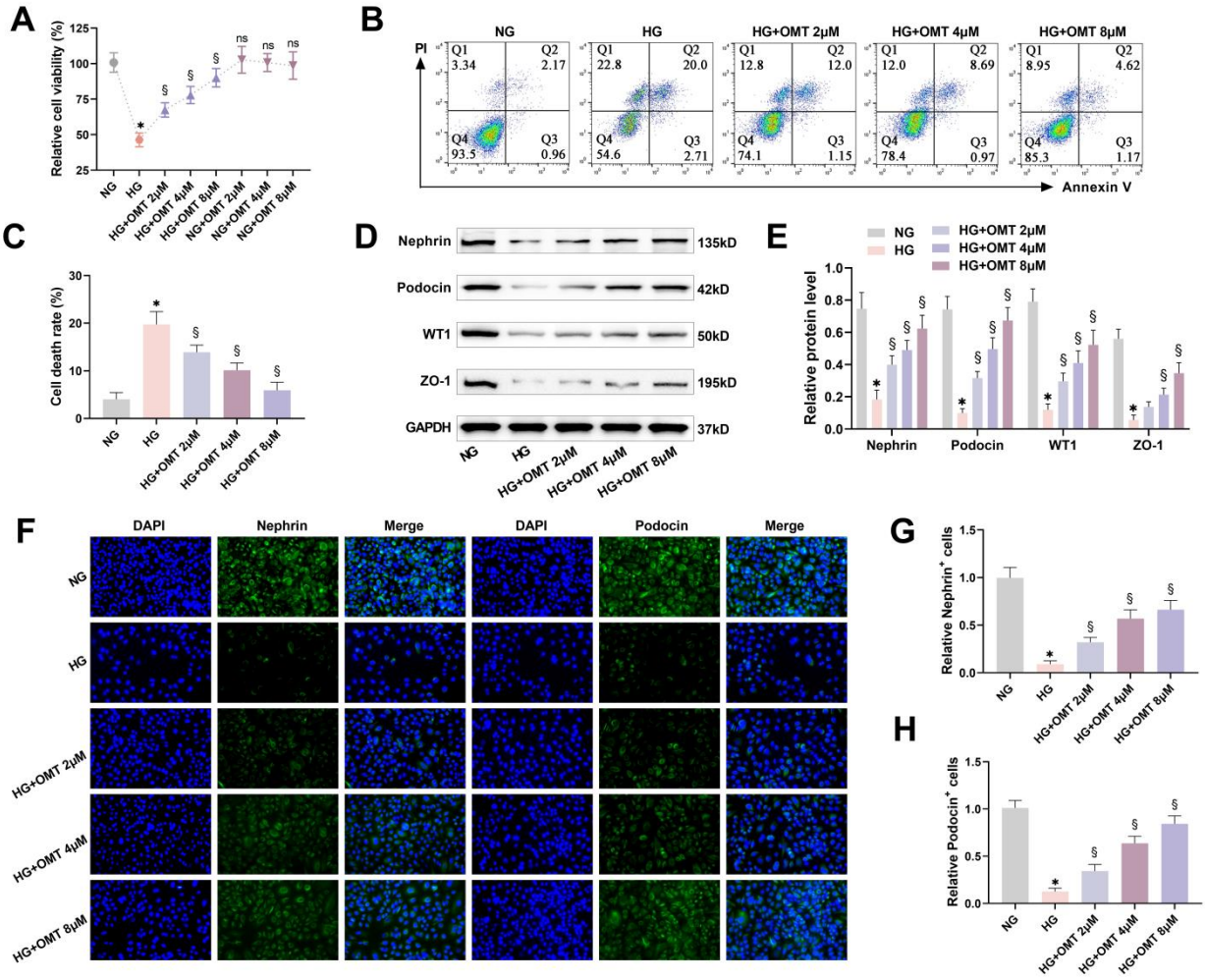


Figure 1. Oxymatrine (OMT) attenuates high glucose (HG)-induced MPC5 cell injury

A: MPC5 cells induced by HG were treated with 2, 4, and 8 μM OMT, respectively, and the proliferation of MPC5 cells under different treatment conditions was tested by cell counting kit-8 (CCK-8). B–C: The apoptosis level of MPC5 cells was tested by flow cytometry. D–E: Nephrin, podocin, Wilms tumor 1 (WT1), and zonula occludens-1 (ZO-1) were detected by Western blot. F–H: Nephrin and podocin were detected by immunofluorescence. *p<0.05 vs NG group; § p<0.05 vs HG group.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

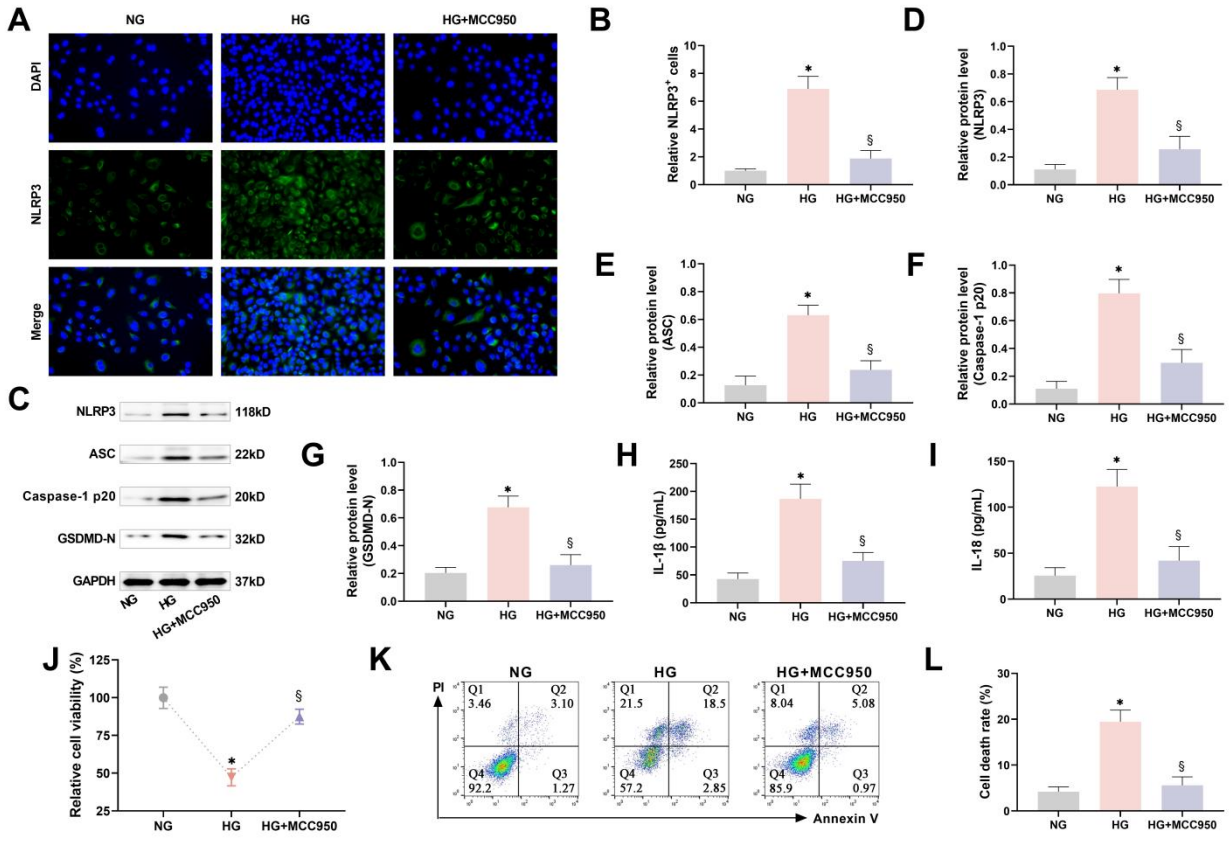


Figure 2. NOD-like receptor protein 3 (NLRP3) inflammasome-dependent pyroptosis mediates high glucose (HG)-induced MPC5 cell injury.

A–B: HG-induced MPC5 cells were treated with 10 μM NLRP3 inhibitor MCC950, and NLRT3 was detected by immunofluorescence. After the application of MCC950, NLRT3 declined compared with the HG group. C–G: NLRT3, apoptosis-associated speck-like protein (ASC), cysteinyl aspartate specific proteinase (Caspase)-1 p20, and gasdermin D (GSDMD)-N were detected by Western blot, which was significantly reduced after the application of MCC950. H–I: interleukin (IL)-1β and IL-18 were tested by enzyme-linked immunosorbent assay (ELISA). The content of proinflammatory factors was significantly decreased after MCC950 treatment. J: cell counting kit-8 (CCK-8) was used to test the proliferation. The cell viability increased after the application of MCC950. K–L: Flow cytometry was used to test the apoptosis level. The apoptosis rate of the MCC950 group was decreased. *p<0.05 vs NG group; § p<0.05 vs HG group.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

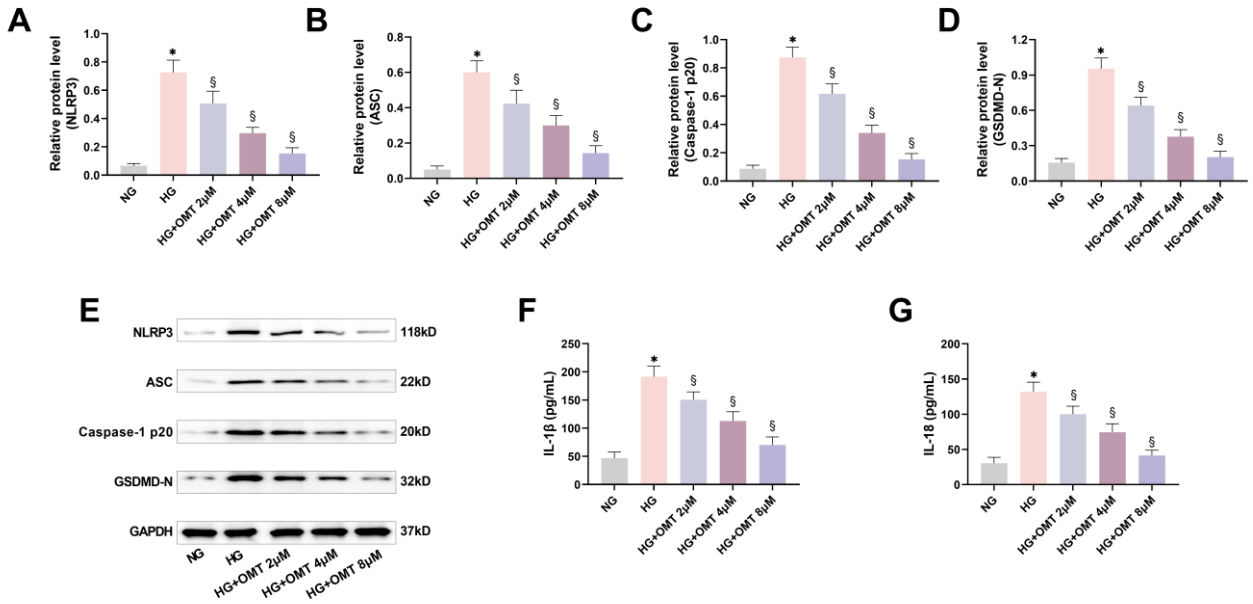


Figure 3. Oxymatrine (OMT) attenuates high glucose (HG)-induced MPC5 cell injury by inhibiting NOD-like receptor protein 3 (NLRP3) inflammasome-dependent pyroptosis. A–E: MPC5 cells induced by HG were treated with 2, 4, and 8 μM OMT, respectively. NLRT3, apoptosis-associated speck-like protein (ASC), cysteinyl aspartate specific proteinase (Caspase)-1 p20, and gasdermin D (GSDMD)-N were tested by Western blot. F–G: interleukin (IL)-1β and IL-18 were tested by enzyme-linked immunosorbent assay (ELISA). *p<0.05 vs NG group; § p<0.05 vs HG group.

The Effect of OMT on SIRT1/NF-κB Pathway

To verify the effect of the SIRT1/NF-κB pathway on podocyte injury, we used immunofluorescence and Western blot techniques. SIRT1 in the HG group was obviously reduced, and p-NF-κB p65 was notably higher. After OMT treatment, the level of SIRT1 was markedly elevated, and p-NF-κB p65 protein was lowered (p<0.05; Figure 4A-E). At the same time, SIRT1 inhibitor EX527 was given to verify the SIRT1/NF-κB pathway. After giving the inhibitor, SIRT1 notably declined, and p-NF-κB p65 protein was significantly increased (p<0.05; Figure 4F–H), suggesting that OMT can regulate podocyte injury induced by activating SIRT1 to inhibit p-NF-κB p65 protein.

Inhibition of SIRT1 Expression Reversed the Inhibitory Effect of OMT on NLRP3 Inflammasome-Dependent Pyroptosis

In order to verify whether SIRT1 has an effect on NLRP3 inflammasome-dependent pyroptosis, we detected NLRP3 inflammasome pyroptosis-related indicators after giving SIRT1 inhibitor EX527. NLRP3, ASC, Caspase-1 p20, and GSDMD-N were upregulated and lowered after OMT treatment (p<0.05; Figure 5A-E), and significantly recovered after the administration of SIRT1 inhibitor EX527. In Figure 5F–G, IL-1β and IL-18 elevated significantly, the HG+OMT group decreased significantly, and the HG+OMT+EX527 group rebounded significantly (p<0.05). The above results indicate that inhibition of SIRT1 expression reverses the inhibitory effect of OMT on NLRP3 inflammasome-dependent pyroptosis. In other words, OMT can regulate HG-induced NLRP3 inflammasome-dependent pyroptosis in podocytes by regulating the SIRT1/NF-κB pathway.

IJPRL

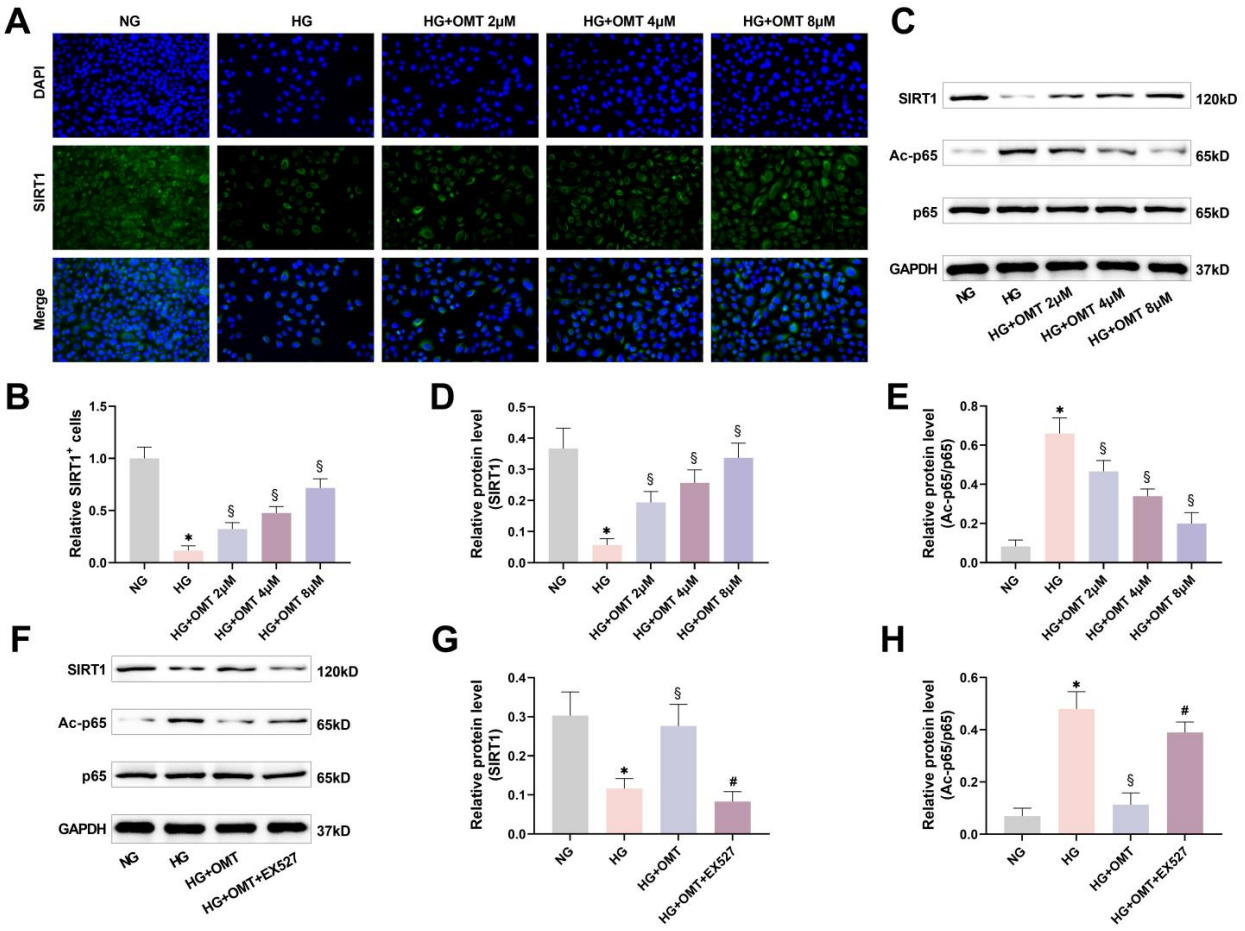


Figure 4. The effect of Oxymatrine (OMT) on sirtuin 1 (SIRT1)/nuclear factor kappa B (NF-κB) pathway.
A–B: MPC5 cells induced by high glucose (HG) were treated with 2, 4, and 8 μM OMT, respectively, and SIRT1 in MPC5 cells was detected by immunofluorescence. C–E: SIRT1, Acetylation of NF-κB (Ac)-p65, and p65 were detected by Western blot. F–H: HG-induced MPC5 cells were treated with 8 μM OMT and 8 μM OMT + 1 μM SIRT1 inhibitor EX527. SIRT1, Ac-p65, and p65 were detected by Western blot. SIRT1 was decreased and Ac-p65 was significantly increased after application of EX527. *p<0.05 vs NG group; § p<0.05 vs HG group; #p<0.05 vs HG + OMT group.

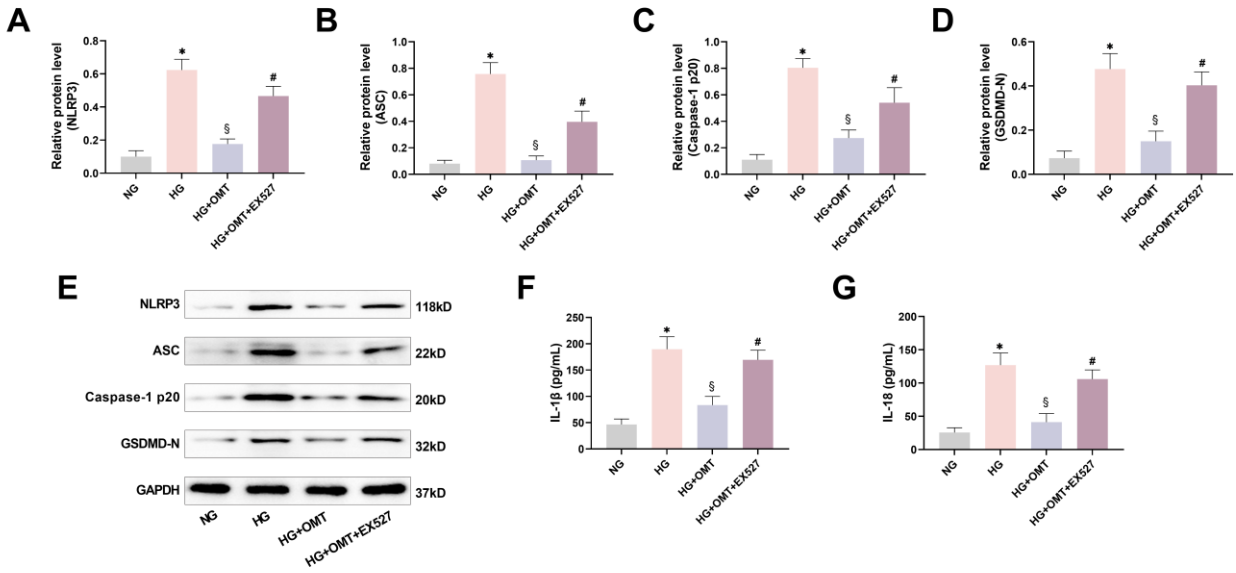


Figure 5. Inhibition of sirtuin 1 (SIRT1) expression reversed the inhibitory effect of Oxymatrine (OMT) on NOD-like receptor protein 3 (NLRP3) inflammasome-dependent pyroptosis.

A–E: High glucose (HG)-induced MPC5 cells were treated with 8 μM OMT and 8 μM OMT + 1 μM SIRT1 inhibitor EX527. NLRT3, apoptosis-associated speck-like protein (ASC), cysteinyl aspartate specific proteinase (Caspase)-1 p20, and gasdermin D (GSDMD)-N were detected by Western blot. The visible protein increased after the application of EX527 compared with OMT alone. F–G: Interleukin (IL)-1β and IL-18 were tested by enzyme-linked immunosorbent assay (ELISA). The content of proinflammatory factors increased after the application of EX527. *p<0.05 vs NG group; § p<0.05 vs HG group; #p<0.05 vs HG + OMT group.

Inhibition of SIRT1 Expression Reversed the Protective Effect of OMT on HG-induced MPC5 Cell Injury

To further verify the effect of SIRT1 on podocyte injury, we detected podocyte injury-related indicators after giving the SIRT1 inhibitor EX527. The cell viability of the HG group was reduced, and the apoptosis rate of podocytes increased. It was reversed in the HG+OMT group, but the viability of podocytes was lowered in the HG+OMT+EX527 group (p<0.05; Figure 6A–C). Nephrin, podocin, WT1, and ZO-1 in the HG group were decreased, which were elevated in the HG+OMT group, but decreased in the HG+OMT+EX527 group (p<0.05; Figure 6D–H), indicating that inhibition of SIRT1 expression reverses the improvement of OMT on HG-induced cell injury. Combined with the above results, it is fully proved that OMT can inhibit HG-induced NLRP3 inflammasome-dependent pyroptosis of podocytes by regulating the SIRT1/NF-κB pathway to improve cell injury.

IN PRESS

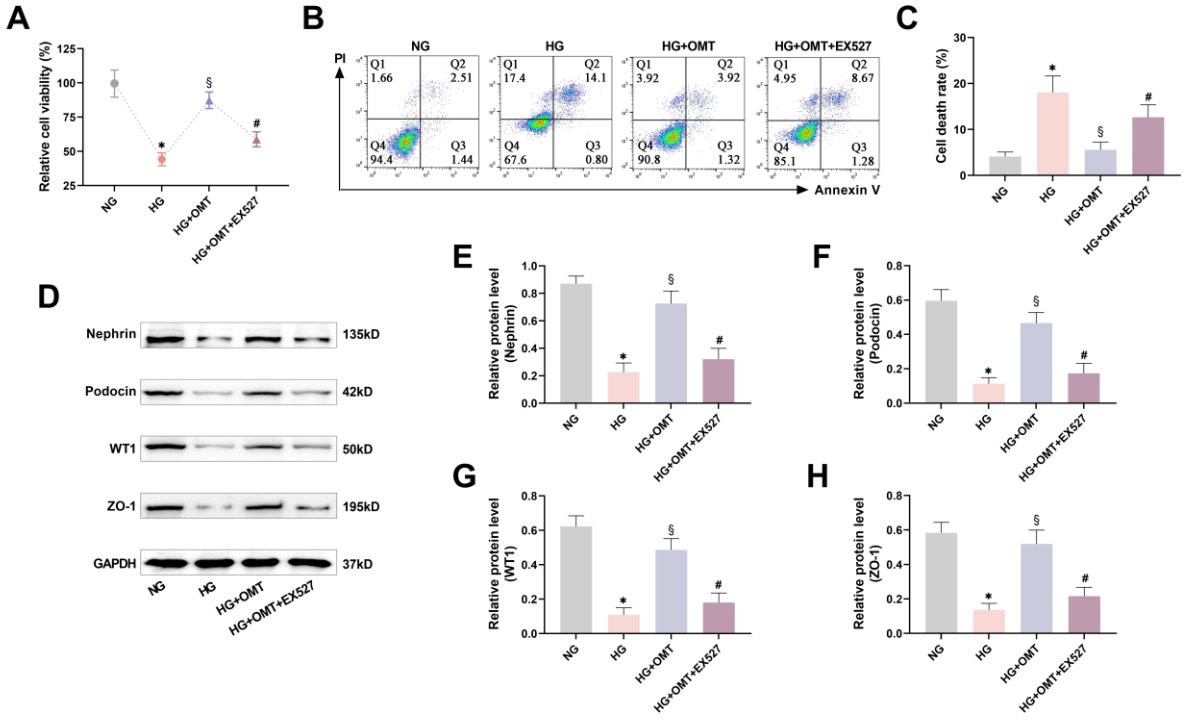


Figure 6. Inhibition of sirtuin 1 (SIRT1) expression reversed the protective effect of Oxymatrine (OMT) on high glucose (HG)-induced MPC5 cell injury

A: MPC5 cells induced by HG were treated with 8 μM OMT and 8 μM OMT + 1 μM SIRT1 inhibitor EX527, and the proliferation of MPC5 cells was tested by cell counting kit-8 (CCK-8). B–C: The apoptosis level of MPC5 cells was tested by flow cytometry, and the apoptosis rate was increased after the application of EX527 compared with OMT alone. D–H: NOD-like receptor protein 3 (NLRT3), apoptosis-associated speck-like protein (ASC), cysteinyl aspartate specific proteinase (Caspase)-1 p20, and gasdermin D (GSDMD)-N in MPC5 cells were detected by Western blot. Visible protein was decreased after the application of EX527 compared with OMT alone. *p<0.05 vs NG group; § p<0.05 vs HG group; #p<0.05 vs HG + OMT group.

DISCUSSION

DN is a disease of microvascular disease caused by persistent hyperglycemia in diabetes,[16] and there is a lack of effective molecular intervention targets and means. Its treatment is still based on comprehensive symptomatic treatment. The drug treatment of DN is still unable to improve the long-term prognosis of most patients, and it is also unable to improve the process of DN injury in the early stages. Therefore, we urgently need to find effective molecular intervention methods.

Podocytes are terminally differentiated cells that provide structural support for glomeruli. They are composed of 3 parts: cell body, primary protrusion, and foot process. They are essential for maintaining a complete glomerular filtration barrier. Furthermore, they can be destroyed due to genetic, drug, infection, and other incentives, and their regeneration and repair capabilities are limited. Therefore, podocyte injury is considered to be a permanent lesion.[17,18] Podocin and nephrin are the marker proteins of the podocyte slit diaphragm. They are important for maintaining the integrity of podocyte structure and function. The downregulation of their expression indicates that podocyte function is impaired, and the permeability of the glomerular filtration membrane is increased.[19] The normal work of podocytes depends on the maintenance of cytoskeleton proteins. ZO-1 can maintain the integrity of the podocyte filtration membrane. The loss of ZO-1–specific expression will damage the formation of the slit diaphragm.[20] WT1 is a specific marker protein of mature podocytes. The change in WT1 expression can reflect the degree of podocyte injury and can be used as a marker for podocyte counting.[21]

In this experiment, the survival rate of podocytes in the HG group was significantly declined, the apoptosis rate was significantly elevated, and the podocyte-related proteins podocin, nephrin, WT1, and ZO-1 were also significantly decreased, indicating that the quantity and density of podocytes were reduced. After OMT treatment, podocin, nephrin, WT1, and ZO-1 were markedly recovered. These results suggest that OMT attenuates HG-induced podocyte injury.

DN is associated with pyroptosis.[22,23] Pyroptosis is a cell death closely related to inflammatory processes that have been discovered and confirmed.[24] At present, the study of the pyroptosis pathway mediated by the NLRP3 inflammasome has attracted the most attention. NLRP3 inflammasome is important for inflammation.[25] As an immunosensor, it can detect a variety of microbial and host-derived signals, and its activation needs to start and activate 2 processes.[26,27] In the initiation stage, microbial molecules or endogenous cytokines trigger NLRP3 through NF-κB, and NLRP3 is assembled into the NLRP3 inflammasome complex, which can induce pyroptosis. NLRP3 inflammasome is activated to promote ASC expression, regulate the hydrolysis of pro-Caspase-1 activation to form Caspase-1, and then convert releases IL-1β and IL-18 to the extracellular. At the same time, it will cause cell rupture, and release of a large amount of content, triggering an inflammatory cascade, inducing the body to cause excessive inflammatory response and tissue damage, and aggravating the pathological process of DN.[28-31]

Caspase-1 has been shown to regulate GSDMD-mediated pyroptosis.[32,33] The Caspase-1 activation cleaves GSDMD to form GSDMD-N, which binds to phosphoinositide and cardiolipin to form pores and release proinflammatory factors, causing cell lysis and death,[34] inducing pyroptosis.[31] Therefore, GSDMD-N is the executor of pyroptosis and has been considered an important indicator for evaluating pyroptosis.[35] At the same time, pyroptosis is accompanied by the formation of inflammatory waterfalls.[36]

In this study, we detected that NLRP3, ASC, Caspase-1 p20, and GSDMD-N in the HG group were significantly elevated, and the contents of IL-1β and IL-18 were also significantly upregulated, indicating that pyroptosis occurs in podocytes, and OMT can significantly reverse podocyte apoptosis. At the same time, we applied the NLRP3 inhibitor MCC950 to HG-induced podocytes. Podocytes treated with inhibitor MCC950 significantly increased cell viability. The pyroptosis-related proteins were notably decreased, and IL-1β and IL-18 were also significantly lowered. In summary, OMT can alleviate HG-induced podocyte injury.

Inflammation is a pathological feature of most kidneys and can lead to glomerulosclerosis, tubular atrophy, vascular damage, and fibrosis.[37] The inflammatory response participates in the pathogenesis of DN.[38] SIRT1 is a member of the deacetylase family.[39] SIRT1 regulates gene expression by deacetylating lysine residues of various proteins. In recent years, DN has been closely related to the SIRT1 pathway.[40] HONG et al found podocytes and SIRT1 protein expression decreased in DN.[41] The podocyte injury was negatively correlated with SIRT1.[42] SIRT1 can be negatively regulated by long noncoding RNA (lncRNA). By upregulating SIRT1, it can activate mitophagy and improve podocyte injury.[43]

However, most of the views believe that the mechanism of SIRT1 inhibiting inflammatory response is related to the inhibition of inflammatory pathways. Among them, the most studied is the inhibitory effect of SIRT1 on the NF-κB signaling pathway.[44] In recent years, the NF-κB p65 subunit itself has needed a series of modifications to exert its function as a transcription factor, and acetylation modification is 1 of the most important modification pathways.[45] SIRT1 can reduce NF-κB, which may prevent the occurrence of an inflammatory response.[46] It can be speculated that SIRT1 expression can inhibit p-NF-κB p65 to inhibit the inflammatory response, delay renal injury, and treat DN.

In order to detect whether the SIRT1/NF-κB pathway participated in the treatment of DN, we detected SIRT1 and NF-κB in this experiment. It was found that OMT significantly increased SIRT1 protein and decreased NF-κB protein. We used the SIRT1 inhibitor EX527 and found that SIRT1 protein was significantly decreased and NF-κB protein was significantly increased after treatment with inhibitor EX527. NLRP3, ASC, Caspase-1 p20, and GSDMD-N were significantly decreased; IL-1β and IL-18 were also significantly decreased; and the podocyte survival rate was notably decreased, indicating that the inhibition of SIRT1 expression reversed the inhibitory effect of OMT on NLRP3 inflammasome-dependent pyroptosis. The protein expression levels of podocyte-related proteins podocin, nephrin, WT1, and ZO-1 were significantly increased, indicating that inhibition of SIRT1 expression reversed the improvement of OMT on HG-induced

IN PRESS

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

MPC5 cell injury. Combined with the results of this study, we can fully and reasonably believe that OMT can promote NF-κB p65 deacetylation by activating SIRT1 and inhibit HG-induced NLRP3 inflammasome-dependent pyroptosis of podocytes to improve cell damage.

We evaluated the therapeutic effect of OMT on HG-induced podocytes. OMT regulates the NLRP3 inflammasome through the SIRT1/NF-κB pathway, inhibits pyroptosis-related proteins, promotes podocyte-related proteins such as nephrin and podocin, and reduces HG-induced podocyte injury. This study provides a reliable reference for targeted therapy and drug screening of podocyte injury. However, there are still some limitations in this study, and the safety and efficacy of OMT in clinical application need to be further evaluated.

STATEMENT OF ETHICS

Since we are conducting in vitro cell experiments, ethical considerations are not applicable.

FUNDING

This study received no external funding.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author, Wei Liu, upon reasonable request.

AI Assistance Disclosure

Not applicable.

REFERENCES

1. Tsai YC, Kuo MC, Hung WW, Wu LY, Wu PH, Chang WA, et al. High Glucose Induces Mesangial Cell Apoptosis through miR-15b-5p and Promotes Diabetic Nephropathy by Extracellular Vesicle Delivery. Mol Ther. 2020;28(3):963-74.
2. Jiang Z, Qian L, Yang R, Wu Y, Guo Y, Chen T. LncRNA TCF7 contributes to high glucose-induced damage in human podocytes by up-regulating SEMA3A via sponging miR-16-5p. J Diabetes Investig. 2023;14(2):193-204.
3. Feng J, Xie L, Yu X, Liu C, Dong H, Lu W, et al. Perilipin 5 ameliorates high-glucose-induced podocyte injury via Akt/GSK-3β/Nrf2-mediated suppression of apoptosis, oxidative stress, and inflammation. Biochem Biophys Res Commun. 2021;544:22-30.
4. Gan P, Ding L, Hang G, Xia Q, Huang Z, Ye X, et al. Oxymatrine Attenuates Dopaminergic Neuronal Damage and Microglia-Mediated Neuroinflammation Through Cathepsin D-Dependent HMGB1/TLR4/NF-κB Pathway in Parkinson's Disease. Front Pharmacol. 2020;11:776.
5. Wang Y, Shou Z, Fan H, Xu M, Chen Q, Tang Q, et al. Protective effects of oxymatrine against DSS-induced acute intestinal inflammation in mice via blocking the RhoA/ROCK signaling pathway. Biosci Rep. 2019;39(7).
6. Xiao X, Hou X, Shi W, Hu C, Cui Y, Hu J, et al. Oxymatrine inhibits proliferation and apoptosis of human breast cancer cells through the regulation of miRNA-140-5P. Am J Transl Res. 2021;13(12):13674-82.
7. Sun Y, Xu L, Cai Q, Wang M, Wang X, Wang S, et al. Research progress on the pharmacological effects of matrine. Front Neurosci. 2022;16:977374.
8. Jin X, Fu W, Zhou J, Shuai N, Yang Y, Wang B. Oxymatrine attenuates oxidized low-density lipoprotein-induced HUVEC injury by inhibiting NLRP3 inflammasome-mediated pyroptosis via the activation of the SIRT1/Nrf2 signaling pathway. Int J Mol Med. 2021;48(4).
9. Sun J, Wang S, Zhao Z, Lu J, Zhang Y, An W, et al. Oxymatrine Attenuates Ulcerative Colitis through Inhibiting Pyroptosis Mediated by the NLRP3 Inflammasome. Molecules. 2024;29(12).
10. Xiao Y, Peng C, Xiao Y, Liang D, Yuan Z, Li Z, et al. Oxymatrine Inhibits Twist-Mediated Renal Tubulointerstitial Fibrosis by Upregulating Id2 Expression. Front Physiol. 2020;11:599.
11. Xiao Y, Liang D, Li Z, Feng Z, Yuan Z, Zhang F, et al. BMP-7 Upregulates Id2 Through the MAPK

IN PRESS

Signaling Pathway to Improve Diabetic Tubulointerstitial Fibrosis and the Intervention of Oxymatrine. Front Pharmacol. 2022;13:900346.
12. Liu L, Wang Y, Yan R, Li S, Shi M, Xiao Y, et al. Oxymatrine Inhibits Renal Tubular EMT Induced by High Glucose via Upregulation of SnoN and Inhibition of TGF-β1/Smad Signaling Pathway. PLoS One. 2016;11(3):e0151986.
13. Jung SW, Moon JY. The role of inflammation in diabetic kidney disease. Korean J Intern Med. 2021;36(4):753-66.
14. Seok JK, Kang HC, Cho YY, Lee HS, Lee JY. Therapeutic regulation of the NLRP3 inflammasome in chronic inflammatory diseases. Arch Pharm Res. 2021;44(1):16-35.
15. Qiu YY, Tang LQ. Roles of the NLRP3 inflammasome in the pathogenesis of diabetic nephropathy. Pharmacol Res. 2016;114:251-64.
16. Zhang B, Zhang X, Zhang C, Sun G, Sun X. Berberine Improves the Protective Effects of Metformin on Diabetic Nephropathy in db/db Mice through Trib1-dependent Inhibiting Inflammation. Pharm Res. 2021;38(11):1807-20.
17. Liu N, Xu L, Shi Y, Zhuang S. Podocyte Autophagy: A Potential Therapeutic Target to Prevent the Progression of Diabetic Nephropathy. J Diabetes Res. 2017;2017:3560238.
18. Li X, Zhang Y, Xing X, Li M, Liu Y, Xu A, et al. Podocyte injury of diabetic nephropathy: Novel mechanism discovery and therapeutic prospects. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2023;168:115670.
19. Jiang L, Cui H, Ding J. Smad3 signalling affects high glucose-induced podocyte injury via regulation of the cytoskeletal protein transgelin. Nephrology (Carlton). 2020;25(9):659-66.
20. Itoh M, Nakadate K, Matsusaka T, Hunziker W, Sugimoto H. Effects of the differential expression of ZO-1 and ZO-2 on podocyte structure and function. Genes Cells. 2018;23(7):546-56.
21. Ettou S, Jung YL, Miyoshi T, Jain D, Hiratsuka K, Schumacher V, et al. Epigenetic transcriptional reprogramming by WT1 mediates a repair response during podocyte injury. Sci Adv. 2020;6(30):eabb5460.
22. Yan M, Li W, Wei R, Li S, Liu Y, Huang Y, et al. Identification of pyroptosis-related genes and potential drugs in diabetic nephropathy. J Transl Med. 2023;21(1):490.
23. Zhang K, Li M, Yin K, Wang M, Dong Q, Miao Z, et al. Hyperoside mediates protection from diabetes kidney disease by regulating ROS-ERK signaling pathway and pyroptosis. Phytother Res. 2023;37(12):5871-82.
24. Rao Z, Zhu Y, Yang P, Chen Z, Xia Y, Qiao C, et al. Pyroptosis in inflammatory diseases and cancer. Theranostics. 2022;12(9):4310-29.
25. Seoane PI, Lee B, Hoyle C, Yu S, Lopez-Castejon G, Lowe M, et al. The NLRP3-inflammasome as a sensor of organelle dysfunction. J Cell Biol. 2020;219(12).
26. Zhao C, Zhao W. NLRP3 Inflammasome-A Key Player in Antiviral Responses. Front Immunol. 2020;11:211.
27. Ge Q, Chen X, Zhao Y, Mu H, Zhang J. Modulatory mechanisms of NLRP3: Potential roles in inflammasome activation. Life Sci. 2021;267:118918.
28. Ma Q. Pharmacological Inhibition of the NLRP3 Inflammasome: Structure, Molecular Activation, and Inhibitor-NLRP3 Interaction. Pharmacol Rev. 2023;75(3):487-520.
29. Shen Y, Xu Y, Shen P, Shen P, Bian Q, Han L, et al. A bifunctional fusion protein protected against diabetic nephropathy by suppressing NLRP3 activation. Appl Microbiol Biotechnol. 2023;107(7-8):2561-76.
30. Chen F, Wei G, Xu J, Ma X, Wang Q. Naringin ameliorates the high glucose-induced rat mesangial cell inflammatory reaction by modulating the NLRP3 Inflammasome. BMC Complement Altern Med. 2018;18(1):192.
31. Wang L, Hauenstein AV. The NLRP3 inflammasome: Mechanism of action, role in disease and therapies. Mol Aspects Med. 2020;76:100889.
32. Ram C, Gairola S, Verma S, Mugale MN, Bonam SR, Murty US, et al. Biochanin A Ameliorates Nephropathy in High-Fat Diet/Streptozotocin-Induced Diabetic Rats: Effects on NF-kB/NLRP3 Axis, Pyroptosis, and Fibrosis. Antioxidants (Basel). 2023;12(5).
33. Xu X, Qin Z, Zhang C, Mi X, Zhang C, Zhou F, et al. TRIM29 promotes podocyte pyroptosis in diabetic nephropathy through the NF-kB/NLRP3 inflammasome pathway. Cell Biol Int. 2023;47(6):1126-35.
34. Wang X, Fan L, Yin H, Zhou Y, Tang X, Fei X, et al. Protective effect of Aster tataricus extract on

IN PRESS

NLRP3-mediated pyroptosis of bladder urothelial cells. J Cell Mol Med. 2020;24(22):13336-45.
35. Burdette BE, Esparza AN, Zhu H, Wang S. Gasdermin D in pyroptosis. Acta Pharm Sin B. 2021;11(9):2768-82.
36. Lu F, Lan Z, Xin Z, He C, Guo Z, Xia X, et al. Emerging insights into molecular mechanisms underlying pyroptosis and functions of inflammasomes in diseases. J Cell Physiol. 2020;235(4):3207-21.
37. Fioranelli M, Roccia MG, Flavin D, Cota L. Regulation of Inflammatory Reaction in Health and Disease. Int J Mol Sci. 2021;22(10).
38. Rayego-Mateos S, Rodrigues-Diez RR, Fernandez-Fernandez B, Mora-Fernández C, Marchant V, Donate-Correa J, et al. Targeting inflammation to treat diabetic kidney disease: the road to 2030. Kidney Int. 2023;103(2):282-96.
39. Jiao F, Gong Z. The Beneficial Roles of SIRT1 in Neuroinflammation-Related Diseases. Oxid Med Cell Longev. 2020;2020:6782872.
40. Wang W, Sun W, Cheng Y, Xu Z, Cai L. Role of sirtuin-1 in diabetic nephropathy. J Mol Med (Berl). 2019;97(3):291-309.
41. Hong Q, Zhang L, Das B, Li Z, Liu B, Cai G, et al. Increased podocyte Sirtuin-1 function attenuates diabetic kidney injury. Kidney Int. 2018;93(6):1330-43.
42. Lassén E, Bouchareb R, Daehn IS. Podocyte as the link between sterile inflammation and diabetic kidney disease. Kidney Int. 2022;102(4):688-90.
43. Jayaraman S, Pazhani J, PriyaVeeraraghavan V, Raj AT, Somasundaram DB, Patil S. PCNA and Ki67: Prognostic proliferation markers for oral cancer. Oral oncology. 2022;130:105943.
44. Zhao K, Zhang H, Yang D. SIRT1 exerts protective effects by inhibiting endoplasmic reticulum stress and NF-κB signaling pathways. Front Cell Dev Biol. 2024;12:1405546.
45. Ghizzoni M, Haisma HJ, Maarsingh H, Dekker FJ. Histone acetyltransferases are crucial regulators in NF-κB mediated inflammation. Drug Discov Today. 2011;16(11-12):504-11.
46. Nadtochiy SM, Yao H, McBurney MW, Gu W, Guarente L, Rahman I, et al. SIRT1-mediated acute cardioprotection. Am J Physiol Heart Circ Physiol. 2011;301(4):H1506-12.

IN PRESS