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Nephroprotective effect of HZF by upregulating IκBα protein expression in DKD mice

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ABSTRACT

HEZHIFNAG (HZF) is a traditional Chinese prescription used to treat diabetes. In this study, a mouse model of diabetic kidney disease (DKD) induced by alloxan was used, and the random blood glucose of diabetic mice decreased significantly after the intervention of HZF, and the morphological results showed that HZF improved the pathological damage of glomeruli, reduced the degree of fibrosis of kidney tissue, and increased the expression level of IKBα in the kidney of diabetic mice, suggesting that HZF has a protective effect on the DKD mice, and the mechanism of action may be related to the up-regulation of IKB-α expression.

KEYWORDS

Nephroprotective; HEZHIFANG; IκBα; Diabetes

1. INTRODUCTION

Diabetic kidney disease (DKD) is one of the most common complications of diabetes, and according to statistics, 30%~40% of patients with diabetes develop DKD [1]. The clinical treatment of DKD is generally based on controlling blood glucose, blood pressure and blood lipids, reducing proteinuria and its complications to maintain or delay the progression of DKD to end-stage kidney disease, and Western medicine generally uses renin-angiotensin-aldosterone system inhibitors and some hypoglycemic drugs that have been shown to be effective in reducing the progression of diabetes and DKD [2], but the above drugs are mostly used in the early stage of DKD and have certain limitations. In recent years, a large number of clinical trials have shown that traditional Chinese medicine has a good clinical effect in the prevention and treatment of DKD [3], and traditional Chinese medicine has the characteristics of multi-target, multi-level comprehensive treatment and functional regulation, and the treatment of diabetes is not only aimed at lowering blood sugar, but also has the outstanding advantage of effectively preventing and delaying the occurrence and development of diabetes comorbidities, improving symptoms, and improving the quality of life of patients. Therefore, it is of great significance to study the mechanism of action of chinese medicine in the treatment of DKD.

IκB is an upstream component of the inflammatory factor signal transduction pathway, and factors such as hyperglycemia, glycation end products, and infection lead to enhanced oxidative stress,

activate IκB kinase, phosphorylate IκB, regulate the binding of nuclear transcription factors to target gene loci, initiate downstream gene expression, and increase the level of inflammatory factors in vivo [4]. Among them, IκBα is the most important and first cloned molecule [5], initiating the transcription and expression of a series of specific proinflammatory factor genes.

Hezhifang was created by Chen Shiduo, a famous doctor in the Qing Dynasty, and was included in the "SHISHIMILU", believing that urine disease is treated from the kidney, and coordinating the yin and yang of the kidney is the fundamental. It is often used in the treatment of diabetes mellitus in traditional Chinese medicine, but there is a lack of animal data. In this study, a mouse model of alloxan-induced diabetes mellitus was used to observe its effect on the expression of IκBα in diabetic nephropathy, and to explore the mechanism of Hezhifang in the treatment of diabetic nephropathy.

2. MATERIALS AND METHODS

2.1. Animal Care

Male KM mice (8 weeks, 37 - 43 g) were purchased from Changzhou Cavens Laboratory Animal Co. Ltd. (Changzhou, China) (laboratory animal license [SCXK (Su) 2021-0013]). The animal experiments were performed in specificpathogen-free barrier laboratory at the Experimental Animal Centre of North China University of Science and Technology (Tangshan, China). All procedures for animal experiments were approved by the Animal Ethics Committee of North China University of Science and Technology.

2.2. Reagents

Chinese herbal medicines Rehmanniae Radix, Common Macrocarpium Fruit, Dwarf Lilyturf Tuber, Plantain Seed and Figwort Root purchased from Beijing Tong Ren Tang Pharmacy in Tangshan, China; alloxan (A 7413, Sigma, USA), IκBα antibody (GeneTex, GTX110521).

2.3. DKD Model Mice

Each mouse is intraperitoneally injected with 100 mg/kg body weight of alloxan at a single dose administration. The mice with fasting blood glucose concentration higher than 11.1mmol/L after 3 days of injection are qualified diabetic model mice.

2.4. Grouping and Administration

Male KM mice (6-8 weeks, 37- 43g) were selected to establish the normal control group (CON). Based on the RBG levels, the 18 diabetic mice were randomly divided into three groups received different treatments: Metformin group (Met, 200 mg/kg/d), Hezhifang group (HZF, the dosage is consistent with the dosage for person) and model control group (Model), The CON and Model mice received an equal volume of pure water. All treatments were administered at 9 a.m. every day, and the duration of the entire experiment was 6 weeks.

2.5. Hematoxylin-eosin (HE) Staining and Masson Staining

Renal tissue was paraffin-embedded, sectioned (4 μm thick), dehydrated, transparent, mounted, and observed the pathological morphology and fibrosis of kidney tissue under light microscopy

2.6. Immunohistochemistry

Immunohistochemistry was performed using a universal two-step test kit (ZSGB-BIO, PV-9000, China), incubated with the following primary antibodies: IκBα antibody (1:200, GeneTex,

GTX110521). The positive proteins were colored brownish yellow, and the expression and distribution of indicated proteins were observed under a light microscope.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS 20.0 software, and the experimental data are expressed as the means ± standard deviation (SD). $P<0.05$ was considered to indicate a statistically significant difference.

3. RESULTS

3.1. Effects of DCI on the Level of RBG in Diabetic Mice

HZF lowers random blood glucose in diabetic mice (Fig. 1). Before administration, compared with the normal group, the random blood glucose of mice in the model group, Met group and HZF group was significantly increased, and there was no statistical difference between the groups. After 6 weeks of administration, compared with the model group, the random blood glucose of mice in the Met group and the HZF group decreased significantly. See Figure 1.

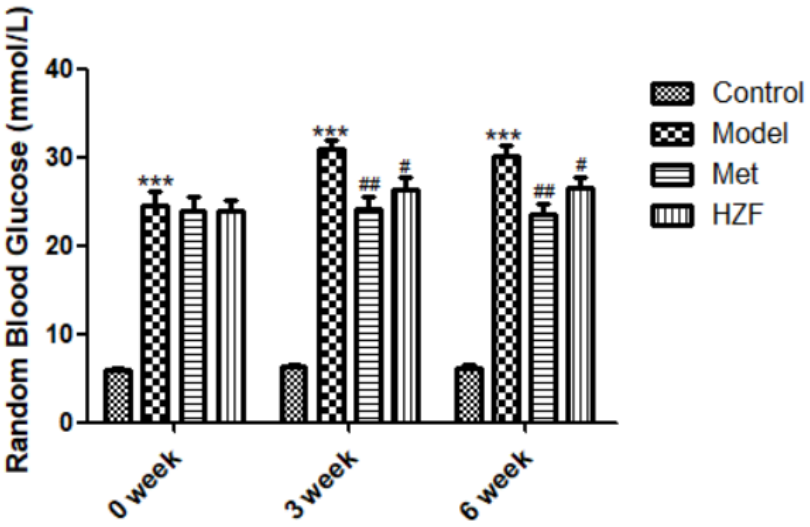

Figure 1. Effect of HZF on random blood glucose in DKD mice(n = 6). All values are expressed as the means ± SD. $^{***}p < 0.001$ vs.CON mice; $^{\#}p < 0.05$. $^{\#\#}p < 0.01$ vs. Model mice.

3.2. Effect of HZF on renal pathology in DKD mice

HZF improved the pathological changes of kidney tissue in diabetic mice (Fig. 2). HE staining showed that the nuclei in kidney tissue in the CON were stained blue, the cytoplasm was stained pink. HE staining showed that the glomeruli, tubule and interstitial structures were normal in the CON, and pathological changes such as moderate proliferation of mesangial cells and stroma and capsular space were seen in the Model, while renal tissue pathological damage in the Met group and HZF group were improved.

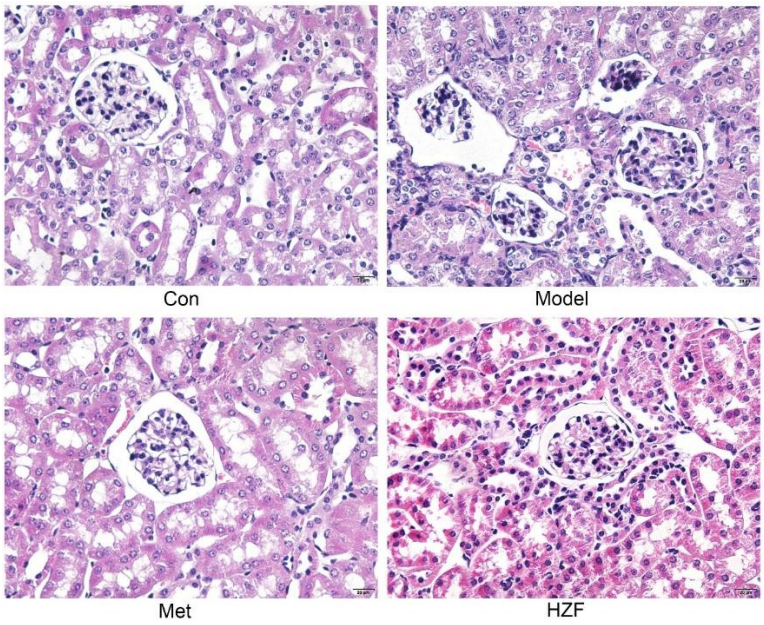

Figure 2. Effect of HZF on renal pathological injury in DKD mice (HE staining, ×400, bar=20μm)

3.3. Effect of HZF on Renal Fibrosis in Diabetic Mice

HZF ameliorates fibrosis of kidney tissue in diabetic mice (Fig. 3). Masson's trichrome staining demonstrated that there was a small amount of collagen deposition in the renal interstitium of CON mice, whereas there were large numbers of collagen fibers in the renal interstitium of Model mice. However, the result showed a significant decreased in glomerular and renal interstitial fibrosis of HZF mice.

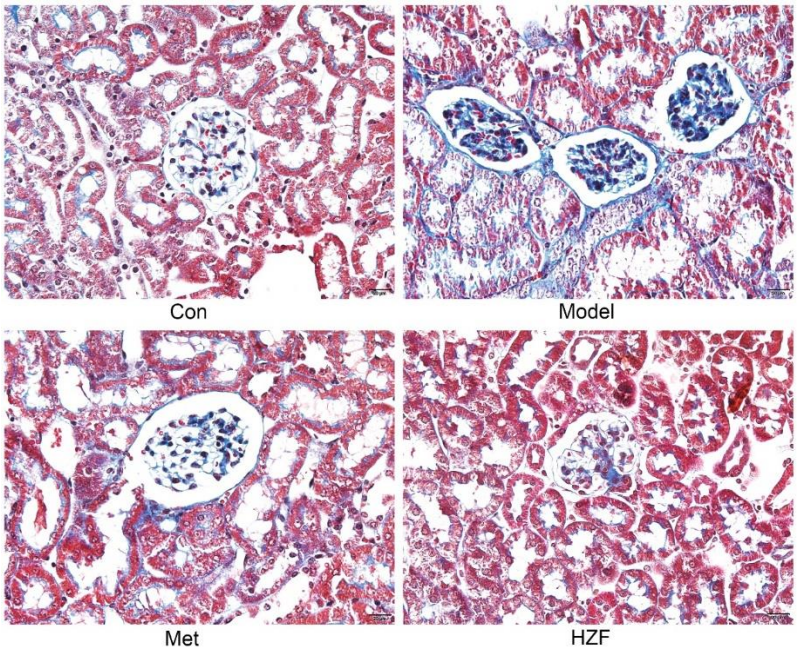

Figure 3. Effect of HZF on renal interstitial fibrosis in DKD mice (HE staining, ×400, bar=20μm)

3.4. Protective Mechanism of Action of HZF In DKD

Iκbα protein plays a role in the protective effect of HZF in diabetic nephropathy (Fig. 4). The immunohistochemical staining analysis are shown in Figure 4. Iκbα protein is expressed on mouse kidney glomerular cells. This protein were highly expressed in the kidney tissue in the CON, and the

positive staining was brownish yellow and evenly distributed. The protein expression Iκbα in the Model was lower than that in the CON. Moreover, the expression levels of Iκbα in the HZF were higher than those in the MCG.

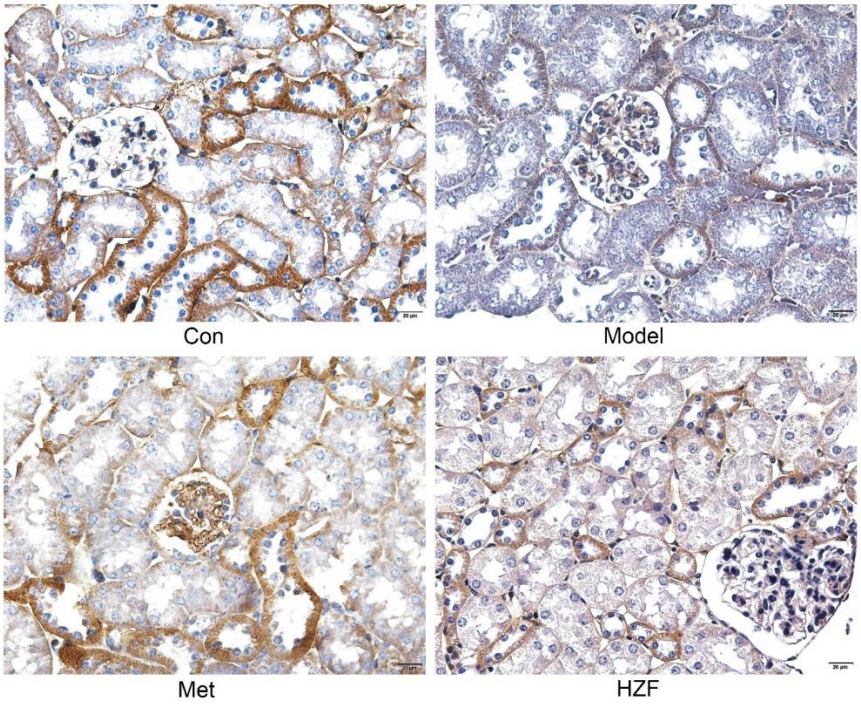

Figure 4. Effect of HZF on the protein expression of Iκbα in DKD mice (IHC, ×400, bar=20μm)

4. DISCUSSION

HZF is derived from Chen Shiduo's classic prescription of "treating from the kidney", which mainly focuses on "kidney" [6]. But there are few experimental research data on its mechanism of action, and there are few reports on the IKB signaling pathway.

IKB is an inhibitory protein of NF-kB, an upstream component of the inflammatory cytokine signal transduction pathway, and in physiological state, the two are combined into inactive complexes in the cytoplasm [7]. Factors such as hyperglycemia, glycation end products, and infection lead to the enhancement of oxidative stress in the body [8], activating IκB kinase (IKK), and the activated IKK phosphorylates IκB, thereby separating it from NF-κB, releasing NF-κB p50/p65, and translocating NF-kB into the nucleus, binding to target gene loci to initiate downstream gene expression, accelerating the stimulation of the expression and transcription of various signaling factors in the body, and increasing the level of inflammatory factors in the body [9]. Among the many inhibitory proteins of NF-kB, IKB-α is the most important and the first to be cloned [10], initiating the transcription and expression of a series of specific proinflammatory factor genes [11].

In animal experiments, HZF was used to intervened in DKD mice, and it was found that HZF could significantly reduce random blood glucose in diabetic mice, and the morphological results showed that HZF protected kidney damage caused by hyperglycemia. Through pathological staining, it was found that HZF significantly improved renal fibrosis in mice, and alleviated mesangial hyperplasia, tubular vacuolar and inflammatory cell infiltration in mice. The results of animal experiments showed that HZF could improve the expression of IκBα protein in kidney tissue of diabetic mice. At present, only a single dose of HZF has been used for experimental studies, and the intervention effect of different doses needs to be further studied. In summary, this study suggests that HZF can improve random blood glucose, alleviate renal tubular damage caused by high glucose, and protect renal function in diabetic mice. The mechanism may be related to the regulation of IκBα expression.

5. CONCLUSIONS

In summary, this study showed that HZF improved the general condition of mice with DKD, reduced random blood sugar levels, alleviatde glomerular damage caused by high glucose, improved the degree of kidney tissue fibrosis, then protected kidney function. The protective mechanism may be related to the IKB signaling pathway, up-regulating the expression of IKB-α protein, antagonizing the inflammatory damage of DKD, thereby slowing down the process of DKD. However, the inflammatory mechanism of kidney cells in DKD is very complex and needs to be further studied.

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