RESEARCH ARTICLE

Effect of Jiawei Shenqi Pill on renal cell apoptosis and PERK/eIF2α pathway in diabetes rats

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Received: February 6, 2025; **accepted:** June 2, 2025.

Diabetes is a chronic metabolic disease characterized by high blood sugar levels. Jiawei Shenqi Pill is a traditional Chinese medicine compound that has the effects of tonifying kidney and qi, promoting blood circulation and removing blood stasis. It has been used to treat various kidney diseases. However, its therapeutic effect on diabetes nephropathy and its mechanism are still unclear. This study explored the effect of Jiawei Shenqi Pill on the control of renal cell apoptosis in diabetes rat model and investigated whether its mechanism related to the inhibition of PERK/eIF2α signaling pathway. A total of 80 Zucker Diabetic Fatty (ZDF) rats, 20 Sprague Dawley (SD) healthy rats, and 20 Goto Kakizaki (GK) rats were involved in this research. ZDF rats were divided into Irbesartan and high, medium, low dose of Jiawei Shenqi Pill groups, while SD healthy rats were set as the Blank Group (BG) and GK rats were set as the model group. The model and BG groups were treated by only pouring the equal volume of distilled water into the stomach for 12 consecutive weeks, while the ZDF group received 0.026g/kg Irbesartan suspension, 51.78 g/kg, 21.6 g/kg, 10.8 g/kg high-, middle-, low-dose of Jiawei Shenqi Pill administered by gavage. Bicinchoninic Acid (BCA) method was used to detect the 24-hour urine protein content of rats, and immunohistochemistry was used to detect blood protein indicators. The general state, renal morphology, urinary protein, and expression of PERK/eIF2α signaling pathway proteins in rats were examined. The HE staining analysis results demonstrated that the high-dose Jiawei Shenqi Pill group had relatively clear cortical and medullary structures in renal tissue, reduced swelling of renal tubular epithelial cells, relatively fewer protein casts, reduced interstitial inflammatory cells, and significant improvement in glomerular structure. The renal cell apoptosis rates of the Irbesartan group, Jiawei Shenqi Pill low-dose group, medium-dose group, and high-dose group were 22.24%, 23.52%, 20.41%, and 16.39%, respectively. The results of this study proved that the high-dose of Jiawei Shenqi Pill had effective intervention on renal cell apoptosis in diabetes rats, which provided guidance for effective intervention and nursing in clinical practice.

Keywords: Jiawei Shenqi pill; diabetes; renal cell apoptosis; signal pathway.

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Introduction

The pathogenesis of diabetes nephropathy is complex, involving a variety of cellular signaling pathways and molecular mechanisms. Endoplasmic Reticulum (ER) stress and related signaling pathways play an important role in the pathogenesis of diabetes nephropathy. The PERK/eIF2α signal pathway is one of the key signal pathways in ER stress, which maintains the

stability of the intracellular environment by regulating the stress response within cells. Therefore, inhibiting the activation of PERK/eIF2α signal pathway may have a protective effect on diabetes nephropathy. Renal cell apoptosis (RCA) has a vital influence on eliminating unnecessary and damaged cells and various normal biological processes, but excessive cell apoptosis will lead to the emergence of neurodegenerative diseases.

Zhang *et al*. explored the role of urinary lithium protein A (UA) in the mouse RIR model. In which, after UA administration, RCA and active oxygen degrees were cut down. The results showed that UA could lessen oxidative stress and improve autophagy, thereby protecting the kidneys from ischemia-reperfusion hurt [1]. Ibaokurgil *et al*. analyzed the impacts of melatonin on kidney inflammation, oxidative stress, cell apoptosis, and inflammatory response through rat experiments. From the analysis of biochemical and inflammatory parameters, histopathology, immunohistochemistry, and other indicators, the research showed that melatonin could significantly reduce the increase of serum urea and creatinine levels caused by acrylamide [2]. Ma *et al*. found that the exposure of triclosan in urine was directly related to albumin, a biomarker of renal function, suggesting that the use of triclosan might lead to renal dysfunction. An *in vitro* experiment was then initiated to investigate the molecular mechanism of triclosan on glomerular cell toxicity [3]. Current research indicates that renal inflammation leads to damage to renal podocytes, which in turn causes a significant amount of urinary protein and edema in patients with kidney disease. Jiawei Shenqi Pill involves multiple biological processes such as inflammation, protein hydrolysis, transcription and translation, redox reactions, and immune responses, and therefore has a therapeutic mechanism in RCA. However, the therapeutic effect and mechanism of Jiawei Shenqi Pill on diabetes nephropathy are still unclear.

According to the aforementioned research outcomes, this study investigated the control effect of Jiawei Shenqi Pill on RCA in diabetes rats and whether its mechanism was related to the inhibition of PERK/eIF2α signal pathway, hoping to cut down the risk of renal injury and renal failure of diabetes rats and improve the treatment and care of diabetes patients by comparing the dosage resulted from this study [4, 5]. This study on the therapeutic effect of Jiawei Shenqi Pill provided a new therapeutic strategy and theoretical basis for the current treatment of diabetes nephropathy.

Materials and methods

Experimental animals

A total of 80 Zucker Diabetic Fatty (ZDF) male rats, 20 Sprague Dawley (SD) healthy male rats, and 20 Goto Kakizaki (GK) male rats (Kavins Experimental Animal Company, Changzhou, Jiangsu, China), 4 - 5 weeks old, average body weight of 180 ± 20 g were involved in this research. All animals were raised in Animal Experiment Center, Chengde Medical College, Chengde, Hebei, China for one week at room temperature of 18 - 24°C, relative humidity of 40 - 50%, and daily light exposure of 12 hours with free access to food and water. All procedures of this research were approved by the Animal Welfare and Ethics Review Committee of Chengde Medical College, Chengde, Hebei, China (Approval No. YSY-DWLL-2022072).

Experimental design

After one week observing for possible abnormality, the healthy animals were assigned to three major groups including experimental group with 80 ZDF rats, model group (MG) with 20 GK rats, and blank group (BG) with 20 SD rats. The experimental group was additionally divided into four subgroups of Irbesartan group, low-dose (LDG), medium-dose (MDG), and high-dose groups (HDG) of Jiawei Shenqi Pill with 20 rats in each group. The suspensions of Jiawei Shenqi Pill granules and Irbesartan were gavage administrated with the concentrations of 10.8

g/kg, 21.6 g/kg, 51.78 g/kg, and 0.026 g/kg for LDG, MDG, HDG, and Irbesartan, respectively, while the BG and MG groups were given the same volume of distilled water. The Irbesartan was obtained from Sanofi Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China). Jiawei Shenqi Pill granule was composed of 24 g cooked *rehmannia*, 12 g *cornus* meat, 12 g yam, 9 g *poria cocos*, 9 g *alisma orientalis*, 9 g *paeonol*, 3 g *cassia bark*, 6 g *monkshood*, 18 g *astragalus membranaceus*, 6 g wine-treated *rhubarb*, 3 g leech, 18 g *rosa laevigata*, 18 g *gorgon* fruit provided by Shenwei Pharmaceutical Group Co., Ltd. (Shijiazhuang, Hebei, China) [6]. The experiments lasted for 12 weeks [7, 8], while the blood was taken from the tail vein every 2 weeks to measure blood sugar. Meanwhile, urine samples were collected using a metabolic cage to detect microalbumin in urine. 12 h after the final administration, the rats were narcotized with 3% Pentobarbital sodium after weighing. The eyeballs were taken for blood, and the serum were preserved after centrifugation. The abdominal cavity was exposed, the kidneys were dissected and weighed, washed with normal saline. The renal cortex was cut off on ice and stored in liquid nitrogen and 4% Paraformaldehyde for standby [9, 10].

Observation of general state of rats

During the experiment, the general states of rats were observed and recorded, which included hair status, behavioral activities, fecal conditions, water consumption, and food consumption. The animal body weight was measured on the 7^{th} and 14^{th} days of drug administration, while the animal anal temperature was also measured using an electronic thermometer.

Detection of biochemical indicators and urine protein quantification

The mouse blood creatinine, urea nitrogen, and urinary trace protein assay kits (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, Jiangsu, China) and a Hitachi 7170A fully automatic biochemical analyzer (Hitachi Corporation, Tokyo, Japan) were used to measure the blood biochemical indicators and urine protein of experimental animals following the manufacturer's instructions.

Morphological observation of rat kidney tissue

Paraffin sections were used to prepare observation samples of rat kidney tissue. The right kidney tissue was placed in a 10% neutral formaldehyde and fixed for 24 hours. After rinsing with distilled water, the tissue was dehydrated in ethanol with gradient concentrations of 75%, 80%, 90%, 95% and 100% in sequence by soaking in each concentration for 30 minutes before placing in xylene I solution for 30 minutes until transparent. The transparent treated kidney tissue was placed in 65℃ paraffin and soaked for 60 minutes before placing in an embedding plate. The solidified kidney tissue wax block was sliced using an RM2015 slicer (Leica Corporation, Wiesler, Hesse, Germany) and trimmed to a thickness of approximately 5 μm slice. The slice was observed using an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan). The slice was further observed using a Hitachi H-7650 projection electron microscope (Hitachi, Tokyo, Japan) after Hematoxylin-Eosin (HE) staining.

Detection of apoptosis in renal tissue cells

Paraffin sections of renal tissue were de-waxed and hydrated and mixed with 20 mg/mL protease K for 20 minutes at 37°C followed by a 3% hydrogen peroxide solution at room temperature for 20 minutes. After incubation with biotin labeling solution in dark at 37°C for 60 minutes, Streptavidin-HRP working solution was added and incubated at room temperature for 30 minutes followed by DAB staining, hematoxylin staining, gradient ethanol dehydration, xylene transparent, and neutral resin sealing [11]. The TUNEL cell apoptosis detection kit and proteinase K (Biyun Tian Company, Shanghai, China) were employed following the supplier's instructions.

Detection of the indicators

The enzyme-linked immunosorbent assay (ELISA) was applied to detect the expression of proteins in the sample. Briefly, renal tissue paraffin sections were de-waxed and hydrated, and the

antigens were repaired using citrate buffer. After incubating with 3% H_2O_2 at room temperature for 15 minutes and sealed with goat serum at 37℃ for 30 minutes, the first antibodies including TGF-β1, E-cadherin, Vimentin, FN, PAI-1, Collage IV at 1:200 dilution, and rabbit anti-p-eIF2 α, eIF2 α, BAX, BCL-2, cleaved caspase-3 (ImmunoWay Biotechnology Company, Plano, TX, USA) were added and incubated at 4℃ overnight. After the addition of a second antibody at 1:1,000 dilution and incubation at 37℃ for 30 minutes, the horseradish enzyme labeled Streptomyces ovalbumin was added and incubated at room temperature for 30 minutes. After diaminobenzidine (DAB) coloring and hematoxylin staining [12], the results were measured using a Varioskan LUX enzyme-linked immunosorbent assay (ELISA) reader (Thermo Fisher, Waltham, MA, USA).

Detection of PERK/eIF2α expression of signal pathway proteins

Rat kidney tissue was ground using a tissue homogenizer and lysed using radio immunoprecipitation assay (RIPA) lysate. After centrifugation, the supernatant was collected, and the protein density was measured using bicinchoninic acid (BCA) method and a NanoDrop 2000 UV visible spectrophotometer (Thermo Fisher, Waltham, MA, USA). Total proteins and 5× loading buffer were mixed and denatured at 100℃ for 10 min before loading on 10% SDS-PAGE gel for 2 h electrophoresis using Bio Rad Mini PROTEAN3 electrophoresis system (Bole Corporation, Hercules, CA, USA) followed by polyvinylidene fluoride (PVDF) membrane conversion for 120 min using Mini Trans Blot transfer system (Bole Corporation, Hercules, CA, USA). The PVDF membrane was then incubated with 5% BSA for 1.5 hours followed by incubating with first antibodies of p-PERK (1:1,000), PERK (1:3,000), ATF4 (1:2,000), CHOP (1:2,000) (Proteintech Corporation, Chicago, IL, USA), p-eIF2 α (1:1,000), eIF2 α (1:2,000) (ImmunoWay Biotechnology Company, Plano, TX, USA), and β-actin (1:5,000) (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd, Beijing, China) overnight. After washing the membrane, the secondary antibody at 1:5,000 dilution was incubated with the PVDF for 1 hour. Enhanced chemiluminescence (ECL) method was applied for color rendering. The image was captured using Kodak 2000MM imaging system (Kodak Company, Rochester, NY, USA) [13]. ImageJ (https://imagej.net/ij/) software was used to analyze the Western blot results.

Statistical analysis

SPSS 21.0 (IBM, Armonk, NY, USA) was employed for statistical analysis of this study. The data was denoted as mean ± standard error (SE). One-way ANOVA was utilized for group comparison, and Tukey method was utilized for multiple comparisons. *P* value less than 0.05 indicated a statistically significant difference.

Results

The general state of experimental animals

The rats of BG showed good mental state, bright fur, normal diet and bowel movements, and no abdominal swelling. However, contrasted to BG, the MG rats demonstrated significant fall in food intake, dry hair, lethargy, chills, decreased urine output, and significant abdominal swelling. After administration of medicines, contrasted to the MG, each medication group had slight increase in food intake, significant improvement in mental health, and relief in abdominal edema. During the experiments, three MG rats and one Irbesartan group rat died. The results showed that the weights of the BG rats continued to rise during experiment, while the weights of the MG rats were lessened obviously at different time periods ($P < 0.01$). Contrast to the MG, the body weights of LDG, MDG, and HDG rats increased significantly on the 7th day of administration. On the 14th day of administration, the weights of rats in each treatment group significantly increased. Compared with the BG, the MG rats showed a significant increase in body weight ($P < 0.01$), while the body weights of rats in the LDG, MDG, and HDG significantly increased compared to that in the MG ($P < 0.01$). The weights of rats in the HDG were obviously larger than that in the

Table 1. General state changes of rats in each group.

Status	Group	Sample size	7th day of administration	14th day of administration
Weight(g)	BG	20	297.92 ± 22.71	344.32 ± 19.33
	MG	17	231.58 ± 39.35**	251.91 ± 20.78**
	Irbesartan	19	255.29 ± 19.69	276.28 ± 28.22^^
	LDG	20	261.19 ± 20.26^	280.07 ± 27.25^^
	MDG	20	270.94 ± 21.15^	290.62 ± 21.43^^
	HDG	20	275.25 ± 26.17^	303.04 ± 19.48^^
Rectal temperature (℃)	BG	20	37.24 ± 0.21	37.16 ± 0.14
	MG	17	35.91 ± 0.62**	35.25 ± 0.56**
	Irbesartan	19	36.10 ± 0.57	35.77 ± 0.74
	LDG	20	36.47 ± 0.56	36.82 ± 0.43^^
	MDG	20	36.59 ± 0.86	36.83 ± 0.31^^
	HDG	20	36.75 ± 0.49^^	37.14 ± 0.45^^

Notes: **: $P < 0.01$ compared to the BG. ^: $P < 0.05$ compared to the MG. ^^: $P < 0.01$ compared to the MG.

Irbesartan group. Overall, the weight growth of rats in each administration group was ranked as HDG > MDG > LDG > Irbesartan group. In contrast to the BG, the anal temperature of the MG rats obviously reduced at various time points ($P < 0.01$). Compared with the MG, the anal temperature of rats in the HDG increased dramatically on the 7th day of administration ($P < 0.01$). On the 14th day of administration, except for the Irbesartan group, the anal temperature of rats in each administration group increased significantly ($P < 0.01$). Overall, the anal temperature levels of rats in each administration group were ranked as HDG > MDG > LDG > Irbesartan group (Table 1).

Morphological observation of rat kidney tissue

There were no abnormal pathological changes in the kidney tissue of BG rats. The structures of the cortex and medulla were clear, and the morphological structures of the glomeruli and renal tubules were normal. MG rats demonstrated unclear boundaries between the cortex and medulla of the renal tissue, swelling of renal tubular epithelial cells, more visible protein and transparent tubular cells, a great deal of inflammatory cell infiltration in the interstitial tissues, glomerular hypertrophy, mesangial hyperplasia, and narrowing of the glomerular capillary lumen. Compared to the MG, the HDG demonstrated relatively clear cortical and medullary structures in the renal tissue of rats, reduced swelling of renal tubular epithelial cells, relatively fewer protein tubular types, reduced interstitial inflammatory cells, and significantly improved glomerular structure. The pathological damages of the kidney tissue in the other treatment groups were improved to varying degrees, but the improvement was not as significant as the HDG (Figure 1).

24-hour urine volume and urine protein levels

The results showed that the 24-hour urine output of the MG rats at all time points was significantly reduced compared to that of the BG ($P < 0.01$). The 24-hour urine output of rats in the LDG, MDG, and HDG significantly increased compared to MG ($P < 0.01$). The 24-hour urine volume in each administration group was ranked as HDG > MDG > LDG > Irbesartan group. The analysis of urinary protein levels showed that the 24-hour urinary protein levels of the MG rats were obviously reduced compared to BG at all time points ($P < 0.01$), while that in each administration group were significantly reduced at all time points compared to the MG ($P < 0.01$). On the 14th day of administration, the 24 h urinary protein level of rats in the HDG was obviously lower than that in the Irbesartan group. The 24-hour urinary protein levels of rats in each administration group were ranked as HDG < MDG < LDG < Irbesartan group (Table 2).

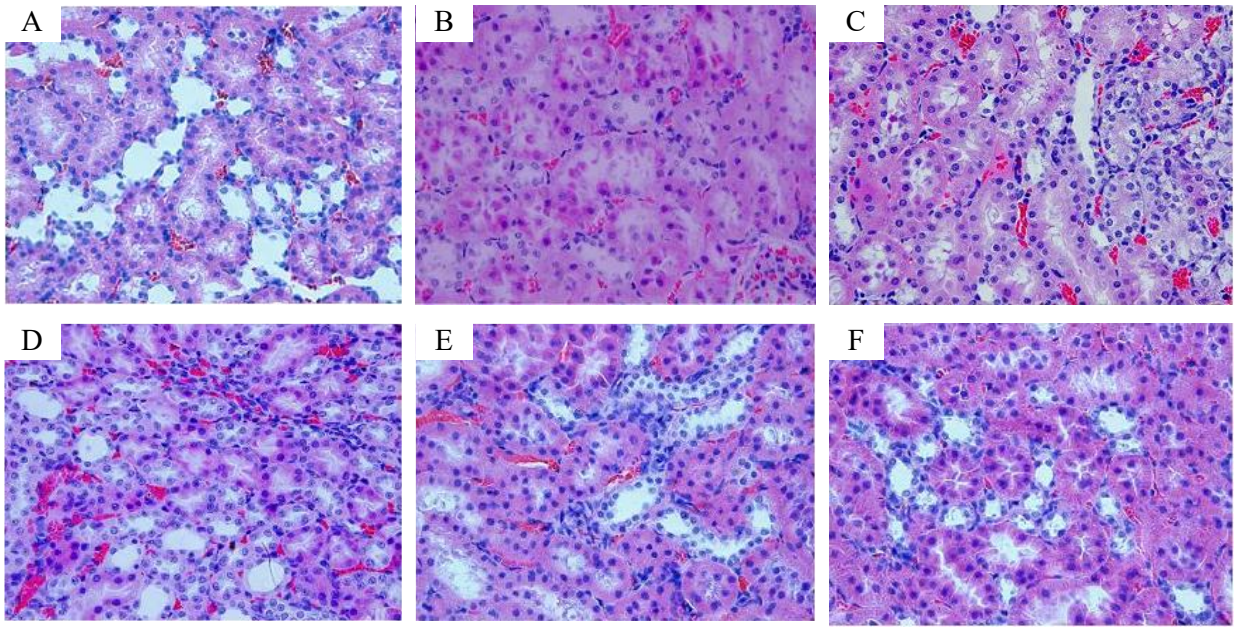

Figure 1. Morphological observation of rat renal tissues (HE staining). **A.** blank. **B.** model. **C.** Irbesartan. **D.** LDG. **E.** MDG. **F.** HDG.

Table 2. 24-hour urine volume and urine protein levels of rats in each group.

Status	Group	Sample size	7th day of administration	14th day of administration
Urine output (mL/24 h)	BG	20	27.82 ± 3.6	32.13 ± 7.54
	MG	17	15.46 ± 2.27**	13.77 ± 1.19**
	Irbesartan	19	17.92 ± 2.13^	20.18 ± 2.74^^
	LDG	20	17.99 ± 2.75^	20.25 ± 2.69^^
	MDG	20	18.2 ± 1.81^	21.1 ± 2.65^^
	HDG	20	19.61 ± 3.61^	23.15 ± 2.94^^
Urinary protein level (mL/24 h)	BG	20	78.51 ± 15.84	84.56 ± 12.8
	MG	17	234.98 ± 52.22**	302.02 ± 45.84**
	Irbesartan	19	154.6 ± 24.97^^	136.24 ± 15.43^^
	LDG	20	148.56 ± 31.3^^	118.76 ± 26.84^^
	MDG	20	142.83 ± 23.77^^	120.24 ± 39.02^^
	HDG	20	133.34 ± 35.64^^	110.55 ± 31.21^^

Notes: **: $P < 0.01$ compared to the BG. ^: $P < 0.05$ compared to the MG. ^^: $P < 0.01$ compared to the MG.

Effect of Jiawei Shenqi Pill on serum Scr, BUN, and UACR

The levels of serum creatinine (Scr), blood urea nitrogen (BUN), and urine albumin-to-creatinine ratio (UACR) in the MG rats were greatly raised compared to that in BG ($P < 0.05$), while them in the Jiawei Shenqi Pill and Irbesartan groups were decreased to different levels compared to the MG ($P < 0.05$) (Figure 2).

Effect of Jiawei Shenqi Pill on rat RCA

The average rate of RCA in the BG was 11.67%, while it was 34.18% in the MG. The RCA rates in the Irbesartan, LDG, MDG, and HDG were 22.24%, 23.52%, 20.41%, and 16.39%, respectively. Compared to the normal group, the RCA rate in the kidney tissue of the MG rats was obviously raised ($P < 0.05$), while that in all treatments and Irbesartan groups were reduced compared to that in the MG ($P < 0.05$) (Figure 3).

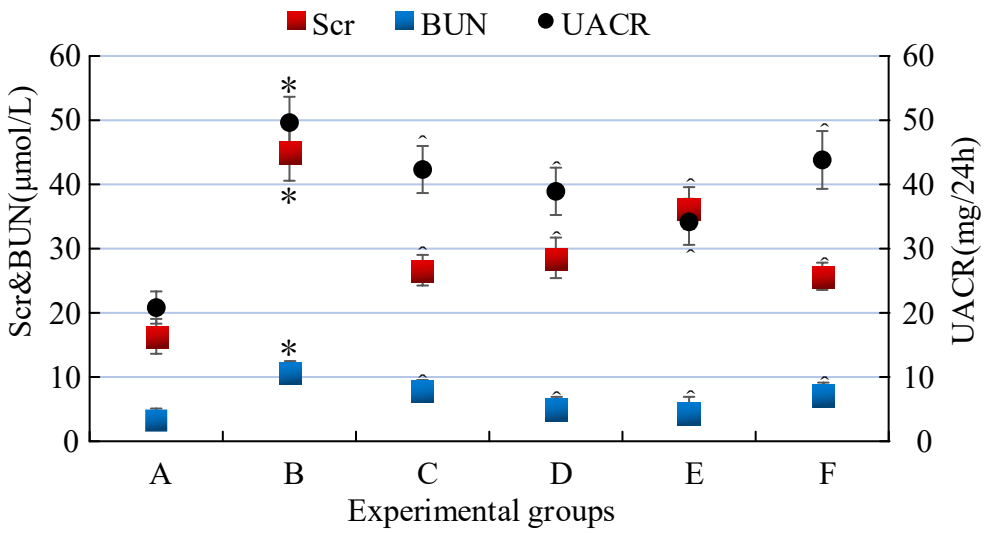

Figure 2. Effects of Jiawei Shenqi Pill on rat serum Scr, BUN, and UACR. **A.** blank. **B.** model. **C.** Irbesartan. **D.** LDG. **E.** MDG. **F.** HDG. *: $P < 0.05$ compared to BG. ^: $P < 0.05$ compared to the MG.

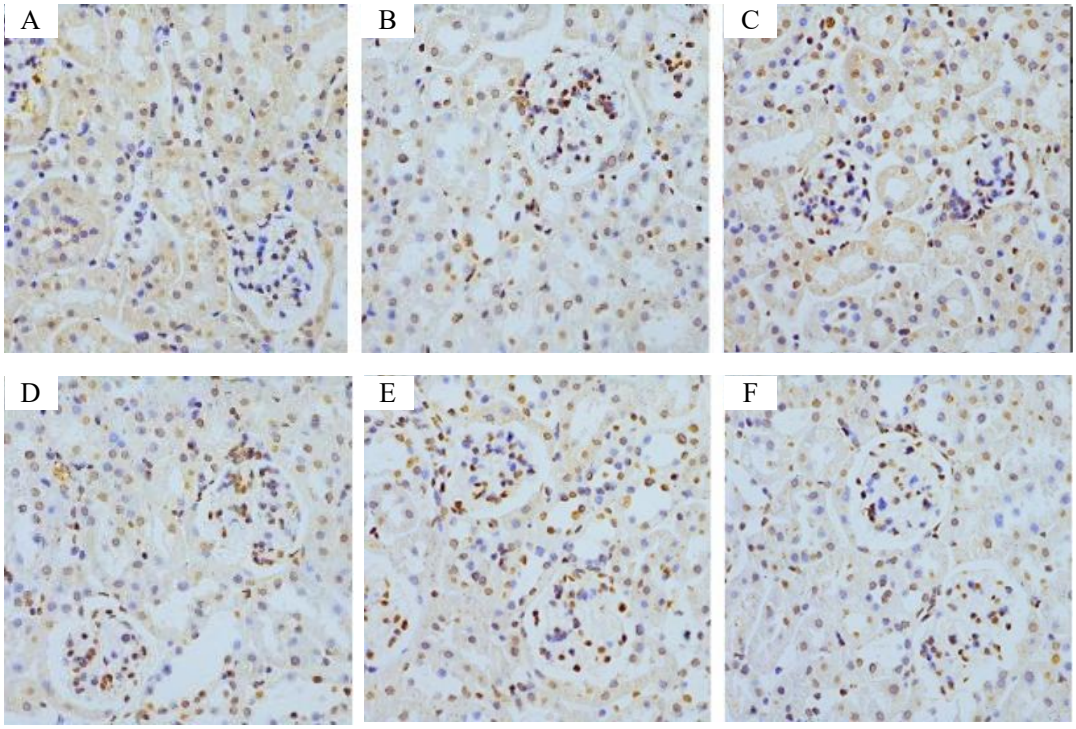

Figure 3. Effect of Jiawei Shenqi Pill on rat RCA. **A.** blank. **B.** model. **C.** Irbesartan. **D.** LDG. **E.** MDG. **F.** HDG.

Effect of Jiawei Shenqi Pill on the expression of indicators

The expression levels of BAX, BLC2, and cleaved caspase3 in each group were detected using immunohistochemistry (Figure 4). The results showed that the expressions of BAX and cleared caspase-3 proteins in the kidneys of the MG rats were increased compared with the BG, while BCL-2 protein expression level was decreased ($P < 0.05$). Further, the expression levels of BAX and

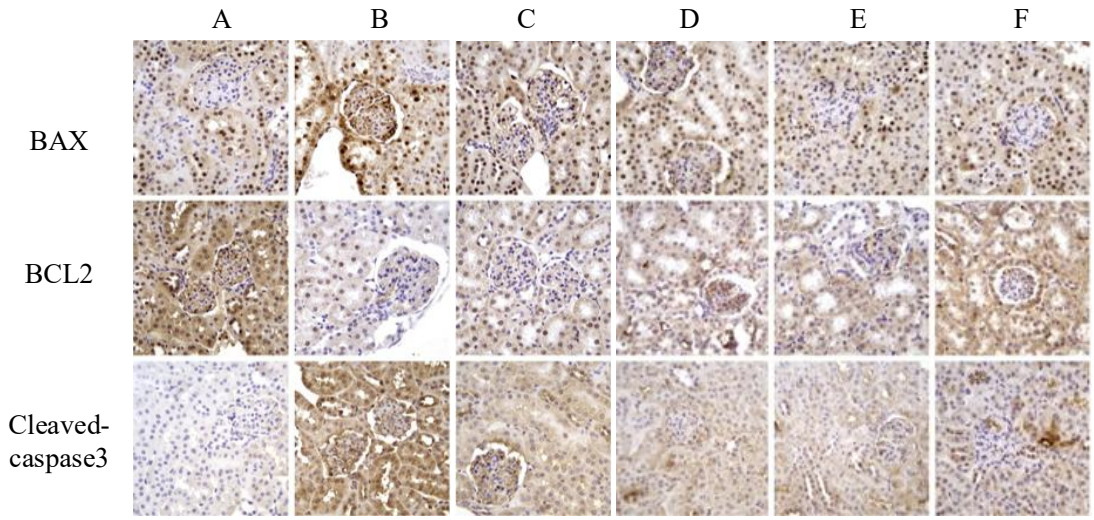

Figure 4. Expressions of BAX, BCL-2, and cleaved caspase3 under optical microscopy. **A.** blank. **B.** model. **C.** Irbesartan. **D.** LDG. **E.** MDG. **F.** HDG.

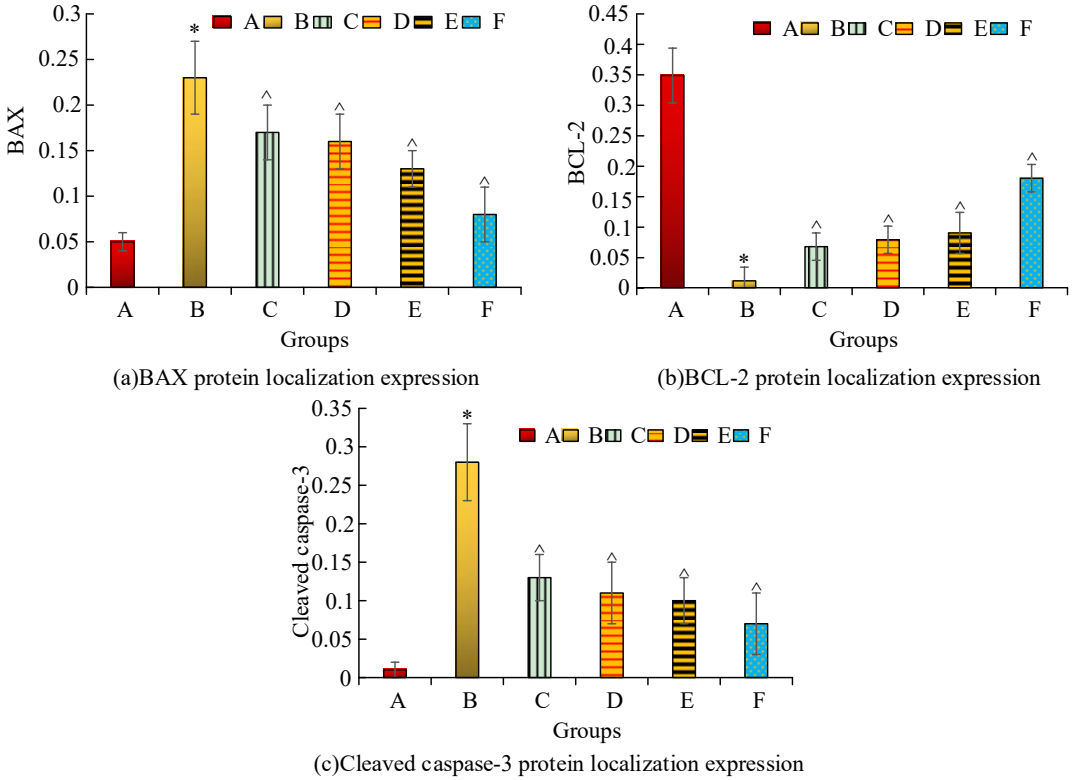

Figure 5. Effect of Jiawei Shenqi Pill on the localization and expression of indicators in rat kidney. *: $P < 0.05$ compared to BG. ^: $P < 0.05$ compared to the MG.

cleaved caspase3 in the kidney of Jiawei Shenqi Pill treatment and Irbesartan groups were reduced varying degrees compared to that in the MG, while the expression of BCL-2 protein raised to different levels ($P < 0.05$). The expression levels of indicators in the kidney tissue of each treatment group were in the same order of HDG < MDG < LDG < Irbesartan group (Figure 5).

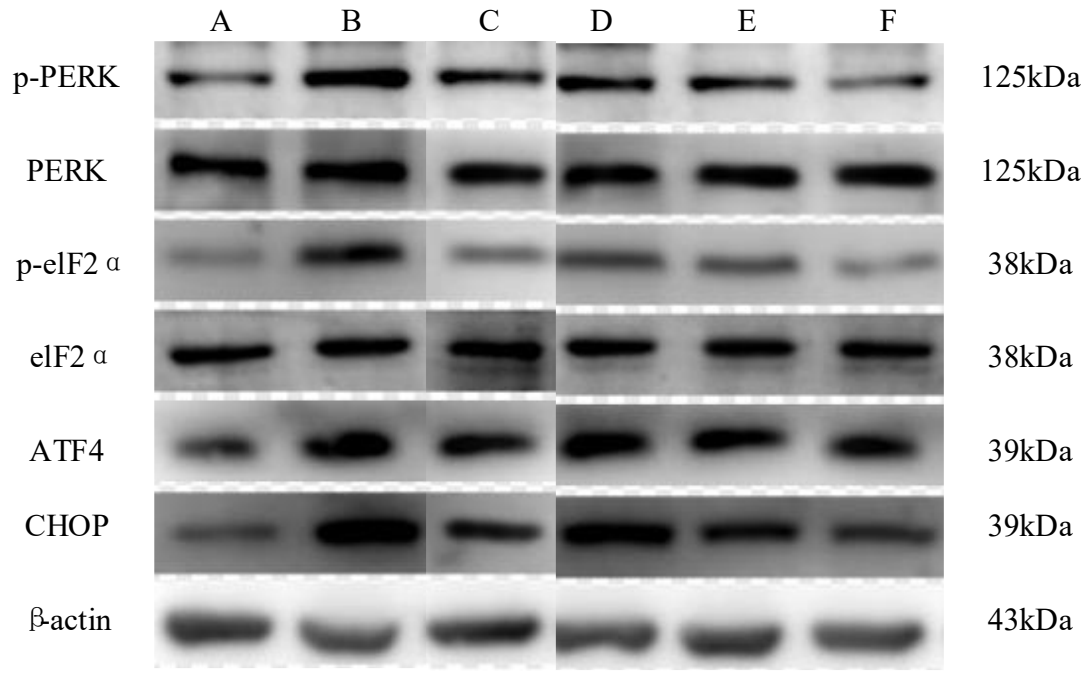

Figure 6. The influence of Jiawei Shenqi Pill on the expression of PERK/eIF2α, ATF4, and CHOP proteins in rat kidney. **A.** blank. **B.** model. **C.** Irbesartan. **D.** LDG. **E.** MDG. **F.** HDG.

Table 3. Effects of Jiawei Shenqi Pill on the expressions of PERK/eIF2α signal pathway proteins.

Group	Sample size	p-PERK/PERK	p-eIF2α/eIF2α	ATF4/β-actin	CHOP/β-actin
BG	20	0.11 ± 0.02	0.02 ± 0.01	0.19 ± 0.04	0.16 ± 0.03
MG	17	0.89 ± 0.13*	0.74 ± 0.09*	1.13 ± 0.16*	1.34 ± 0.15*
Irbesartan	19	0.59 ± 0.08^	0.26 ± 0.05^	0.73 ± 0.09^	0.62 ± 0.09^
LDG	20	0.56 ± 0.07^	0.40 ± 0.06^	0.85 ± 0.10^	0.98 ± 0.11^
MDG	20	0.32 ± 0.05^	0.34 ± 0.07^	0.64 ± 0.07^	0.71 ± 0.08^
HDG	20	0.17 ± 0.04^	0.09 ± 0.03^	0.52 ± 0.08^	0.39 ± 0.06^

Notes: *: $P < 0.01$ compared to the BG. ^: $P < 0.05$ compared to the MG.

Effect of Jiawei Shenqi Pill on the expression of PERK/eIF2α signal pathway proteins

The expression levels of PERK/eIF2α signal pathway proteins showed that all the tested proteins in the MG rats were dramatically increased compared to that in BG ($P < 0.05$), while the Jiawei Shenqi Pill treatments and Irbesartan groups demonstrated decreased expression levels compared to the MG ($P < 0.05$) (Figure 6). Overall, the rat renal tissues in each treatment group showed significant changes in PERK/eIF2α with the protein expression levels in the same order of HDG < MDG < LDG < Irbesartan group (Table 3).

Discussion

The kidney is the most critical excretory organ in an animal body, which can remove unnecessary substances in plasma and maintain the homeostasis of water, electrolyte, and pH in the body. Diabetes kidney disease (DKD) is a crucial microvascular complication of diabetes and chronic kidney disease. During the development of diabetes, due to the existence of hyperglycemia, lipid metabolism disorder, and other pathological factors, it is often accompanied by corresponding kidney damage, which accumulates to cause kidney function

failure. Apoptosis is an active and physiological process of cell death under certain physiological or pathological conditions, which is taken by cells to better adapt to the living environment. The abnormal RCA can lead to the occurrence of various diseases, so the research on the treatment of RCA has become a hot topic. Sun *et al*. found that mouse methylCpG binding domain protein 2 could effectively attenuate rhabdomyolysis (RM) induced acute renal failure and RCA. The study demonstrated that MBD2 had impact on RM induced AKI by activating Tox4 and represented a potential goal for the therapy of RM related AKI [14]. Zhao *et al*. explored intervention and treatment methods for acute renal failure and heatstroke complications through rat experiments. The results showed that curcumin treatment reduced the levels of blood urea nitrogen and others. Therefore, the extraction of curcumin from turmeric was effective in intervening the RCA and protecting the kidney from damage caused by heat stress [15]. Guo *et al*. conducted an intervention experiment on RCA in nephrosis through diabetes rats to analyze the way of calcitriol administration and apoptosis. The findings illustrated that the treatment of calcitriol could improve the severity of proteinuria in diabetic nephropathy (DN) rats and reduce RCA. In addition, calcitriol treatment obviously improved the expression of renal VDR gene [16].

Jiawei Shenqi Pill was recorded in the Traditional Chinese Medicine literature "Ji Sheng Fang", which was described as the treatment of kidney deficiency, waist and foot swelling, and poor urination, and was derived from the Jingui Shenqi Pill. Li *et al*. investigated the therapeutic effect of Shenqi Pill on kidney yang deficiency in rat using MetaboAnalyst tool to analyze the serum metabolic spectrum and pattern recognition of Kidney-yang Deficiency Syndrome (KYDS) model [17]. Kao *et al*. analyzed the intervention effect of Shenqi Pill on alleviating asthma symptoms induced by recurrent house dust mite (Der p) stimulation in chronic asthma mice and its immune regulatory mechanism. The results showed that Shenqi Pill inhibited the infiltration and exhibited a specific immune regulatory effect [18].

This study analyzed the intervention effect of different doses of Jiawei Shenqi Pill and Irbesartan on diabetes rats. From the observation of rats' body weight, anal temperature, and other general conditions, the study found that, contrasted with the MG, the body weight of rats in each administration group on the 14^{th} day improved significantly. Meanwhile, the body weight of rats in the HDG was greatly bigger than that in the Irbesartan group. Contrasted with the MG, on the 14^{th} day of administration, except for the Irbesartan group, the anal temperature of rats in each administration group rose dramatically ($P < 0.01$). In the observation of renal tissue morphology, the results demonstrated that the rats in HDG had relatively clear cortical and medullary structures in the renal tissue, reduced swelling of renal tubular epithelial cells, relatively fewer protein tubular types, reduced interstitial inflammatory cells, and significantly improved glomerular structure [19]. In the observation of RCA, the results showed that the RCA rates of rats in the Irbesartan group, LDG, MDG, and the HDG were 22.24%, 23.52%, 20.41%, and 16.39%, respectively. The study found that compared with the MG, the expressions of BAX and cleared caspase-3 proteins in the kidneys of rats in the Jiawei Shenqi Pills group and Irbesartan group were decreased to varying degrees, while the expressions of BCL-2 protein were increased to varying degrees ($P < 0.05$). Overall, the expression levels of BAX and cleared caspase-3 proteins in the renal tissues of rats in each treatment group were consistently ranked from low to high, while the protein expression levels in the HDG were the lowest. The study showed that the expression levels of p-PERK and other genes in the kidneys of rats in the Jiawei Shenqi Pill and Irbesartan groups were reduced to varying degrees ($P < 0.05$). The expression levels of PERK/eIF2α pathway proteins in each treatment group were always ranked from low to high, with HDG being the lowest.

This study suggested that Jiawei Shenqi Pill had a good intervention effect on RCA in diabetes rats. The results showed that, in the detection of urinary protein levels, Jiawei Shenqi Pill could effectively reduce the expression of BAX, achieving the effect of inhibiting RCA. In the expression of PERK/eIF2α signal pathway protein, Jiawei Shenqi Pill could reduce the protein expression and inhibit the activation of the pathway to protect the kidney of diabetes rats and reduce kidney damage.

Acknowledgements

The research was supported by Special Research Projects on Traditional Chinese Medicine in Henan Province (Grant No. 20-21ZY2313).

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