Int J Clin Exp Pathol 2016;9(2):620-626
www.ijcep.com /ISSN:1936-2625/IJCEP0010711

Original Article

Effect of total glucosides of paeonia on TGF-β1 and ICAM-1 expression in the kidney of diabetic rats

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Received May 26, 2015; Accepted June 29, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Intercellular adhesion factor 1 (ICAM-1) and transforming growth factor (TGF-β1) expression enhanced following the diabetes extension. They may be involved in the pathogenesis of diabetic nephropathy. Total glucosides of paeonia (TGP) have anti-inflammation and immune regulation effect. We discussed TGP effect on TGF-β1 and ICAM-1 expression and its renal protection mechanism on diabetic rats. 7 weeks old healthy male SD rats were randomly divided into control group (C), diabetes group (B), and TGP group H, M, and L with 10 in each group. Fasting blood glucose (BG), creatinine (Scr), urea nitrogen (BUN), and 24 h urine trace albumin excretion rate (UAER) were measured and renal pathological morphology was observed. Immunohistochemistry was applied to measure TGF-β1 and ICAM-1 expression in the kidney. TUNEL assay was performed to observe renal cortical cell apoptosis. Compared with group C, BG, Scr, BUN, and UAER significantly increased in group B ($P < 0.05$). Their levels in TGP group H, M, L were higher than the group C but lower than group B ($P < 0.05$). Histological changes of the lesions were improved obviously in TGP group. TGF-β1 and ICAM-1 expression in the kidney presented similar trends. Cell apoptosis index increased markedly in group B compared with group C, it showed different degree of decrease in TGP group ($P < 0.05$).TGP can improve early renal damage in diabetic rats, which might be related to its reducing TGF-β1 and ICAM-1 expression in the kidney to inhibit cell apoptosis.

Keywords: Total glucosides of paeonia, diabetic nephropathy, ICAM-1, TGF-β1

Introduction

Diabetic nephropathy (DN) is a type of diabetes complication that is the leading cause of end-stage renal failure. Its incidence increased in recent years. However, its etiology and pathogenesis still have not been fully elucidated [1]. Studies have shown that [2, 3] diabetes is associated with oxidative stress and inflammation, while intercellular adhesion factor-1 (ICAM-1) is the important reason for macrophages and monocytes infiltration in renal tissue. Animal experiments showed that [4, 5] ICAM-1 overexpressed in the kidney of the type 1 diabetes model induced by streptozocin (STZ), which is consistent with glomerular structure changes. High glucose can increase the extracellular matrix hyperplasia by inducing transforming growth factor β (TGF-β) gene and protein expression in glomerular mesangial cells. Several TGF-β and related receptors overexpressed in diabetic rats renal tubular. Following duration extension, TGF-β1 protein expression gradually upregulated and its overexpression played an important role in diabetic kidney injury [6, 7]. Immune/inflammatory inhibitors and antioxidants can reduce renal tissue inflammation cells infiltration and oxidative stress reaction, reduce urinary albumin levels, and improve renal structure and function in diabetes models [8]. Total glucosides of paeonia (TGP) are extracted from the dry root of paeonia lactiflora Pall, which contains hydroxy paeoniflorin, paeoniflorin, and other active ingredients. Radix paeoniae alba can protect blood and liver. Pharmacological studies showed that [9, 10] TGP has anti-inflammatory, anti-stress and immune regulation function, and it was an anti-inflammatory immune regulatory drug in clinic. In this paper, we aimed to investigate the possible mechanism of TGP protection effect on diabetic rats by observing TGP impact on the TGF-β1 and ICAM-1 expression in the kidney.

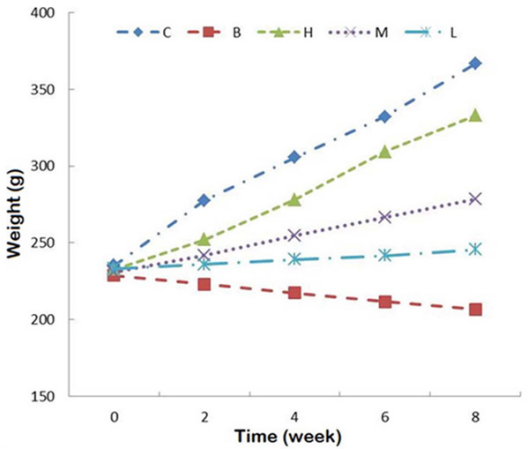

Figure 1. TGP effect on diabetic rat's weight.

Materials and methods

Experimental animals and grouping

7 weeks old healthy male SD rats weighted 200~240 g were provided by the Chinese academy of medical sciences animal experiment center (license SYXK-2013-0025) and maintained in SPF laboratory. The rats were randomly divided into control group (C), diabetes group (B), and TGP group H, M, L with 10 in each group.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of our hospital.

Drugs and reagents

TGP was purchased from Hunan KinglongBio Co., LTD. and suspended in 1% carboxymethyl cellulose. Streptozocin (STZ) was provided by Sigma and suspended in citrate buffer. Blood glucose, urea nitrogen, serum creatinine detection kits were got from Nanjing Jiancheng biological technology co., LTD. TGF-β1 and ICAM-1 primary antibodies, DAB chromogenic agent were provided by Wuhan Boster Biological Co., LTD.

Modeling

STZ were used to establish diabetic rat model. After fasting for 12 h, the rats in the model group received 60 mg/kg 1% STZ solution intraperitoneal injection at one-time, while the control group received equal amount of citric acid buffer injection. Diabetes model was evaluated success when fasting blood glucose ≥ 16.7 mmol/L after 72 h [11]. After modeling, 24 h urine was collected to test urinary protein level.

Drug delivery

TGP at 50, 100, 200 mg/(kg•d) were given through stomach for 8 weeks. While the rats in group C and B received equal amount of saline solution.

Measurement

Rat's general information such as mental state, eating and drinking, stool and urine were observed daily. The weight was measured every 2 weeks. Blood glucose was tested once a month. At the end of 8 weeks' test, urine was collected after 24 h fasting and urine protein level was detected. The rat abdominal venous blood was collected to inspect BG, BUN, and Scr levels. The left kidney was used for weighting, while the right kidney was fixed in 10% formaldehyde. Glucose oxidase method was applied to determine BG. Scr and BUN were measured using automatic biochemical analyzer. Kidney weight index KI = left kidney weight/body weight. Kidney pathological morphology was observed under optical microscope. TGF-β1 and ICAM-1 expression were evaluated by immunohistochemistry, and the positive result was evaluated by mean absorbance in the positive area. TGF-β1 mainly expressed in renal tubular epithelial cells and part expressed in glomerular. ICAM-1 mainly expressed in glomerular endothelial cells and mesangial cells. They both located in the cytoplasm.

TUNEL assay

After stained with TUNEL, apoptotic cells were observed under microscope. Five fields under 400× were enrolled for calculation. Apoptosis index (AI) = apoptotic cell number/total cell number × 100%.

Statistical analysis

All statistical analyses were performed using SPSS17.0 software (Chicago, IL). Numerical data were presented as means and standard deviation (± SD). Differences between multiple groups were analyzed using one-way ANOVA and LSD-t test. $P < 0.05$ was considered as significant difference.

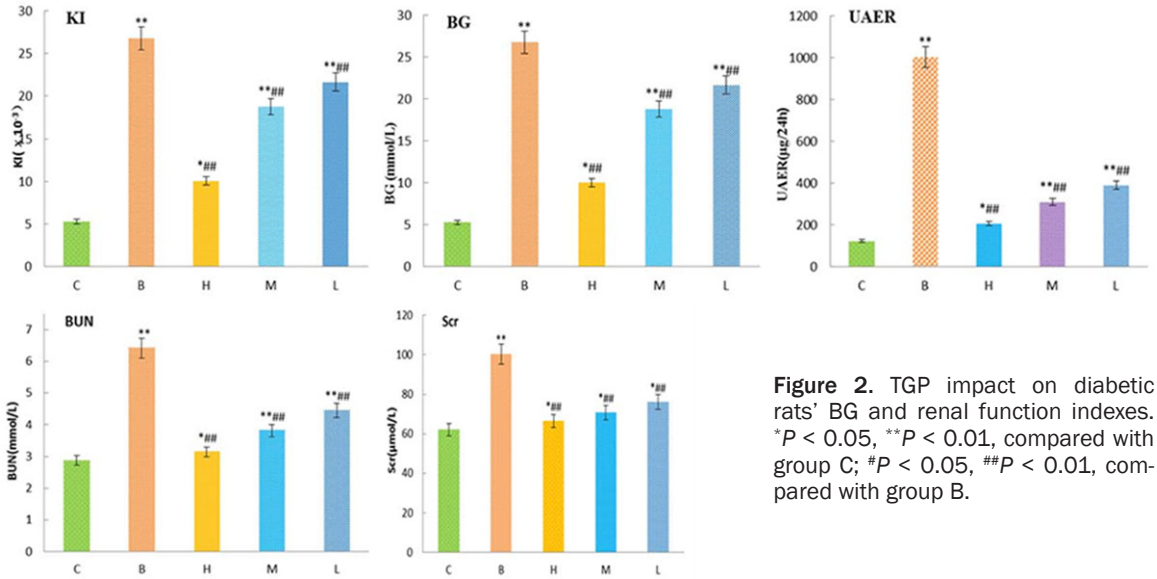

Figure 2. TGP impact on diabetic rats' BG and renal function indexes. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with group C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with group B.

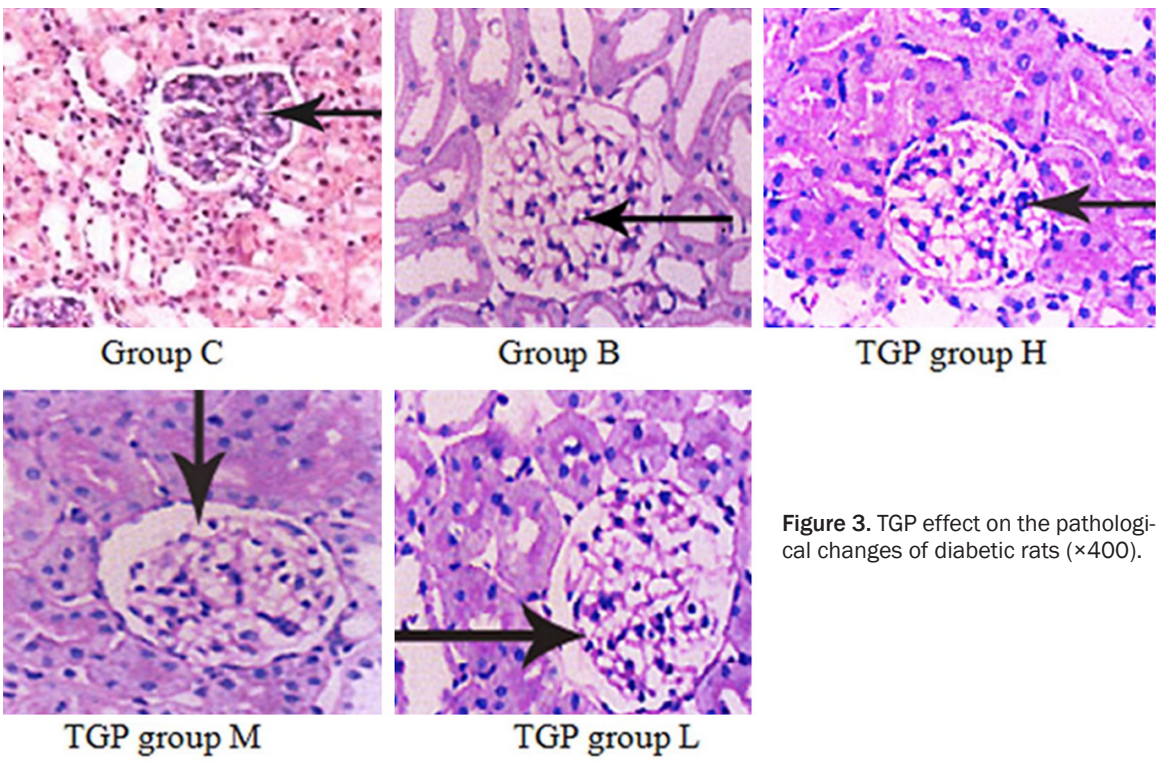

Figure 3. TGP effect on the pathological changes of diabetic rats (×400).

Results

General information comparison

Rats in group C exhibited significant weight gain, responsive, smooth and shining furs. Rats in group B appeared more eating and drinking, polyuria, with weight loss, unresponsive, and lackluster furs. Rats in H, M, and L groups presented weight gain, good spirit. They were more sensitive than in group B, but worse than group C (**Figure 1**).

TGP impact on BG and renal function

Compared with group C, BG, Scr, BUN, UAER, and KI in group B significantly increased ($P < 0.01$). Their levels in TGP group H, M, L were higher than the group C but lower than group B ($P < 0.01$ or $P < 0.05$) (**Figure 2**).

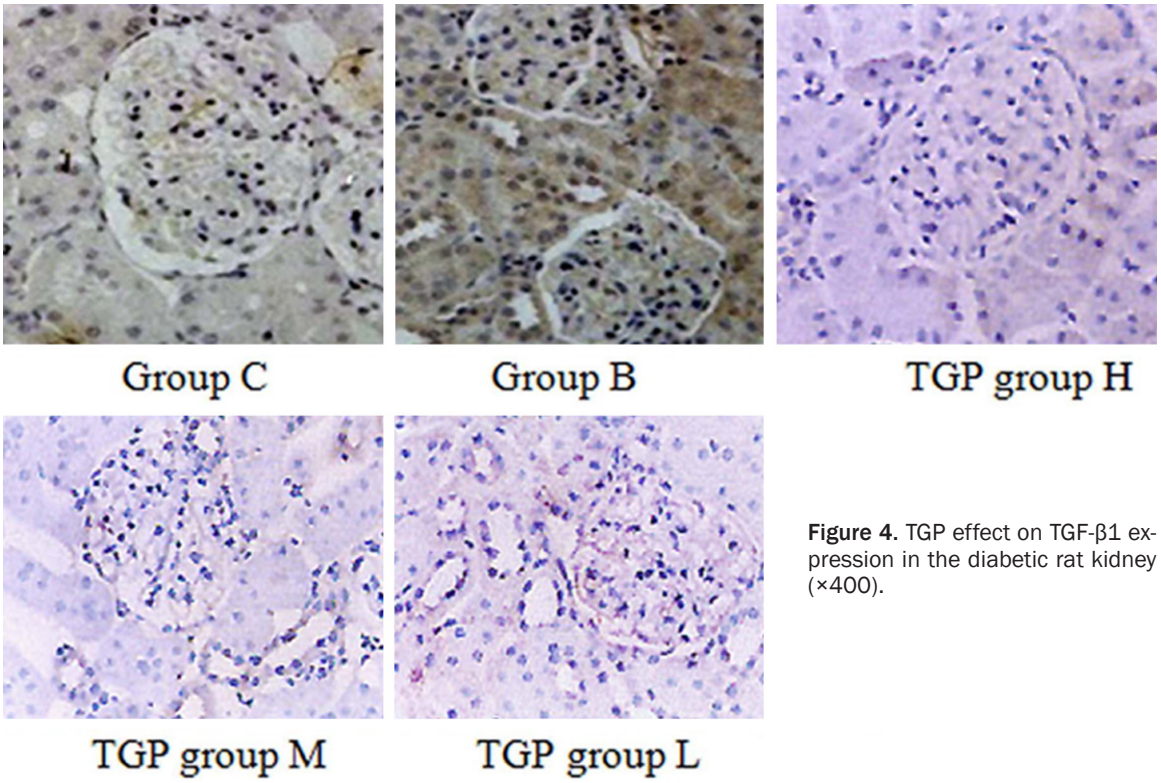

Figure 4. TGP effect on TGF-β1 expression in the diabetic rat kidney (×400).

TGP effect on diabetic rat pathological changes

HE staining revealed that rat glomerular structure in group C was clear with no obvious abnormality. Glomerular volume increased in group B. Glomerular mesangial cells appeared hyperplasia and cystic wall adhesion. TGP groups showed histological lesions improved significantly compared with group B, and the improvement degree showed dose dependent (**Figure 3**).

TGP effect on TGF-β1 and ICAM-1 expression in the diabetic rat kidney

TGF-β1 and ICAM-1 expression were weak in group C, and they obviously overexpressed in group B ($P < 0.01$). Their expression significantly reduced in TGP group H, M, L compared with group B ($P < 0.05$) (**Figures 4**-**6**).

TGP impact on diabetic rat renal cortical cell apoptosis

Cell apoptosis index increased markedly in group B compared with group C, it showed different degree of decrease in TGP group ($P < 0.05$) (**Figure 7**).

Discussion

Inflammatory reaction runs through the whole process of diabetes. Inflammatory factor is closely associated with diabetic nephropathy (DN). The pathological basis of DN is kidney high filtration, high perfusion, glomerular mesangial cell proliferation, basement membrane thickening, and extracellular matrix hyperplasia, following with nodular or diffuse sclerosis, eventually leading to hypertension, proteinuria and renal failure [12, 13]. Mononuclear macrophages infiltration existed in the renal tissue in both type 1 and type 2 DN at early stage. Infiltrated mononuclear macrophages may cause kidney damage through secreting inflammatory factors and oxygen free radicals, promote glomerular sclerosis. Kidney under pathological condition can produce inflammatory cytokines such as TNF-α and NO itself. Secreted inflammatory cytokines can augment inflammation through paracrine or autocrine and trigger the inflammatory cascade reaction [14, 15]. Inflammation might be a downstream of DN to cause renal damage. Macrophages mediated kidney inflammation plays a significant role in the progression of DN. Inflammation/immune inhibitor application in diabetes model can inhibit macrophage infiltra-

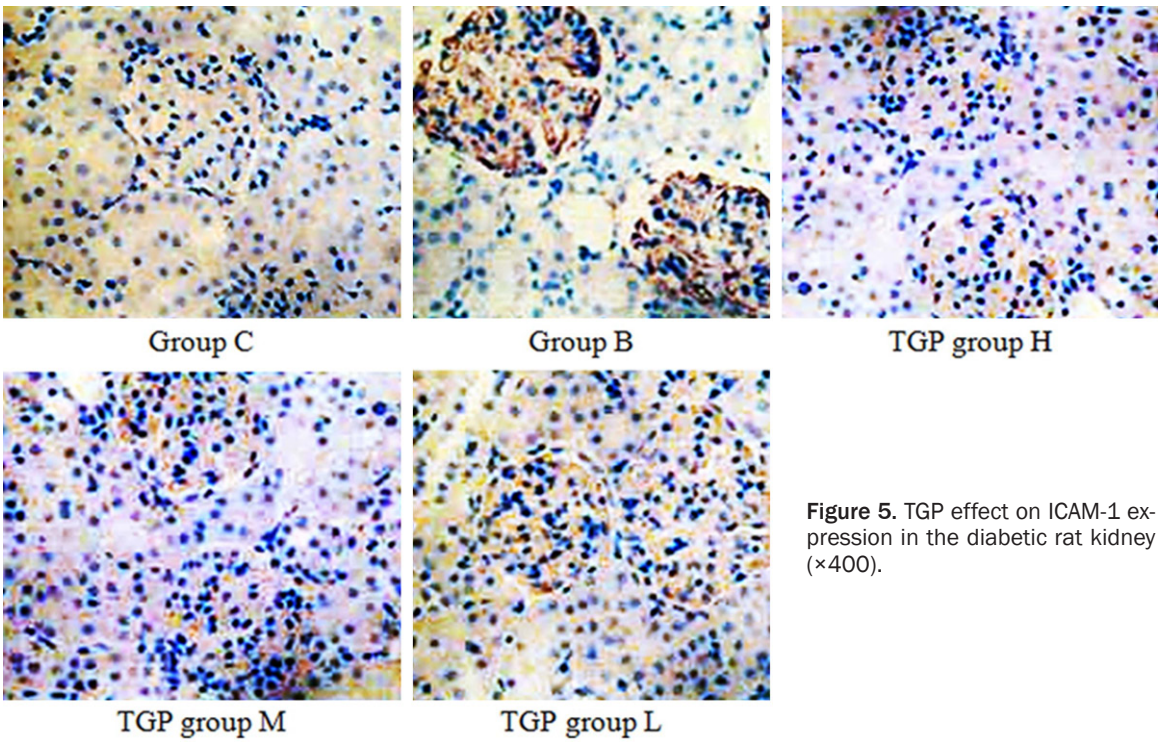

Figure 5. TGP effect on ICAM-1 expression in the diabetic rat kidney (×400).

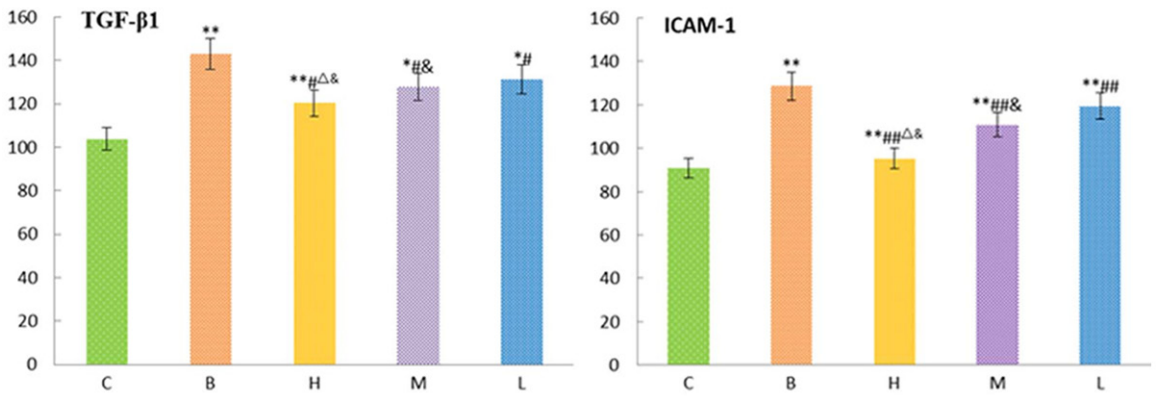

Figure 6. TGP effect on ICAM-1 expression in the diabetic rat kidney (×400). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with group C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with group B; $^{\&}P < 0.05$, compared with group L; $^{\Delta}P < 0.05$, compared with group M.

tion found in the kidney tissue, significantly reduce UAER, and improve the renal pathological tissue damage [16]. TGF-β1 and ICAM-1 overexpressed following the extension of diabetes. It was found that they can increase cell apoptosis and lead to renal function deterioration [14-16]. TGP has multiple pharmacological activities such as anti-inflammation and anti-stress. In vivo animal experiment results showed that [17] in adjuvant arthritis rats, TGP can inhibit basic fibroblast growth factor and vascular endothelial growth factor expression by downregulating macrophage mediated TNF-α and IL-1 expression. This paper discussed TGP effect on TGF-β1 and ICAM-1 expression in the kidney of diabetic rats to investigate its protection mechanism to diabetes.

We used STZ to damage islet β cells and the rats in model group appeared polydipsia, polyphagia, and polyuria, elevated BG, UAER, BUN,

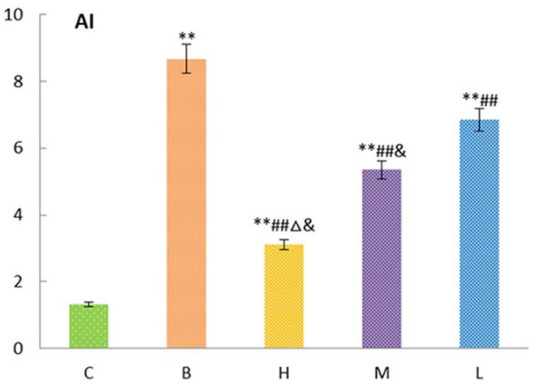

Figure 7. TGP impact on renal cortical cell apoptosis in diabetic rats. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with group C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with group B; $^{\&}P < 0.05$, compared with group L; $^{\Delta}P < 0.05$, compared with group M.

Scr and KI, suggesting that diabetes caused renal damage. They decreased at different degree in TGP group H, M, L compared with group B, but still higher than in group C. Histological lesions improved obviously in TGP group, indicating that TGP can alleviate early renal damage, reduce proteinuria, and improving renal function in diabetic rats.

Studies have shown that [18, 19] ICAM-1 can mediate WBC migration and adhesion. It mainly expressed in renal tubule-mesenchymal cells and vascular epithelial cells. ICAM-1 knockout diabetic rat model showed obvious alleviated kidney macrophages infiltration, albumin excretion rate, glomerular hypertrophy, and mesangial matrix expansion. Our results revealed that ICAM-1 overexpressed in group B, while it reduced in TGP group H, M, L with dose dependent, indicating that TGP can inhibit ICAM-1 protein expression in renal tissue, which may be related to inhibiting macrophages infiltration.

Animal and clinical trials demonstrated that [20, 21], TGF-β1 plays an important role on glomerular-interstitial damage in DN process. It was an important cytokine for cell growth and producing extracellular matrix. Its expression level increased in the kidney of diabetes patients and animal models. TGF-β1 can induce renal pathological changes by promoting kidney hypertrophy, inducing extracellular matrix hyperplasia, and inhibiting cell proliferation. Our study showed that TGF-β1 expression increased in group B, while it decreased in TGP group H, M, L with dose dependent, indicating that TGP can protect kidney by inhibiting TGF-β1 expression in renal tissue.

In addition, apoptosis index increased significantly in model group, and it decreased at different degree in TGP group H, M, L. This study suggested that TGP can improve diabetic rats general information and decrease BG. TGP may reduce renal damage through lowering BG to reduce the over deposited glycosylation products. Scr, BUN and UAER decreased significantly after TGP treatment, indicating that inflammatory cytokines and adhesion factors may damage glomerular filtration barrier and cause proteinuria. TGP treatment can decrease TGF-β and ICAM-1 expression, suggesting that TGP can improve early renal damage and reduce proteinuria through decreasing TGF-β and ICAM-1 expression in the kidney of diabetic rats.

Above all, TGP can significantly improve early renal damage in diabetic rats, which may be related to its reducing TGF-β and ICAM-1 expression in kidney and inhibiting cell apoptosis.

Disclosure of conflict of interest

None.

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