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The protective effects of qihuang jianpi zishen decoction on mrl/lpr mice and its mechanism

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Abstract: Systemic lupus erythematosus (SLE) is a chronic disease of the autoimmune system with multiple damages, most commonly renal damage. The aim of the present study is to examine the therapeutic ability of Qihuang Jianpi Zishen decoction (QJZ) on MRL/lpr mice and uncover its mechanism preliminarily. Twenty-four female MRL/lpr mice were assigned into the model, prednisolone, mycophenolate mofetil and QJZ groups randomly. Six C57BL/6 mice were considered as controls. Each group was treated with corresponding drugs for 4 weeks, anti-dsDNA autoantibodies, C3 and C4, renal function and renal histopathological changes were observed. The expression of GAS5/miR-21/sprouty1 axis and ERK/CREB pathway in kidney was identified by western blotting and qRT-PCR. Compared with MRL/lpr mice, anti-dsDNA autoantibodies of mice treated with QJZ were significantly down-regulated, C3 and C4 were significantly up-regulated. QJZ also alleviated proteinuria, decreased SCr and BUN levels and minimized renal histopathological changes. In addition, QJZ affected the expression of GAS5/miR-21/sprouty1 axis and the phosphorylation of ERK/CREB pathway in renal tissues. QJZ bears therapeutic ability on healing renal injury in MRL/lpr mice. These effects may be achieved by regulating the GAS5/miR-21/sprouty1 axis and inhibiting the ERK/CREB pathway, thus improving the excessive proliferation of glomerular mesangial cells.

Keywords: Systemic lupus erythematosus, MRL/lpr mice, Qihuang Jianpi Zishen decoction.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by excessive production of multiple autoantibodies and excessive inflammatory response. Antibodies against autoantigens, accumulation of immune complexes in tissues and excessive complement activation are all hallmarks of SLE. The production of such autoantibodies affects multiple organ systems, including the kidneys, heart, blood vessels and the central nervous system (Kiriakidou and Ching, 2020, Pons-Estel *et al*., 2017). The incidence of Lupus nephritis (LN) is approximately 50% among SLE patients and is one of the important factors determining the prognosis of SLE patients (Parikh *et al*., 2020). The first pathological damage in LN is mesangial cell overproduction (Kong *et al*., 2019), which is closely related to the binding of glomerular cells with the ds-DNA antibody and the deposition of mesangial immune complexes (Karasawa *et al*., 2022, Liu *et al*., 2022). Therefore, one of the ways that drugs alleviate lupus nephritis may be to inhibit the excessive proliferation of mesangial cells.

The current drug therapy does not completely control the progression of SLE because it is tricky to quantify the effects of SLE on various organ systems and all kinds of complications (Mohamed *et al*., 2019, Steiger *et al*., 2022). Traditional Chinese medicine has attained significant effects in treating SLE, such as Zhibai Dihuang pill, Lang Chuang Wan, Liu-Wei-Di-Huang Wan, Lang Chuang Fang and Dan-Chi San (Dai *et al*., 2020, Gao *et al*., 2020, Liao *et al*., 2011, Huang *et al*., 2016). Qihuang Jianpi Zishen decoction (QJZ) is derived from the clinical experience of Professor Huang, a famous Chinese rheumatologist. It is a traditional Chinese medicine prescription whose effects include strengthening the spleen, nurturing the kidney, replenishing qi and strengthening the body and is made from the classic Liuwei Dihuang pill. Its prescriptions include Astragalus, Rehmannia, Dodder, Chinese Yam, Poria, Atractylodes, Raspberry and Fructus Rosae Laevigatae, mainly used for SLE. Professor Huang has applied QJZ in treating SLE for many years, which can effectively relieve SLE and improve kidney damage. However, its effect and mechanism for improving SLE still need to be fully elucidated.

As we all know, glomerular mesangial cells often proliferate abnormally in LN (Gouda *et al*., 2022, Yuan *et al*., 2017). Cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) is a transcription factor that controls various cellular processes including cell proliferation (Sapio *et al*., 2020). Extracellular signal-regulated kinase (ERK) signal transduction is a crucial intracellular signal transduction pathway, including ERK1 and ERK2. ERK1/2 has a wide

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range of catalytic activities and its activated form is phosphorylated ERK. The nuclear transport the downstream transcription factors of ERK can be encouraged once it becomes activated, these factors include CREB c-Myc, c-Ets, hence the transcription of corresponding target genes are also improved (Zhu *et al*., 2016). CREB is located downstream of ERK and its phosphorylation is controlled by the activation of ERK (Koga *et al*., 2019, Xi *et al*., 2022). And researchers (Zhang *et al*., 2020) have confirmed that the ERK/CREB pathway regulates mouse glomerular mesangial cells when they proliferate excessively.

So far, the cause and pathogenesis of SLE are not yet clear. LncRNA GAS5 is a specific transcription factor 5 for growth inhibition. It can be used as a biomarker for SLE and its expression level is abnormal in SLE patients (Wu *et al*., 2019). GAS5 has been confirmed to induce growth stagnation and apoptosis of human peripheral blood T lymphocytes and has been found to be involved in disease progression in SLE (Wu *et al*., 2021). Experiments have shown that microRNA-21 (miR-21) is the miRNA targeted by lncRNA GAS5 and there is a binding site for miR-21 in exon 4 of lncRNA GAS5 structure, enabling GAS5 to control the expression of miR-21 via targeted binding (Tao *et al*., 2017). Additionally, sprouty1 is an important downstream target gene of miR-21 and overexpressed miR-21 can reduce the level of sprouty1 (Gao *et al*., 2019). At the same time, sprouty1 has the effect of inhibiting the phosphorylation effect of ERK (Gao *et al*., 2019). At present, it has not been reported that GAS5/miR-21/sprouty1 axis can regulate ERK/CREB pathway to mediate excessive proliferation of glomerular mesangial cells, leading to renal damage in SLE. Therefore, the present study aims to examine the renal-protective ability of QJZ on MRL/lpr mice and its effects on GAS5/miR-21/sprouty1 axis and ERK/CREB pathway, so as to preliminarily explore its mechanism.

MATERIALS AND METHODS

Experimental animals

The present study was performed at the Key Laboratory of Anhui Province (Hefei, China). Eight-week-old female MRL/lpr mice ((total, 24; weighing 18-20g) and C57BL/6 mice (total, 6; weighing 18-22g) were provided by Silaike Experimental Animal Limited Liability Company (Certificate No. SCXK [HU] 2017-0005). A specific pathogen-free (SPF) animal room housed all experimental mice and the temperature was maintained at 24 ± 1°C with a regular cycle of light/dark, each lasting for 12 h. Water and food supply was made readily available to mice for the course of the study. All animal experimental protocols were carried out following the international guidelines and approved by the Ethics Committee of Anhui University of Traditional Chinese Medicine.

Reagents

Reagents used for experiment include mouse anti-dsDNA autoantibody ELISA kit, mouse C3 ELISA kit, mouse C4 ELISA kit (Wuhan Genomei Technology Co., LTD, Lot: GR2021-08, GR2021-08, GR2021-08 respectively), Creatinine (CRE) Determination Kit, Urea nitrogen (BUN) test kit, Urine protein quantitative test kit (Nanjing Jiancheng Institute of Biological Engineering, Lot: 20211014, 20210908, 20210730 respectively), RIPA cell lysate (Beyotime, Lot: 09271919023), Sprouty1 antibody (Bioss, Lot: AD08156610), ERK1/2 antibody, p-ERK1/2 antibody (CST, Lot: 9, 11 respectively), CREB antibody (Bioworld, Lot: CJ36131), p-CREB antibody (Bioss, Lot: AD19568820), GAPDH antibody (Zsbio, Lot: 210040421), Goat anti-mouse IgG (Zsbio, Lot: 140193), Goat anti-rabbit IgG (Zsbio, Lot: 202700514), ECL hypersensitive luminescence Kit (Thermo, Lot: VC298015), Trizol (Life technogies, Lot: 332607), Novostart SYBR qPCR Super Mix Plus (Novoprotein, Lot: 0520331), Prime Script™RT reagent Kit with gDNA Eraser (TaKaRa, Lot: AKF1919A), Xylene (Tianjin Kaitong Chemical Co., LTD, Lot: 20210619), Hematoxylin dye, Alcohol-soluble eosin dye, Masson trichromatic dyeing solution, PAS staining kit (Ebiogo, Lot: 06252114, 06242112, 06282115, 06272109 respectively).

Drugs preparation

The drugs used in the experiment are Qihuang Jianpi Zishen decoction (QJZ), prednisolone acetate tablets and mycophenolate mofetil capsules. QJZ is composed of eight herbs of Astragalus (10g), Rehmannia (10g), Dodder (10g), Chinese Yam (15g), Poria (10g), Atractylodes (10g), Raspberry (10g) and Fructus Rosae Laevigatae (10g). All herbs were purchased from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Hefei, China). According to the traditional Chinese medicine decocting method to prepare QJZ, the process is as follows: put all Chinese medicine into a ceramic pot, add about 500ml water to soak for 30 minutes, then boil with high fire, then simmer with low heat for 30 minutes, filter out the liquid; For the rest of the drug, the process is repeated again and the liquid from the two filters is mixed. 100mL of the final concentrated solution was yielded (pure solution, an adult's daily dose).

Prednisolone acetate tablets were bought from Tianjin Tianyao Pharmaceutical Co., LTD. (Tianjin, China, Lot: H12020689). Mycophenolate mofetil capsules were bought from Wuxi Fuqi Pharmaceutical Co., LTD. (Wuxi, China, Lot: H20080642). Prednisolone acetate tablets and Mycophenolate mofetil capsules were dissolved in normal saline.

Experimental protocol

Twenty-four female MRL/lpr mice were fed adaptively for one week before they were divided into different groups at random, which include the model group,

prednisolone group (PED), mycophenolate mofetil group (MMF) and QJZ group (QJZ). Another six C57BL/6 mice were considered as controls. Each group consists of 6 mice (n=6). Mice in the QJZ group received QJZ (13ml/kg/d), those in the PED and MMF groups received prednisolone acetate tablets (2.73mg/kg/d) and mycophenolate mofetil capsules (0.26g/kg/d), in accordance with pharmacology experiments performed in the Key Laboratory of Anhui Province. Equal amount of normal saline was administered to mice in the model and control groups. Mice received the indicated drugs at a fixed time once a day for continuous 4 weeks. During the course of oral gavage, standard laboratory chow and clean water were easily accessible to experimental mice.

Before the first administration and after administration on day 27, a metabolic cage was used to house the mice in order to collect urine sample for 24 hours. Four weeks later, mice were deeply anesthetized by 1% pentobarbital sodium (50mg/kg). Then blood samples were drawn from the orbit and immediately centrifuged at 3000 rpm for 15 min to separate the serum for detecting the anti-dsDNA autoantibody, C3, C4, SCr and BUN levels. Afterwards, the mice were euthanized by cervical dislocation. Kidney samples were quickly dissected and part of them treated with 10% neutral formaldehyde for fixation. Liquid nitrogen was used for freezing the rest of the kidney samples, which were then kept at -80^{o}C.

Histopathological examination (HE)

Neutral formaldehyde of 4% concentration was used to fix kidney tissue, which were then treated with ethanol for dehydration and embedded in paraffin; afterwards, the samples were cut into sections of 4μm, then stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) and Masson. The results of H&E, PAS and Masson staining were observed under a light microscope (magnification, x400).

The pathological specimens were scored with activity index (AI) and chronic index (CI) according to Austin scoring criteria (Austin *et al*., 1983) by experienced pathologists blinded to the treatment conditions. According to Austin scoring criteria, AI observed six indicators, including glomerular endothelial cell proliferation, cellular crescent body, inflammatory cell infiltration, nuclear fragmentation and necrosis, clear thrombus or platinum ear change, interstitial inflammatory cell infiltration. Each indicator was scored by 0,1,2,3 according to the degree of lesion (-)~(+++). The scores of cytologic crescent formation and fibrinoid necrosis could be double scored and the total AI score was 24. CI mainly included four indicators: glomerulosclerosis, fibrous crescent formation, interstitial fibrosis and tubular atrophy. The score of each was 0,1,2,3 according to the severity of lesion, mild, moderate and severe and the total score was 12.

Evaluation of renal function

SCr (serum creatinine), BUN (blood urea nitrogen) and 24hPRO (24-hour urine protein) levels were quantified using Creatinine (CRE) Determination Kit, Urea nitrogen (BUN) test kit and Urine protein quantitative test kit following the guideline supplied by the manufacturer to assess renal function.

Anti-dsDNA autoantibody, C3, C4 detection

ELISA was used for establishing the serum anti-dsDNA autoantibody levels. Purified mouse anti-double stranded DNA (dsDNA) antigen was coated with 96-well microplate and standard well 10 was set on the plate. Serum was diluted and samples were added. 50ul hrp-conjugate reagent was added to individual well and cultured for 30 min at 37^{o}C after sealing with sealing plate membrane. After washing, add color developing agent to each well and develop color at 37^{o}C for 10 min away from light. The absorbance at 450 nm is reported as the result. ELISA was used for quantifying serum levels of C3 and C4, the process adhered to the guideline supplied by the manufacturer.

Real-time quantitative PCR (qRT-PCR) for Sprouty1, ERK1/2, CREB mRNA and LncRNA GAS5, miR-21-5p expression

Total RNA was obtained from frozen renal cortical tissues with the use of TRIzol reagent adhering to standard instruction given by the manufacturer. The PrimeScript RT reagent Kit was used for reversely transcribing the total RNA was into cDNA. Amplification of the target cDNA fragment was achieved with the ordinary PCR instrument (Hangzhou Lattice Scientific Instrument Co., LTD, Hangzhou, China, Type: K960). Sangon Biotech constructed the qPCR primer sequences (Sangon Biotech, Shanghai, China) which is illustrated in table 1. Each experiment was done in triplicate to enhance the accuracy and reliability. After amplification, β-actin was adopted as an internal reference, however, standardization of the relative expression level of miR-21-5p was conducted with respect to U6 expression level. The $2^{-\Delta\Delta Ct}$ method was used to establish the gene expression levels.

Western blot analysis

The protein expressions of Sprouty1, p-ERK1/2, ERK1/2, p-CREB and CREB in renal cortical tissues were established using Western blot analysis. All proteins were extricated on ice. Put about 0.1g of renal tissue homogenate in a 1-ml Eppendorf (EP) tube and directly add RIPA lysis buffer which contains 0.6mM PMSF. After full lysis, centrifugation was performed at 12000 rpm for 15 min to collect the protein sample. Protein concentration was quantification by the BCA method. Protein samples of the same amount were subjected to SDS-PAGE before they were transferred onto polyvinylidene difluoride membranes (Millipore,

Billerica, USA, Lot: R7SA9081E). After blocking at room temperature with 5% skimmed milk for 2h, membranes were incubated with primary antibodies overnight at 4°C, the antibodies were specific for Sprouty1 (1:1500), ERK1/2 (1:1000), phospho-ERK1/2 (1:1000), CREB (1:1000), phospho-CREB (1:2000) and GAPDH; then, membranes were incubated with secondary antibody at room temperature for 1.2h. ECL luminescence kit was used to detect proteins according to the instruction supplied by the producer. The bands were analysed using the Image J software.

Ethical approval

All animal experimental protocols were carried out following the international guidelines and approved by the Ethics Committee of Anhui University of Traditional Chinese Medicine.

STATISTICAL ANALYSIS

All data were assessed by SPSS 21.0 and GraphPad Prism version 8.0. The results were illustrated as means ± SDs. The student's t-test was used to assess the differences between two groups. One-way ANOVA was used to evaluate the dissimilarities between multiple groups and multiple comparisons were made within the groups. $P<0.05$ was adopted as the threshold of statistically significant differences.

RESULTS

Effect of QJZ on pathological damage in kidney tissues of MRL/lpr mice

As shown in fig. 1, histologically, there was no inflammatory exudation and connective tissue hyperplasia in kidneys of normal mice and the glomerulus is well structured. The renal tissue of MRL/lpr mice showed the broken glomerular structure, proliferation of cells in capillaries and mesangial area, thickening of basement membrane and increasing of matrix. Masson staining showed a few blue areas, indicating that renal fibrosis was mild in MRL/lpr mice. After treatment with mycophenolate mofetil, prednisolone and QJZ, renal pathological changes were significantly reduced, glomerular structure tended to be normal, mesangial cell proliferation was reduced and the blue areas were not obvious. Moreover, as depicted in fig. 1 (a) and 1 (b), the score of activity index (AI) and chronic index (CI) of renal tissue decreased significantly ($P<0.01$).

The effect of QJZ on renal function in MRL/lpr mice

As shown in fig. 2, in comparison with the control group, the SCr, BUN and 24hPRO levels in the model group were considerably higher ($P<0.01$). In comparison with the model group, the SCr, BUN and 24hPRO levels in the PED, MMF and QJZ groups were decreased considerably ($P<0.01$).

The effect of QJZ on anti-dsDNA autoantibody, C3, C4 levels in MRL/lpr mice

As fig. 3 demonstrates, in contrast to the control group, the model group exhibited a much higher anti-dsDNA autoantibody level ($P<0.01$) and a much lower C3, C4 levels ($P<0.01$). Contrasting to the model group, the anti-dsDNA autoantibody levels in the PED, MMF and QJZ groups were greatly reduced ($P<0.01$) and C3, C4 levels in those groups were substantially higher ($P<0.01$).

The Effect of QJZ on expression of lncRNA GAS5/miR-21-5p/Sprouty1 axis in the kidney tissues of MRL/lpr mice

As shown in fig. 4, in comparison with the control group, a substantial decrease in the expression of lncRNA GAS5, sprouty1 mRNA and sprouty1 protein was observed in the model group ($P<0.01$) and the expression of miR-21-5p in the model group was considerably elevated ($P<0.01$). Compared with the model group, QJZ significantly increased the expression of lncRNA GAS5, sprouty1 mRNA and sprouty1 protein ($P<0.01$), reduced the expression of miR-21-5p ($P<0.01$), similar to the effect produced by the use of prednisone and mycophenolate mofetil (fig. 4).

The effect of QJZ on expression of ERK/CREB pathway in the kidney tissues of MRL/lpr mice

As fig. 5 displays, the mRNA expression of ERK1, ERK2 and CREB in the model group rose considerably in comparison to the control group ($P<0.01$), the phosphorylation level of ERK and CREB detected by western blotting in the model group surged, too ($P<0.01$). Relative to the model group, the QJZ, PED and MMF groups presented with substantially declined mRNA expression of ERK1, ERK2 and CREB ($P<0.01$), reduced phosphorylation level of ERK ($P<0.05$), as well as considerably decreased phosphorylation level of CREB (fig. 5).

DISCUSSION

In this experiment, renal pathology of our MRL/lpr mice showed excessive proliferation of mesangial cells, inflammatory infiltration and increased fibrous tissue. It is well known that the primary renal histological features of early LN are proliferation of mesangial cells, followed by an increase in extracellular matrix and infiltration of inflammatory cells (Wang *et al*., 2018a). Our study showed that QJZ treatment could ameliorate these pathological features and decrease the activity index (AI) and chronic index (CI) scores of kidney inflammation in MRL/lpr mice.

Table 1: Primer sequence of RT-qPCR.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
β-actin	AGTGTGACGTTGACATCCGT	TGCTAGGAGCCAGAGCAGTA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
CREB	CTTGTACCACCGGTATCCAT	ACTGGATAACTGATGGCTGG
LncRNA GAS5	AGGAAGCTGGATAACAGAGC	TGAGGTGACCCATTAATACCT
Sprouty1	AGAGCTCTGCGGGCTAA	TAGTGCAGAGCCTGGGG
ERK2	CCCTCCTCCCACTCGTAG	GAGGACAACACAGAGGAGAG
ERK1	CTGGAAGCCATGAGAGATGT	TATATACTTGAGGCCCCGGA
miR-21-5p	ACACTCCAGCTGGGTAGCTTATCAGACTGA	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACA
miR-21-5p-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACA	

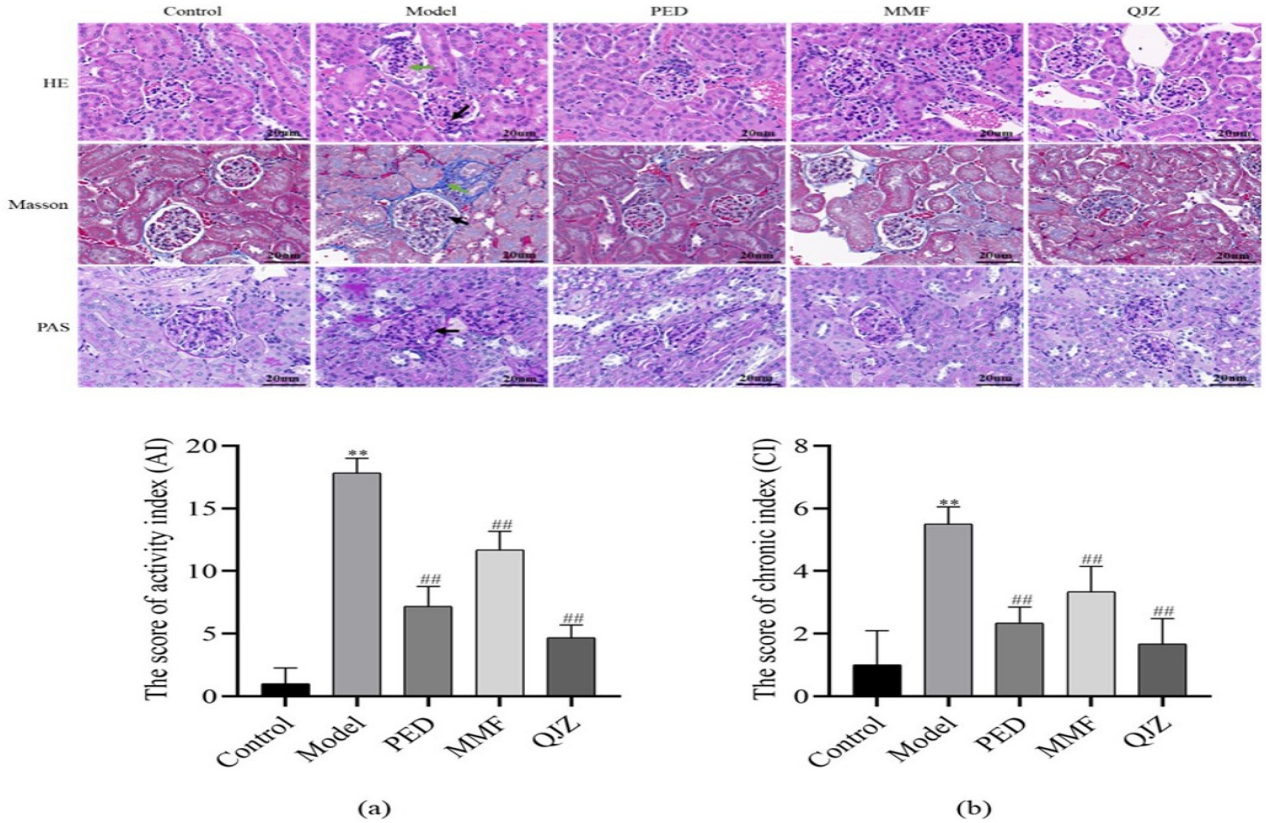

Fig. 1: The effect of QJZ on pathological damage in kidney tissues of MRL/lpr mice. H&E, Masson and PAS staining were used to visualize kidney tissues; the magnifying scale is 400. (a) The score of activity index (AI). (b) The score of chronic index (CI).*P<0.05 and **P<0.01 versus the control group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus the model group.

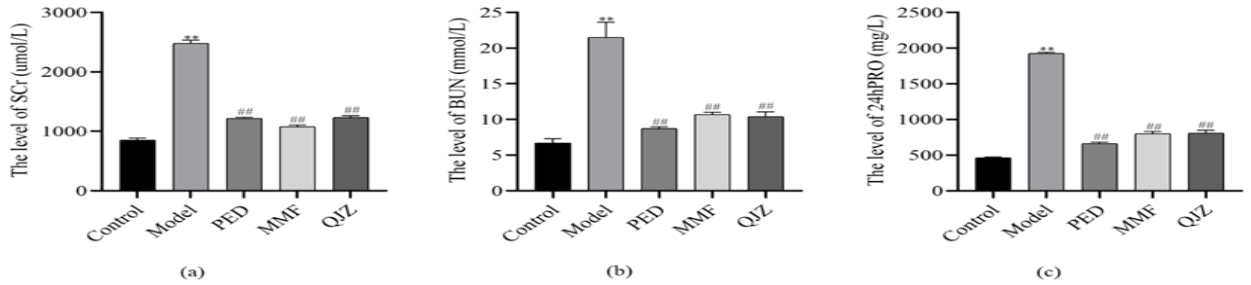

Fig. 2: The effect of QJZ on renal function in MRL/lpr mice. (a) The SCr level. (b) The BUN level. (c) The 24hPRO level. *P<0.05 and **P<0.01 versus the control group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus the model group.

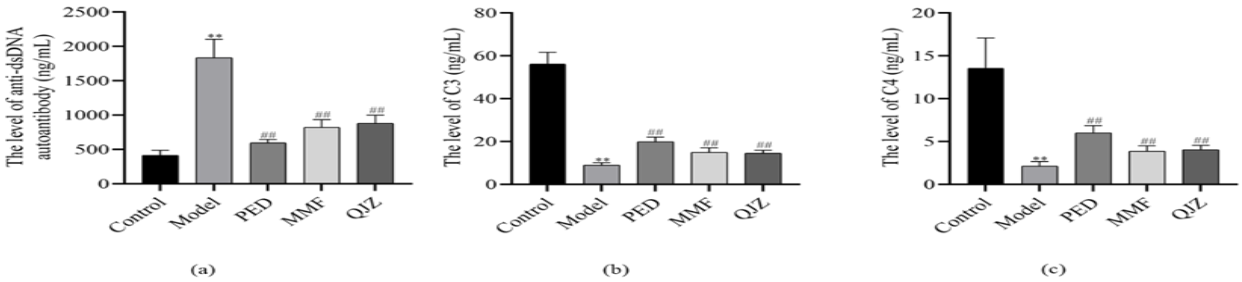

Fig. 3: The effect of QJZ on anti-dsDNA autoantibody, C3, C4 levels in MRL/lpr mice. (a) anti-dsDNA autoantibody level. (b) C3 level. (c) C4 level. **P*<0.05 and ***P*<0.01 versus the control group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus the model group.

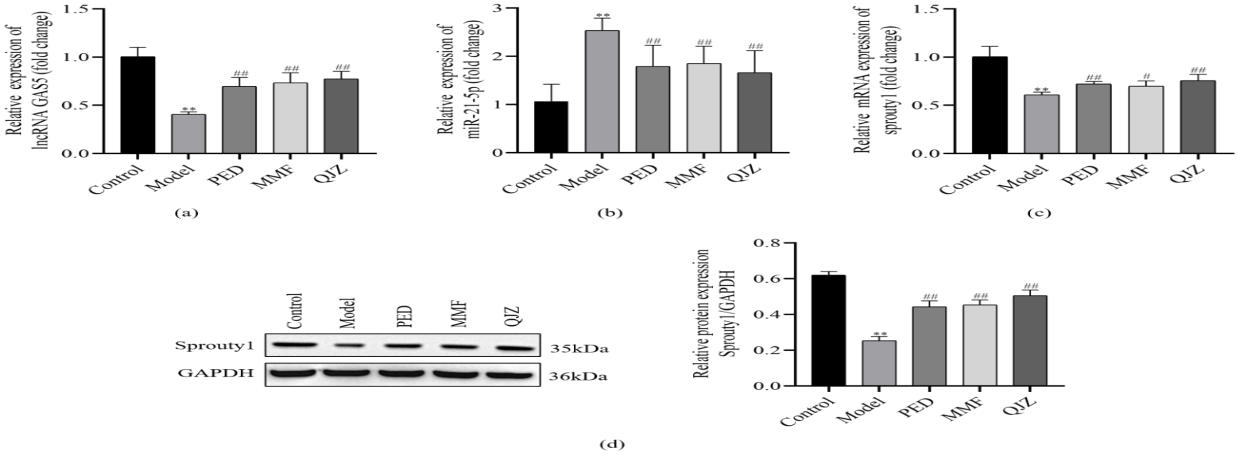

Fig. 4: The effect of QJZ on expression of lncRNA GAS5/miR-21-5p/sprouty1 axis in the kidney tissues of MRL/lpr mice. (a) qRT-PCR quantified the expression of lncRNA GAS5 from renal tissues. (b) qRT-PCR also assessed the level of miR-21-5p from renal tissues. (c) The mRNA expression of sprouty1 from renal tissues was determined by qRT-PCR. (d) Western blotting was used to assess the protein expression of sprouty1. **P*<0.05 and ***P*<0.01 versus the control group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus the model group.

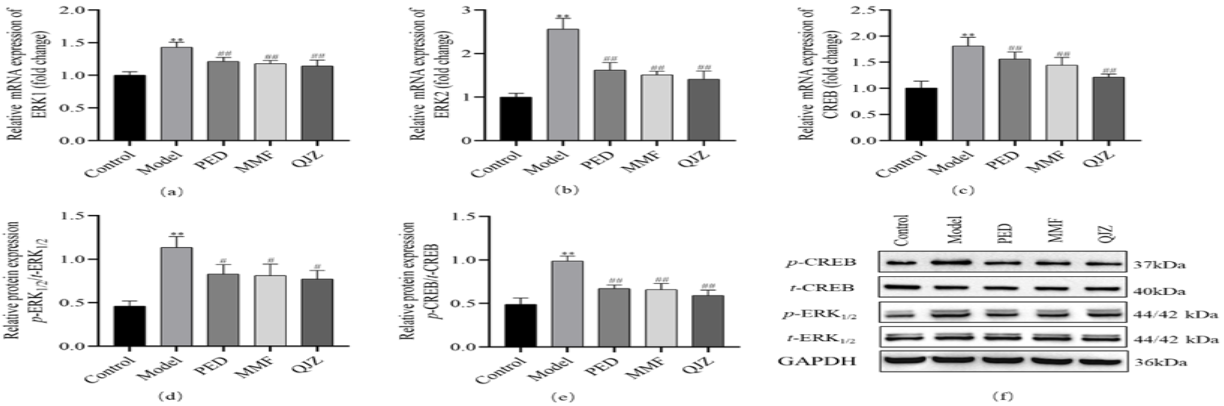

Fig. 5: The Effect of QJZ on expression of ERK/CREB pathway in the kidney tissues of MRL/lpr mice. (a) The mRNA expression of ERK1 from renal tissues was assessed by qRT-PCR. (b) The mRNA expression of ERK2 from renal tissues was established by qRT-PCR. (c) The mRNA expression of CREB from renal tissues was determined by qRT-PCR. (d) Western blotting was adopted to scrutinize the phosphorylation level of ERK. (e) Western blotting was utilized to assess the phosphorylation level of CREB. (f) The protein expression of t-ERK1/2, p-ERK1/2, t-CREB and p-CREB from renal tissues was investigated using western blotting. **P*<0.05 and ***P*<0.01 versus the control group; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus the model group.

Mesangial cells have special functions, including producing cytokines, secreting cellular matrix, supporting the glomerular capillary network; they are also involved in clearing macromolecular substances and phagocytosis, both of which are crucial for maintaining normal glomerular structure and function. An uncontrolled and excessive mesangial cell proliferation can provoke the release of surplus inflammatory factors and encourage the secretion of extracellular matrix, induce glomerular lesions and renal inflammation (Chen *et al*., 2020). Glomerular lesions may lead to persistence of proteinuria and abnormal renal function. Our study showed that the SCr, BUN and 24hPRO levels decreased significantly in QJZ group. These results indicated that QJZ could improve glomerular lesions and renal function in MRL/lpr mice.

In SLE, deposition of complement associated immune complexes leads to mild or substantial proliferation of glomerular mesangial cells. This progression would induce numerous types of immune complex glomerulonephritis (Tortajada *et al*., 2019, Yoshida and Nishi, 2022, Nowling, 2022). Furthermore, anti-DNA antibodies in SLE can induce excessive proliferation of glomerular mesangial cells by secreting IL-1β, IL-6 and TNF-α and participate in the occurrence of LN (Wang *et al*., 2021). These are due to immune system dysfunction in SLE. This experiment displayed that the QJZ helped to lower anti-dsDNA autoantibody levels and raise C3 and C4 levels, which proved the role of QJZ in regulating immune function and contributed to improving kidney disease.

The results showed that QJZ could significantly improve glomerular lesions and protect renal function in MRL/ LPR mice. We also made a preliminary exploration on the mechanism of its action. A large number of studies have shown that lncRNA GAS5 and miR-21 are both abnormally expressed in SLE patients and lupus mice and lncRNA GAS5 can target and bind miR-21 (Ge *et al*., 2019, Yu *et al*., 2020, Liu *et al*., 2020, Liu *et al*., 2021, Suo *et al*., 2018, Xie *et al*., 2022, Fan *et al*., 2021). Sprouty1 is a downstream target gene of miR-21 (Wang *et al*., 2018b, Chai *et al*., 2018) and sprouty1 protein is also recognized as a classical inhibitor of ERK and other signal transduction pathways, regulating cell proliferation (Li *et al*., 2020). We have reason to speculate that GAS5 can target miR-21 to regulate the expression of sprouty1 protein and play a role in regulating ERK signal transduction pathway as GAS5/miR-21/sprouty1 axis. More importantly, the ERK/CREB pathway was demonstrated to inhibit the proliferation of glomerular mesangial cells induced by high glucose (Sugiura *et al*., 2000, Haneda *et al*., 1997). Therefore, in order to preliminarily explore the mechanism of QJZ, in this experiment we observed the expressions of GAS5/miR-21/sprouty1 axis and ERK/CREB pathway in MRL/lpr mice kidney. Our results showed that QJZ can regulate the abnormal expression of GAS5/miR-21/sprouty1 axis and reduce the high phosphorylation level of ERK/CREB pathway in MRL/lpr mice renal, which may be the pathway through which QJZ can improve glomerular lesions and protect renal function in MRL/lpr mice.

CONCLUSIONS

In summary, our experiment indicates that QJZ has a certain therapeutic effect on renal injury in MRL/lpr mice. Its mechanism may be closely related to regulating the expression of GAS5/miR-21/sprouty1 axis, inhibiting the ERK/CREB pathway and inhibiting the proliferation of glomerular mesangial cells. However, these results are only preliminary and need further experimental verification, such as preparing QJZ drug-containing serum and human glomerular mesangial cell experiment.

DATA AVAILABILITY

The data used to support the findings of this study are included within the article and are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

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