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Original Article

Effect of Quyu Chencuo Formula (去菀陈莝方) on Renal Fibrosis in Obstructive Nephropathy Rats*

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ABSTRACT **Objective:** To observe the effect of Quyu Chencuo Formula (去菀陈莝方, QCF) on renal fibrosis in rats with obstructive nephropathy. **Methods:** Twenty-four rats were randomly divided into three groups, 4 for sham operation as the control group, 10 for unilateral ureteral obstruction (UUO) model group, and the rest 10 for QCF treating UUO model group. All rats were sacrificed under 3% pentobarbital (50 mg/kg) anesthesia on the 14th day after surgery, then the right kidney samples of rats were harvested for hematoxylin eosin (HE) staining and Masson staining to observe the renal pathological changes. Immunohistochemistry and Western blotting were used to examine the expression of transforming growth factor β1 (TGF-β1), and real-time polymerase chain reaction (RT-PCR) was employed to examine the expressions of TGF-β1, α-smooth muscle actin (α-SMA) and E-cadherin mRNA. **Results:** HE and Masson staining showed that the renal interstitial of the rats in the control group had no significant fibrotic lesion; in the model group, there were obvious interstitial fibrosis; for the QCF group, there were epithelial cell necrosis, infiltration of lymphocytes and mononuclear cells, aggravated interstitial fibrosis in varied degrees, but the pathological changes were less in the QCF group than in the model group. The immunohistochemistry and Western blotting results showed that the TGF-β1 expression was increased significantly in the model group, while decreased significantly in the QCF group ($P<0.05$); RT-PCR showed that the mRNA expression of α-SMA and TGF-β1 increased significantly in the model group, while both were significantly decreased in the QCF group compared with the model group ($P<0.05$). The mRNA expression of E-cadherin was decreased significantly in the model group, and it was significantly increased in the QCF group as compared with the model group ($P<0.05$). **Conclusion:** QCF may improve renal fibrosis by regulating the expressions of TGF-β1, α-SMA and E-cadherin, and prevent the progress of kidney fibrosis.

KEYWORDS unilateral ureteral ligation, Quyu Chencuo Formula, E-cadherin, transforming growth factor β1, α-smooth muscle actin, kidney fibrosis, Chinese medicine

The renal tubulointerstitial fibrosis is a common pathological change of all chronic kidney diseases, and it is the major pathological basis of end-stage renal disease.[1,2] In recent years, studies have shown that the renal tubular epithelial-mesenchymal transdifferentiation (TEMT) is one of the most important reasons for the excessive accumulation of extracellular matrix and renal interstitial fibrosis.[3,4] Substantial evidence indicates that the transforming growth factor β1 (TGF-β1) is one of the major growth factors inducing TEMT,[5-7] appearance of α-smooth muscle actin (α-SMA) protein is a sign of TEMT.[8,9] These key cytokines are believed to be related with renal fibrosis. Our clinical practice proved that the application of Quyu Chencuo Formula (去菀陈莝方, QCF) achieved good therapeutic effect in the treatment of chronic kidney disease.[10,11,12] A further research found that QCF could reduce serum fibrosis indices of patients with chronic renal insufficiency, such as serum human laminin (LN), serum collagen type Ⅳ (CL-Ⅳ), etc.[10] This study aimed to observe the effect of QCF on renal fibrosis and the related mechanism.

METHODS

Drug Preparation

Salvia miltiorrhiza Bunge 15 g, cooked *Radix et Rhizoma Rhei* 15 g, *Cortex Moutan* 15 g, *Semen Persicae* 12 g, *Radix Puerariae* 15 g, *Poria cocos* (Schw.)

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Wolf 15 g, *Semen Plantginis Asi-aticae* 20 g, *Arecae Pericarpium* 15 g, *Rehmannia glutinosa* (Gaertn.) Li-bosch 20 g, *Cornus officinalis* Sieb. Et Zucc 15 g, totally 157 g, were provided by the Traditional Chinese Medicine Pharmacy in Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, China. Firstly, these drugs were immersed by the distilled water which was equivalent to 5 times the weight of the medicinal materials for 2 h, boiled by conflagration, and then decocted with soft fire for 30 min. The liquid was harvested; secondly, the drugs were decocted once again with the same volume of water; at last, the boiled liquid at the first and second time were mixed together and concentrated to form crude drug at 1 g/mL liquid.

Animal Grouping

Totally 24 Wistar rats, male and female, aged 2-month old, weighing 200±10 g, were purchased from the Laboratory Animal Center in Tongji Medical College, Huazhong University of Science and Technology. The rats were housed in cages with 45%±2% humidity at 22±2 ℃, and noise controlled within 60 dB, free access to water and food, ventilation, and litter discarded every other day. These rats were randomly divided into sham operation (control, *n*=4), unilateral ureteral obstruction (UUO) model (*n*=10), and QCF (*n*=10) groups after 1-week acclimation.

In the model group, according to our previous established modeling method,[13] after anesthesia with intraperitoneal injection of 3% pentobarbital (50 mg/kg), the skin of the rats was disinfected with iodine, and then the abdomen was cut open with about 2-cm long incision along the midline layer by layer. The right ureter in the paravertebral gutter was found and separated. The right ureter was doubly ligated in the upper 1/3 with 4–0 suture thread, and cut off between the two ligation ends to prevent retrograde infection. Then we checked whether there was hemorrhage and infiltration, sutured the abdomen, and disinfected the wound. The pre-operative surgical infection prevention measures were as follows: ultraviolet light was used to irradiate surgical instruments, dressings and operation platform for 30 min; surgery was carried out in strict accordance with aseptic principle to avoid contamination; the abdominal cavity was washed with gentamicin physiological saline before suturing the abdominal cavity; the wound was covered with sterile gauze, and infection was prevented by using antibiotics for 2 days. In the control group (*n*=4), the renal capsule was explored and the right ureter was separated but not ligated; the remaining steps were the same with those of the model group. In the QCF group, the modeling process was the same as that of the UUO mode group. The treatment group was intragastrically administrated with 4 mL/kg QCF every day. However, rats in the model and control groups were intragastrically administrated with the same volume of physiological saline.

Experimental Specimen Collection

The rats were sacrificed under anesthesia with 3% pentobarbital (50 mg/kg) on the 14th day after the surgery. The rats lied in supine position, iodine was used for routine disinfection, the abdomen was cut open along the midline, the right kidney tissue was removed immediately, the renal capsule was separated, and the kidney was split in coronal plane and washed with physiological saline. Part of the kidney tissues were fixed in 10% formalin solution and embedded in paraffin for subsequent hematoxylin eosin (HE) staining, Masson staining and immunohistochemical staining. The remaining kidney tissues were kept in –80 ℃ refrigerator after subpackaging with the EP tubes prepared for polymerase chain reaction (PCR).

HE Staining

The 4-μm-thick paraffin sections were routinely dewaxed in xylene, hydrated in graded ethanol, washed with distilled water 3 times for 5 min each time, and, immersed in hematoxylin solution for 5 min, and then the sections were rinsed by water till they became blue. The sections were then put into 1% hydrochloric acid ethanol solution till they turned to red. Subsequently, they were rinsed with water to get blue again. After gradient ethanol dehydration, the sections were stained with eosin ethanol solution (0.05%).

Masson Staining

After dewaxing and gradient ethanol hydration, the sections were stained with Weiger 's hematoxylin solution for 10 min, rinsed in distilled water for 5 min, stained with Ponceau acid fuchsin solution for 10 min, rinsed in distilled water for 2 min, placed in 1% phosphomolybdic acid solution for 5 min, and immersed in aniline blue solution for 5 min. Collagenous fiber was stained blue by Masson staining. The sections were single-blindly observed under microscope (400× magnification). From each section, ten non-repeated renal interstitium

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fields were selected. By using IPP image analysis software, positive staining area of renal interstitium was determined in each high power field, and percentage of positive staining area was calculated. The staining was scored according to a 5-point scale: 0, no color; 1, mild, percentage of staining area <10%; 2, moderate, percentage of staining area between 11% and 25%; 3, severe, percentage of staining area between 26% and 50%; 4, extremely severe, percentage of staining area >50%

Immunohistochemistry Detection of TGF-β1

Freshly kidney tissues were fixed with 10% neutral formalin and embedded in paraffin for sectioning. Tissue sections of 4-μm thick were cut. Immunohistochemical staining for TGF-β1 was performed using a diaminobenzidine (DAB) kit (Beijing Zhongshan Golden Bridge Biotechnology, China). Briefly, tissue sections were deparaffinized in xylene and rehydrated through a gradient alcohol series to water. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol. Slides were incubated with anti-TGF-β1 antibodies at 1:100 dilution (Boshide, China) overnight at 4 ℃, followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Boshide, China) for 2 h. The antibody binding sites were visualized by incubating with DAB-H_2O_2 solution. The slides were counterstained with hematoxylin, and tissue sections were mounted. Immunofluorescence microscopy (IF) for TGF-β1 was performed using fluorescence-labeled rabbit anti-human antibodies. Control sections were processed as described above without adding primary antibody.

Immunohistochemistry results were considered positive when brown particles appeared in the cells after DAB staining. An Image-Pro Plus 4.5 microscopic image analyzing system was used to measure the density of the positive products. Five visual fields in each section were randomly selected to measure the labeling index. The labeling index was expressed as a percentage of positive stained cells (i.e., TGF-β1 labeling index = number of TGF-β1 positive cells/number of total cells per high power field × 100). A higher labeling index value indicates a greater expression of the corresponding proteins.

Determination of mRNA Expression of TGF-β1, α-SMA and E-cadherin by Real-time PCR

Total RNA of rat kidney was extracted according to the the manufacturer's instructions (RNeasy Mini Kit, QIAGEN Inc., Japan). Kidney tissues nearby left renal cortex were harvested. Reverse transcription with up to 4 μg of total RNA was carried out in a total volume of 20 μL reaction system containing 250 pmol of random primer, 100 U of Super-Script Ⅱ RNase H-reverse transcriptase (Invitrogen, USA) in 50 mmol/L Tris-HCl (pH 8.3), 40 mmol/L KCl, 6 mmol/L $MgCl_2$, 1 mmol/L DL-Dithiothreitol, and 10 mmol/L deoxy-ribonucleoside triphosphate. Initially, total RNA solution mixed with random primer was heated to 70 ℃ for 10 min and immediately chilled on ice, and then the other reagents were added. First-strand cDNAs were obtained after 50 min reaction at 42 ℃ and after 5 min at 98 ℃. Power SYBR Green PCR Master Mix (Applied Biosystems Inc.) was used. Primers were as follows: TGF-β1 sense primer: 5'-CAACCCGGGTGCTTCCGCAT-3'; antisense primer: 5'-CTTGGGCTTGCGACCCACGT-3'. α-SMA sense primer: 5'-AGAGTGGAGAAGCCCAGCCAGTC-3'; antisense primer: 5'-CATCACCAGCAAAGCCCGCCTT-3'. E-cadherin sense primer: 5'-CCGCGGCGCACTACTGAGTT-3'; antisense primer: 5'-CGGGCAAACGCCGAGCAAAC-3'. β-actin sense primer: 5'-GTTGCGTTACACCCTTTCTTG-3'; antisense primer: 5'-CTGCTGTCACCTTCACCGTT-3'. All of the primers were provided by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd., China.

Quantification of target cDNA and an internal reference gene β-actin was performed in 96-well plates on the ABI PRISM7700 Sequence Detection System (ABI, USA). Data collection and analyses were carried out by using a software package that came with the machine. The PCR was performed in a final volume of 25 μL reaction system containing cDNA template 2.5 μL, sense primer and antisence primer 0.25 μL, respectively, SYBR Green PCR Master Mix 12.5 μL and DNA water 9.5 μL. Each sample was analyzed in triplicate. Thermal cycler conditions were 95 ℃ for 10 min, followed by 40 cycles at 95 ℃ for 15 s and 60 ℃ for 1 min. The comparative CT method of data analysis was used to analyze the data. CT is the PCR cycle at which an increase in reporter fluorescence above the baseline level was first detected. CT of target gene and CT of internal reference gene were calculated for each sample. $^{\triangle}$CT was the difference in CT between target gene and reference gene. $^{\triangle\triangle}$CT was the difference in $^{\triangle}$CT between sample and calibrator sample. The expression level of target gene,

normalized to an internal reference and relative to calibrator, was calculated by $2^{-\triangle\triangle CT}$.

Western Blot Analysis

Kidney tissue samples for Western blot were homogenized in radio immunoprecipitation assay buffer. Protein (60 μg) was loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and the separated proteins were transferred to nitrocellulose membranes. The blots were incubated in blocking buffer tris-buffered saline with Tween-20 for 1 h at room temperature. The membranes were then incubated with primary antibodies overnight, and washed by TBS 3 times for 10 min each time, and subsequently were incubated with anti-TGF-β1 antibodies at 1:100 dilution (Boshide, China) in TBS with 0.1% Tween-20, 5% nonfat milk for 1 h. ECL Plus kit (Amersham Biosciences, Sweden) was used to detect protein.

Statistical Analysis

Data were expressed as mean ± standard deviation. $P<0.05$ was considered statistically significant. The statistical analysis was performed by one-way analysis of variance using SPSS 12.0 software.

RESULTS

Gross Observation

No rats died in all groups during the experiment. In the control group, the vitality, activity, response to external stimulus, diet and fur color of the rats were normal and the weight increased significantly. For the model group, the rats were listless, their activities decreased, they ate and drank less, and their fur color became dark. Moreover, this condition aggravated after operation. In the QCF group, the vitality, activity, response to external stimulus, diet and fur color of the rats were mildly abnormal, and their weights increased.

For the kidney in the control group, the size was normal, the color was dark red, the capsule was complete and smooth, there were no significant granules on it, and the boundary between cortex and medulla was clear. For the kidney in the model group, the size was increased, the color became gray and dark, there were granules on it, volume of the renal pelvis increased, renal papillae became flat, cortex got thin, the boundary between cortex and medulla was unclear, and there was brown turbid urine inside. Compared with the model group, granules on the capsule of the treatment group were decreased, the color was dark red, the boundary between cortex and medulla was clear, and the volume was slightly increased.

Pathologic Results of Kidney

The kidneys in the model and QCF groups were damaged to different extent except the control group, among them, the degree of damage was higher in the model group, and lower in the QCF group. In the control group, there were no significant pathological changes in kidney tissue, structures of glomeruli and tubules were normal, arrangement of tubules was close, cortex had no obvious change, and there were no degeneration and necrosis of tubular epithelial cells and no infiltration of inflammatory cells. In the model group, under the light microscope, the majority of tubular epithelial cells were degenerated and necrotic, basilar membrane was damaged, partial interstitial fibrous tissues were hyperplastic, there was infiltration of chronic inflammatory cells and proliferation of fibroblasts, mesenchyme was obviously broadened, indicating accumulation of a large number of collagenous fibers and success of modeling. Pathological changes were less in the QCF group than in the model group, and there were only degeneration of renal tubular epithelial cells and monocytes were rarely seen (Figure 1).

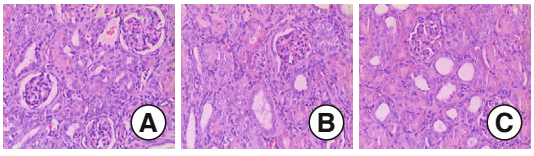

Figure 1. Pathological Changes in Kidney Slice of Rats among Groups by HE Staining (× 400)
Notes: A: control group; B: model group; C: QCF group

The kidney tissue of the rats in the model and QCF groups had varied degrees of fibrosis tissue hyperplasia except the control group, among them, the degree of fibrosis tissue hyperplasia was higher in the model group, and lower in the QCF group. In the control group, there were no significant changes in renal tubules, and few inflammatory cells in mesenchyme and no hyperplasia of fibrosis tissues. In the model group, a large area of renal tissue was stained blue, indicating collagen was stained. Hyperplastic interstitial fibrous tissues were bunchy, reticular and flaky, or multifocal; tubules and collecting tubes were atrophic and collapsed. Fibrosis was

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slighter and stained collagen area was significantly smaller in the QCF group than in the model group (P<0.05). And bunchy hyperplastic interstitial fibrous tissues were seldom seen. After analyzing with image analysis software, stained collagen area was significantly increased in the model group as compared with the control group (P<0.05) and significantly decreased in the QCF group as compared with the model group (P<0.05, Figure 2).

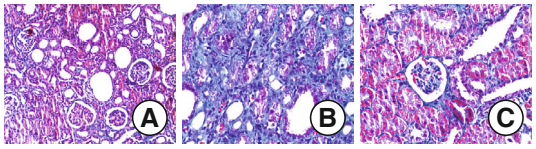

Figure 2. Pathological Changes in Kidney Slice of Rats among Groups by Masson Staining (×400)
Notes: A: control group; B: model group; C: QCF group

Immunohistochemical Results

Expression of TGF-β1 in glomerulus and renal tubular in normal control group was weak positive. While in the model group, there was large brown stained area in renal tubular epithelial cells and mesenchyme, indicating positive TGF-β1 expression; it also expressed in glomerulus. In the QCF group, brown stained area in renal tubular epithelial cells and mesenchyme was decreased as compared with the model group. Brown stained area was significantly increased in the model group as compared with the control group (P<0.05); and significantly decreased in the QCF group as compared with the model group (P<0.05, Figure 3).

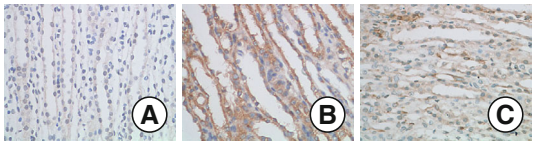

Figure 3. TGF-β1 Expression in Kidney Slice of Rats among Groups by Immunohistochemistry (×400)
Notes: A: control group; B: model group; C: QCF group

TGF-β1 labeling index was 4.25%±1.59% in the control group, while, it was higher in the model group (30.85%±5.98%, P<0.01 *vs*. control group), and 12.43%±2.19% in the QCF group (P<0.01 *vs*. model group).

Expressions of TGF-β1, α-SMA and E-cadherin mRNA

Figure 4 shows the expressions of TGF-β1, α-SMA and E-cadherin in kidney of the three groups. As compared with the control group, the expression levels of TGF-β1 and α-SMA in the model group were significantly increased, while the expression of E-cadherin mRNA was declined. In the QCF group, the expression levels of TGF-β1 and α-SMA were down-regulated, and up-regulated for E-cadherin.

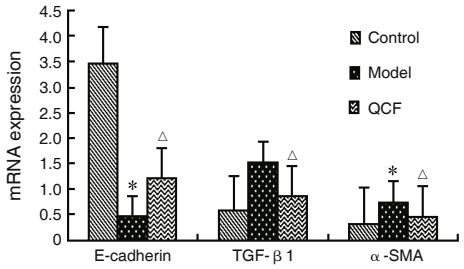

Figure 4. mRNA Expression of E-cadherin, TGF-β1, α-SMA and in Different Groups
Notes: *P<0.05, compare with the control group; △P<0.05 compare with the model group

Western Blot Analysis of TGF-β1 Protein Expression

Western blot analysis of TGF-β1 protein level in the whole kidney homogenate confirmed the measure of TGF-β1 mRNA levels. TGF-β1 protein was detectable at very low levels in rats of the control group (0.2104±0.0394). However, the TGF-β1 protein was induced in the model group and the level of this protein increased obviously (0.6499±0.0480, P<0.01 *vs*. control group), while it was significantly decreased in the QCF group (0.3559±0.0205, P<0.01 *vs*. model group, Figure 5).

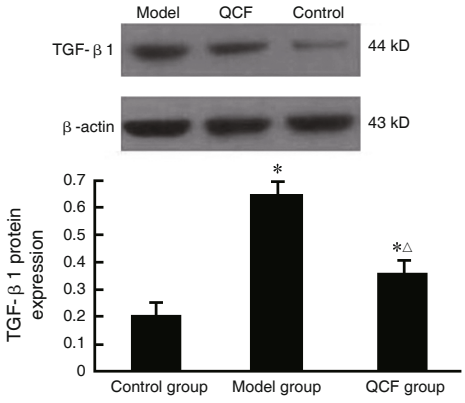

Figure 5. Comparison of TGF-β1 Protein Expression in Whole Kidney Homogenate in Rats among Groups by Western Blot Analysis
Notes: *P<0.05, compare with the control group; △P<0.05, compare with the model group

DISCUSSION

Renal interstitial fibrosis occurs in the progression of chronic kidney disease, characterized by accumulation of a large number of fibroblasts and

extracellular matrix accompanied with gradual loss of normal renal function.[1,14] Recent studies have shown that TEMT is one of the important pathways leading to the excessive accumulation of extracellular matrix and renal interstitial fibrosis.[3] Interstitial fibrosis leads to end-stage renal disease (ESRD), therefore we can prevent ESRD by interrupting or reversing the process of renal interstitial fibrosis.

TEMT mainly includes the following four key steps:[15,16] (1) epithelial cells lose the characteristics of intercellular connection, such as expression of intercellular adhesion molecules E-cadherin, (2) the expression of newly synthesized α-SMA, as well as reconstruction of the cytoskeletal proteins actin, (3) damage of organization of basement membrane integrity, (4) enhancement on cell migration and invasion capacity.

TGF-β1 has been the most frequently studied and the most important fibrogenic cytokines so far, which can stimulate fibroblasts to increase the composition of extracellular matrix (ECM),[17] including proteoglycans, collagen Ⅰ, Ⅲ, and Ⅳ, fibronectin and laminin, etc.; it also inhibits activity of the ECM degrading enzyme and causes decreasing degradation of fibronectin and laminin;[18] it can also stimulate the interaction in cell-cell and cell-matrix, thus inhibit the degradation of ECM; besides, it can induce apoptosis of renal tubular epithelial cells or TEMT, etc.

α-SMA is a cytoskeletal protein, and it is a characteristic protein of myofibroblasts which is widely distributed in smooth muscle, myoepithelial cells, pericytes and some basal cells. There are almost no myofibroblasts in normal kidney, and the expression of α-SMA is only found in the middle layer of renal blood vessels. The myofibroblasts appear in the development of tubulointerstitial fibrosis and are excessively deposited in ECM. The cells mainly express α-SMA, which is a specific marker indicating activation of myofibroblasts, and its expression is a very important event in the development of kidney interstitial fibrosis.[17,19]

A UUO model was established in this study, which is a mature renal fibrosis model nowadays, characterized by no immune injury, no hypertension, no hyperlipidemia-induced damage and so on, and it is simple to operate and easy to observe during experiment with low mortality. In this study, through HE staining we found that in the model group, under the light microscope, pathological changes of tubular epithelial cells were dominated by degeneration and necrosis, renal tubules were necrotic and atrophic, and damages of the basement membrane appeared, part of interstitial fibrous tissues were hyperplastic, chronic inflammation cells infiltrated and fibroblasts proliferated, and mesenchyme was broaden significantly; by Masson staining, a large area of renal tissue was stained blue, indicating collagen was stained. Hyperplastic interstitial fibrous tissues were bunchy, reticular and flaky, or multifocal; tubules and collecting tubes were atrophic and collapsed.

Chronic renal insufficiency is categorized as "edema" in Chinese medicine. Quyu Chencuo method was firstly proposed for treating edema in *Huangdi's Internal Classic • Plain Question* (Huang Di Nei Jing • Su Wen). YANG Shang-shan said in "Tai Su Zhi Tang Yao": "Yuchen means accumulation of extravasated blood". QCF not only includes attacking the water evil, but also involves removing the extravasated blood in body. Strictly following the Quyu Chencuo method, we have created QCF. In this prescription, *Salvia miltiorrhiza Bunge*, cooked *Radix et Rhizoma Rhei*, *Cortex Moutan*, *Semen Persicae*, *Radix Puerariae* were used to remove blood stasis, *Poria cocos* (Schw.) Wolf, *Semen Plantginis Asi-aticae* and *Arecae Pericarpium* were used to eliminate the accumulation of water, and *Rehmannia glutinosa* (Gaertn.) Li-bosch and *Cornus officinalis* Sieb. Et Zucc were used to recover healthy qi. We attained satisfied therapeutic effects of QCF on the treatment of Shen (Kidney) insufficiency in our previous clinical practice. In the experiment, it was observed that QCF can inhibit the renal fibrosis, which may be related to up-regulation the expression of E-cadherin and downregulation the expression of TGFβ1, α-SMA in renal tissue. Nevertheless, the present study is limited by lacking of positive control group and *in vivo* experiment. Moreover, further exploration on the deeper mechanism is needed.

REFERENCES

1. Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med 2008;233:109-122.
2. Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt KU, et al. Chronic kidney disease as a global

public health problem: approaches and initiatives—a position statement from Kidney Disease Improving Global Outcomes. Kidney Int 2007;72:247-259.
3. Liu Y. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 2004;15:1-12.
4. Carew R M, Wang B, Kantharidis P. The role of EMT in renal fibrosis. Cell Tissue Res 2012;347:103-116.
5. Park SH, Choi MJ, Song IK, Choi SY, Nam JO, Kim CD, et al. Erythropoietin decreases renal fibrosis in mice with ureteral obstruction: role of inhibiting TGF-beta-induced epithelial-to-mesenchymal transition. J Am Soc Nephrol 2007;18:1497-1507.
6. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nature Rev Mol Cell Biol 2014;15:178-196.
7. Zhou Q, Zeng R, Xu C, Liu L, Chen L, Kou P, et al. Erbin inhibits TGF-β1-induced EMT in renal tubular epithelial cells through an ERK-dependent pathway. J Mol Med (Berl) 2012;90:563-574.
8. Hinz B. Formation and function of the myofibroblast during tissue repair. J Investig Dermatol 2007;127:526-537.
9. Qi W, Chen X, Poronnik P, Pollock CA. The renal cortical fibroblast in renal tubulointerstitial fibrosis. Internat J Biochem Cell Boil 2006;38:1-5.
10. Gao Z, Ku BQ, Qin YF, Liu JG. Effect of Paidu Mixture on fibrosis parameters and renal function of patients with chronic renal failure. J New Chin Med (Chin) 2007;39:90-92.
11. Ku BQ, Liu JG. Clinical study on change in level of t-lymphocyte subpopulation of the patients with chronic renal failure belongs in blood stasis syndrome and intervening action by Yishen Huoxue Decoction. Hubei J Tradit Chin Med (Chin) 2007;29:5-7.
12. Zhang B, Liu JG. Clinical efficacy observation of "Jixue Paidu Tang" in treating 33 cases of chronic renal failure. J Sichuan Tradit Chin Med (Chin) 2007;25:47-49.
13. Zhang B, Wang QS, Zhu R, Wang YH, Zhang LX, Xu M, et al. Expression of serum-and glucocorticoid-induced kinase 1 in renal glomeruli of patients with obstructive nephropathy and its response to curcumin. J Med Postgrad (Chin) 2008;21:245-257.
14. Eddy AA, Neilson EG. Chronic kidney disease progression. J Am Soc Nephrol 2006;17:2964-2966.
15. Yang J, Liu Y. Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. Am J Pathol 2001;159:1465-1475.
16. Burns W C, Kantharidis P, Thomas M C. The role of tubular epithelial-mesenchymal transition in progressive kidney disease. Cells Tissues Organs 2007;185:222-231.
17. Ning X, Ge X, Cui Y, An HX. Ulinastatin inhibits unilateral ureteral obstruction-induced renal interstitial fibrosis in rats via transforming growth factor β (TGF-β)/Smad signalling pathways. Int Immunopharmacol 2013;15:406-413.
18. Cernaro V, Lacquaniti A, Donato V, Fazio MR, Buemi A, Buemi M. Fibrosis, regeneration and cancer: what is the link? Nephrol Dial Transplant 2012;27:21-27.
19. Dudas PL, Argentieri RL, Farrell FX. BMP-7 fails to attenuate TGF-β1-induced epithelial-to-mesenchymal transition in human proximal tubule epithelial cells. Nephrol Dialysis Transplant 2009;24:1406-1416.

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