Huang et al., Afr J Tradit Complement Altern Med. (2014) 11(5):16-27
http://dx.doi.org/10.4314/ajtcam.v11i5.3

BENEFICAL THERAPEUTIC EFFECT OF CHINESE HERBAL XINJI′ERKANG FORMULA ON HYPERTENSION-INDUCED RENAL INJURY IN THE 2-KIDNEY-1-CLIP HYPERTENSIVE RATS

Ling-ling Huang[1,2†], Chen Pan[3†], Ting-ting Yu[1], Kun Guo[1], Xing-hui Wang[4], Jun-Yan Zhang[1], Hong-zhi Wang[2*], Shan Gao[1*]

[1]Department of Pharmacology, Basic Medical College, Anhui Medical University, Hefei 230032, China, [2]Cancer Hospital, Hefei Institutes of Physical Science, Chinese Academy of Science, Hefei 230031, China, [3]Department of Clinical of Pharmacy, Lishui People's Hospital, Zhe Jiang 323000, China, [4]Department of Pharmacy, the Second People's Hospital of Hefei, Hefei 230011, China.

*Correspondence to: Shan Gao, e-mail: aydgs@126.com; Hong-zhi Wang, e-mail:wanghz@hfcas.ac.cn

† Ling-ling Huang and Chen Pan contributed equally to this work

Abstract

Background: Increase in evidence shows that the role of kidney injury in hypertension is important. Xinji′erkang (XJEK), a Chinese herbal formula, has been identified as an effective preparation in the treatment of coronary heart disease and myocarditis. We have previously demonstrated that XJEK attenuate oxidative stress and hypertension target organ damage. The aim of this study was to assess the renal protective function of XJEK.

Materials and Methods: Two Kidney One Clip (2K1C) model was adopted to induce hypertension in rats. We submitted male Sprague Dawley (150-180) g rats to either renal artery clipping or sham operation. Renal hypertension was established after four weeks of surgery. Rats were randomized divided into the four groups: sham-operated group (Sh-Op) (*n*=10), two-kidney, one-clip hypertension group (2K1C) (*n*=10), Xinji′erkang treatment group (XJEK) (*n*=10) and Fosinopril (*n*=10) treatment group. Drugs were administered orally daily for four weeks. Systolic pressures were measured every week using the tail-cuff apparatus. 24h before death, urine samples were collected for detect of urinary proteins. The kidney weight (KW) index was expressed as kidney weight/body weight (KW/BW). The histological changes were investigated by hematoxylin and eosin and Van Gieson staining. Immunohistochemical assay was employed to observe the intra-renal transforming growth factor-β_1 (TGF-β_1) protein expression. Serum creatinine (SCR) and blood urea nitrogen (BUN) were assayed by automatic biochemical analyzer. ELISA kit was used to assay Angiotensin II (Ang II) and TGF-β_1 content in serum.

Results: Administration of XJEK markedly alleviated the rise in blood pressure and declined LKW/BW ratio. Histo-pathological injuries including hypertrophic glomerular, glomerular sclerosis, glomerular and interstitial fibrosis were attenuated. XJEK also decreased SCR, BUN, urinary proteins in 24h urine, serum Ang II and TGF-β_1 concentrations and the intra-renal TGF-β_1 protein expression.

Conclusion: XJEK therapy in the 2K1C hypertensive rats affects the rise in blood pressure and ameliorates the severity of kidney injury. The protective effect is most likely due to the ability of XJEK to affect the Renin-Angiotensin-Aldosterone System (RAAS) and the TGF-β systems.

Keywords: renal injury; 2K1C hypertensive; Xinji′erkang (XJEK) formula; transforming growth factor-β_1 (TGF-β_1); Angiotensin II (Ang II)

Abbreviations: End-organ damage (EOD); blood pressure (BP); end-stage renal disease (ESRD); Xinji′erkang (XJEK); two-kidney, one-clip (2K1C); sham-operation (Sh-Op); cardiovascular remodeling (CR); renovascular hypertension (RVH); angiotensinogen II (Ang II); Ang II type 1 receptor (AT_1R); renin-angiotensin-aldosterone system (RAAS); transforming growth factor-β_1 (TGF-β_1); serum creatinine (SCR); blood urea nitrogen (BUN); enzyme linked immunosorbent assay (ELISA); right kidney-to-body weight (RKW/BW); left kidney-to-body weight (LKW/BW); extracellular matrix proteins (ECM); oxidative stress (OS); endothelial dysfunction (ED).

Introduction

The relationship between hypertension and cardiovascular diseases is unequivocal now. Cardiovascular diseases are leading causes of deaths globally and hypertension is the major factor contributing to such diseases (Fuster et al. 2011). The reason is that hypertensive complications such as stroke, heart failure, renal failure and myocardial infarction are often lethal. However, end-organ damage (EOD) is the early phase of these complications of hypertension, including the damage to heart，brain，lung，kidney and so on (Morris et al. 2008; García-Donaire et al. 2011). Moreover the kidney plays a relevant role in the control of body fluids and blood pressure (BP) and derangement of renal function could lead to

http://dx.doi.org/10.4314/ajtcam.v11i5.3

the development of hypertension (Stringer et al. 2013). So the linkage between the kidney and hypertension has been considered as a villain-victim relationship because of the potential two-way causality between BP and kidney diseases. Renal structure and function changes caused by hypertension are called hypertensive renal damage in clinic. Undoubtedly, control of blood pressure is the basis of the inhibition of hypertensive renal damage. However, many antihypertensive drugs were developed to provide more effective treatments to hypertension, the incidence of end-stage renal disease (ESRD) induced by hypertension has been increasing in recent years. In decades, the annual growth rate of ESRD incidence was 13%, and the hypertension-induced ESRD accounted for 28% of all (Zuo et al. 2010). These findings suggest that the optimal strategies so far have not been sufficient for the treatment of hypertension to prevent renal damage. The protect effect of renal by using antihypertensive agents, in addition to their efficacy for hypertension, medical therapy has become an increasingly important target.

Xinji′erkang (XJEK) is a Chinese herbal formula, contain fourteen herbal medicines such as *astragali radix, ginseng radix et rhizoma, notoginseng radix et rhizoma, polygonat odorat rhizoma, angelicae sinensis radix* and so on. Both clinical study and basic research have revealed the curative effect of XJEK on coronary heart disease, virus myocarditis and toxic myocarditis (Wang et al.1998; Wang et al.2012). Recently the potential effects of XJEK on the function of the kidney in two-kidney, one-clip (2K1C) hypertension model indicate that XJEK can reduce myocardial fibrosis against isoproterenol-induced ventricular remodeling in mice (Gao et al. 2012) and prevent 2K1C-induced hypertension and cardiovascular remodeling (CR) in 2K1C hypertension rats (Gao et al. 2012;Yu et al. 2013).

2K1C is a model of renovascular hypertension (RVH). Although the clipped kidney is the major driving force behind hypertension, the non-clipped kidney plays an important role in the maintenance of hypertension as well (Lawrentschuk et al.2012). Long-term 2K1C hypertension is followed by the damage of the non-clipped kidney with urinary protein excretion increased, glomerular filtration rate declined, glomerular, tubular, and vascular impaired (Helle et al. 2009; Toklu et al. 2010). The renin level in the non-clipped kidney was decreased (Chugh et al. 2013), however, angiotensinogen II (Ang II) concentration was enhanced (Ploth and Fitzgibbon 1994). The high level of Ang II in kidney seems to depend on admission of Ang II from plasma via an Ang II type 1 receptor (AT_1R)-dependent pathway (Guo et al. 2013; Balla et al. 2011). Transforming growth factor-β_1 (TGF-β_1) is a potent cytokine that promotes cell proliferation and regulates the synthesis of the matrix-associated protein fibronectin and is also implicated in fibrosis and hypertrophy (Pardali and Ten Dijke. 2012). Ang II and TGF-β_1 are found to be involved in renal injury in essential hypertension.

We studied the effect of XJEK on renal function and histology of the non-clipped kidney in rats with RVH, compared with Fosinopril to further explore its antihypertensive mechanism and provide a theoretical basis for its clinical application.

Materials and Methods

Preparation of XJEK extract

XJEK consist of 14 medicinal plants as is shown in Table 1. 14 raw herbs for XJEK were purchased from Hefei Company of Traditional Crude Drugs (Hefei, China). Aqueous extract of XJEK was prepared before experiment and the extracting method could be found in the supplement data. Finally, XJEK was re-suspended in distilled water. The solution was stored in aliquots at –20°C prior to use.

Animal and surgical procedure

All procedures were performed in accordance with the protocol outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication no. 85–23, revised 1996) and approved by the Committee on the Ethics of Animal Experiments of Anhui Medical University. 40 male Sprague-Dawley (SD) rats with the weights of (150-180) g were purchased from Laboratory Animal Center of Nanjing Medical University, housed at 21°C with 12:12-h light-dark cycles, fed with standard laboratory chow, and had access to drinking water ad libitum. After 1 week of acclimatization, 2K1C hypertension of 30 rats was performed as we previously described (Gao et al. 2012; Huang et al. 2013). Briefly, the right kidney of the animal was exposed through a dorsal flank incision, and a 0.25 mm-silver clip was placed on the renal artery under sodium pentobarbital anesthesia (15 mg/kg, intra-peritoneal injection). The other 10 rats were performed without clip application. After operation, rats were intra-peritoneally injected with cefradine for 3 days as an anti-infection measure. The 40 rats remained on the normal chow diet in the following 4 weeks.

The right renal artery clamping (2K1C) for four weeks, which the 30 rats were randomly assigned into 3 groups (n=10): the 2K1C group (2K1C): distilled water was intra-gastrically administrated to rats daily for 4 weeks; the XJEK group (XJEK): XJEK (24g/kg/day) was

http://dx.doi.org/10.4314/ajtcam.v11i5.3

intra-gastrically administrated to rats daily for 4 weeks; the Fosinopril group (Fosinopril): Fosinopril (Bristol-Myers Squibb, China, 15mg/kg/day) was intra-gastrically administrated to rats daily for 4 weeks. The other 10 rats which had undergone a similar surgery procedure without clip application served as the sham-operated group (Sh-Op): distilled water was intra-gastrically administrated to rats daily for 4 weeks.

Table 1: Components of XJEK

Components	Latin name	Ratio (w/w, %)
Sheng shai sheng	*Ginseng radix et rhizoma*	7.03
Yu zhu	*Polygonat odorat rhizoma*	7.80
San qi	*Notoginseng radix et rhizoma*	3.09
Xie bai	*Allii macrostemonis bulbus*	7.80
Dang gui	*Angelicae sinensis radix*	7.80
Mai dong	*Ophiopogonis radix*	7.80
Wu wei zi	*Schisandrae chinensis fructus*	3.93
Dan shen	*Salviae miltiorrhizae radix et rhizoma*	7.80
Ku shen	*Radix Sophorae Fiavescentis*	7.80
Zhi gan cao	*Glycyrrhizae radix et rhizoma*	7.80
Huang qi	*Astragali radix*	15.60
Yin yang huo	*Epimedii folium*	7.80
Gua lou	*Trichosanthis fructus*	7.80
Bing pian	*Borneolum syntheticum*	0.15

The Latin names of the 14 herbs are derived from Chinese Pharmacopoeia.

Measurement of systolic blood pressure

Systolic blood pressures were measured in the middle two days of every week using the tail-cuff apparatus (ALC-NIBP, Shanghai Alcott Biotech Co. Ltd., China), so that the circadian rhythms of the animals were identical throughout the whole study. Rats were pre-warmed on a pad at 40 °C and placed in restrainers individually prior to the measurement. The average of five readings was then recorded. Duration of the study was 9 weeks, marked as weeks 0, 1, 2, 3, 4, 5, 6, 7, 8 (7 days a week, the 2K1C surgery procedure was taken between the last day of week 0 and the first day of week 1).

Analysis of urinary proteins

24 hr before the end of the experiment, the animals were free access to water, and urine samples were collected to calculate the urine volume and to detect the content of urinary protein. The urinary protein was determined by BCA Protein Assay Kit (Beyotime of Institute of Biotechnology).

Analysis of serum creatinine (SCR), blood urea nitrogen (BUN), Ang II and TGF-β_1 content in serum

Rats were killed by exsanguination and the blood was collected in chilled 5-ml tube containing heparin, and then centrifuged at 3000rpm at 4°C for 10 min. The plasma was immediately removed and dispensed into sample tubes for SCR, BUN, Ang II and TGF-β_1 concentration measurement. SCR and BUN were detected by automatic biochemistry analyzer (HITACHI 7020). Ang II and TGF-β_1 were measured by Enzyme Linked Immunosorbent Assay (ELISA) kits (Boster Biotechnology Co. Ltd., Wuhan, China).

http://dx.doi.org/10.4314/ajtcam.v11i5.3

Analysis of kidney hypertrophy

The kidney weight-to-body weight (KW/BW) ratios are a widely accepted index for kidney hypertrophy (Guo et al. 2006). Body weights were recorded before the death of the animals. After death, the bilateral kidneys were rapidly removed and placed in ice-cold 0.9% NaCl solution, trimmed off fat and connective tissue, blotted with water, and weighed separately using an analytic balance (JA2603B electronic analytical balance, Shanghai precision scientific instrument Co. Ltd., China). Then the right kidney-to-body weight (RKW/BW) and left kidney-to-body weight (LKW/BW) ratios were calculated and recorded.

Histological and immunohistochemical analyses of the kidney

The kidneys were paraffin-embedded and sectioned at a 5μm thickness by Leica slicer (LEICARM2035). Some sections were stained with hematoxylinand eosin and Van Gieson for histological studies while others were used for immunohistochemical analyses. From the cortical region of kidney sections, 30 glomeruli per rat were randomly selected, the area was determined by means of a computerized program using NIH Image 1.61 software (National Institutes of Health Service Branch), and the average glomerular areas were compared among different groups.

Immunohistochemical assay was performed by incubating sections with goat serum(10% v/v) to block nonspecific staining, followed by overnight incubation with rabbit polyclonal antibody TGF-β_1 (Boster Biotechnology Co. Ltd., Wuhan, China，1:200). Subsequently, sections were washed by PBS and incubated at room temperature for 30 min with goat anti-rabbit IgG (Boster Biotechnology Co. Ltd., Wuhan, China, 1:500). The specimens were then photographed and semi-quantitatively analyzed by NIH Image 1.61 software as our previous report (Wang et al. 2013).

Statistical analysis

All data are expressed as mean ± SD. For all the statistical tests, multiple comparisons were performed by one-way ANOVA with Tukey–Kramer exact probability test. The least-squares method was used for linear correlation between selected variables. Statistical significance was accepted at $P<0.05$.

Results

During the study, all control rats were survived, 4 rats in other three groups were died. The data of Sh-Op group (n=10), 2K1C group (n=8), XJEK (n=9) and Fosinopril (n=9) treated group were evaluated respectively.

Effect of XJEK on SBP development

There were no significant difference in rats' systolic blood pressure (SBP) among the groups before the experiment started and no significantly changes of SBP were seen in Sh-Op group during the experiment (Figure 1, $P>0.05$). Significantly increases of SBP were found in 2K1C group 1 week after renal artery was narrowed and SBP remained increasing with minor fluctuations all through the experiment. However, lower SBP levels were detected in hypertensive rats treated with XJEK and Fosinopril compared 2K1C group, and this difference was significantly from the 6^{th} week (Fosinopril group) and the 7^{th} week (XJEK group) until the end of the treatment (Fig1, $P<0.05$, $P<0.01$).

Effect of XJEK on right kidney-to-body weight (RKW/BW) and left kidney-to-body weight (LKW/BW)

Body and organ weights are shown in Table 2. Prior to clipping as well as at the 8_{th} week, there was no significant difference in body weight among the 4 groups.

As shown in Table 2, the 2K1C hypertension rats had higher LKW and LKW/BW ratios and lower RKW and RKW/BW ratio compared with the Sh-Op rats ($P< 0.01$).The administration of XJEK and Fosinopril reduced LKW ratios by 17.92%, 12.34% and LKW/BW ratios by 9.49%, 12.20% compared with the 2K1C group, respectively ($P< 0.05$, $P< 0.01$). There was a tendency of increasing RKW in XJEK, Fosinopril treatment groups in comparison with 2K1C group ($P>0.05$), however, with treatment of XJEK and Fosinopril improved RKW/BW ratios by

http://dx.doi.org/10.4314/ajtcam.v11i5.3

13.04% and 13.47%, respectively ($P< 0.05$, $P< 0.01$).

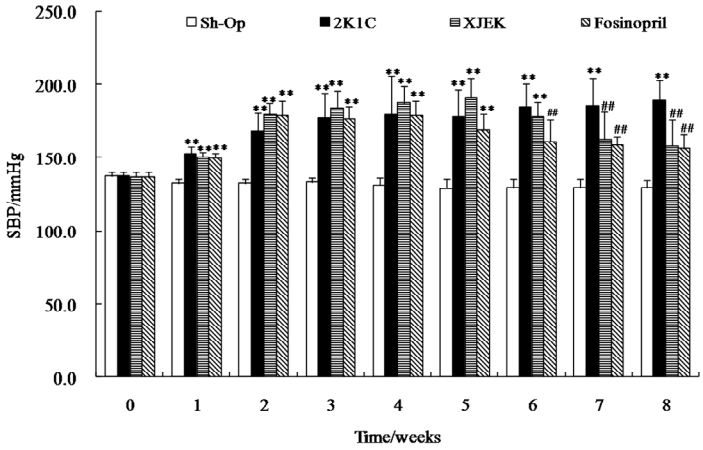

Figure 1: Hypertension induced by 2K1C during the study. Nine time points of SBP were measured using tail-cuff apparatus measurement in each group. Data are expressed as the mean ± SD, n = 8 ~10.$^{**}P$<0.01 compared to Sh-Op group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to 2K1C group.

Table 2: Anthropometric parameters of BW, LKW, LKW/BW, RKW, RKW/BW in different groups (mean ± SD).

Group	n	BW_0 (g)	BW_8 (g)	LKW(mg)	LKW/BW(mg/g)	RKW(mg)	RKW/BW(mg/g)
Sh-Op	10	225.0±7.0	387.5±39.2	1070.7±124.9	2.81±0.26	1080.2±135.9	2.83±0.24
2K1C	8	252.1±11.9	396.0±48.3	1454.3±212.9**	3.69±0.48**	896.2±186.1*	2.30±0.38**
XJEK	10	239.9±2.6	357.4±43.7	1193.7±164.8$^{\#\#}$	3.34±0.28$^{\#}$	907.4±144.5	2.60±0.38$^{\#}$
Fosinopril	9	219.3±9.1	392.8±41.4	1274.9±102.4$^{\#}$	3.24±0.18$^{\#}$	1013.8±110.6	2.61±0.30$^{\#}$

$BW_{0:}$ body weight at 0th week; $BW_{8:}$ body weight at the 8th week; LKW: left kidney weight; LKW/BW: left kidney weight/ body weight ratio; RKW: right kidney weight; RKW/BW: right kidney weight/ body weight ratio. $^{*}P < 0.05$, $^{**}P$<0.01 compared to Sh-Op group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to 2K1C group.

Effect of XJEK on renal function

SCR, BUN and 24h urinary protein content were detected to evaluate renal function. The data revealed that SCR, BUN and 24h urinary protein concentrations had increased by 1.81 fold, 1.79 fold and 1.60 fold in 2K1C group in comparison with Sh-Op group (Table 3), indicated 2K1C had caused marked renal damage. XJEK and Fosinopril treatments had improved renal function confirmed by the decreases in the content of SCR (36.74%, 37.96%), BUN (23.75%, 29.20%) and 24h urinary protein (61.07%, 41.42%) compared with 2K1C group, respectively ($P<$ 0.05, $P<$ 0.01).

Table 3: Effects of XJEK on SCR, BUN content and urinary protein content in 24h urine samples in 2K1C hypertensive rats (mean ± SD)

Group	n	SCR (μmol/L)	BUN(mmol/L)	Urinary protein(mg/24h)
Sh-Op	10	55.9±11.5	6.3±1.1	82.19±31.3
2K1C	8	100.9±11.1**	11.3±1.6**	131.1±41.2*
XJEK	10	77.9±24.6$^{\#\#}$	9.4±1.1$^{\#\#}$	84.2±19.3$^{\#}$
Fosinopril	9	62.6±15.2$^{\#\#}$	8.0±2.2$^{\#\#}$	76.8±33.0$^{\#\#}$

SCR: serum creatinine; BUN: blood urea nitrogen. $^{*}P$<0.05, $^{**}P$<0.01 compared to Sh-Op group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to 2K1C group.

Effect of XJEK on renal histopathological changes

Histopathological examination of the kidney of 2K1C rats revealed tissue injury characterized by hypertrophic glomeruli（Fig2）and

http://dx.doi.org/10.4314/ajtcam.v11i5.3

extensive glomerular damage consisting of global or segmental sclerosis（Fig3）, glomerular fibrosis（Fig4A）, as well as interstitial fibrosis（Fig 4B）. The mean glomerular area was increased by 93.79% in 2K1C group in comparison with that in Sh-Op group（Fig2B）. Such damage was effectively ameliorated by the treatment with XJEK and Fosinopril, and glomerular area was decreased by 48.24% and 37.29%, respectively ($P<$ 0.01).

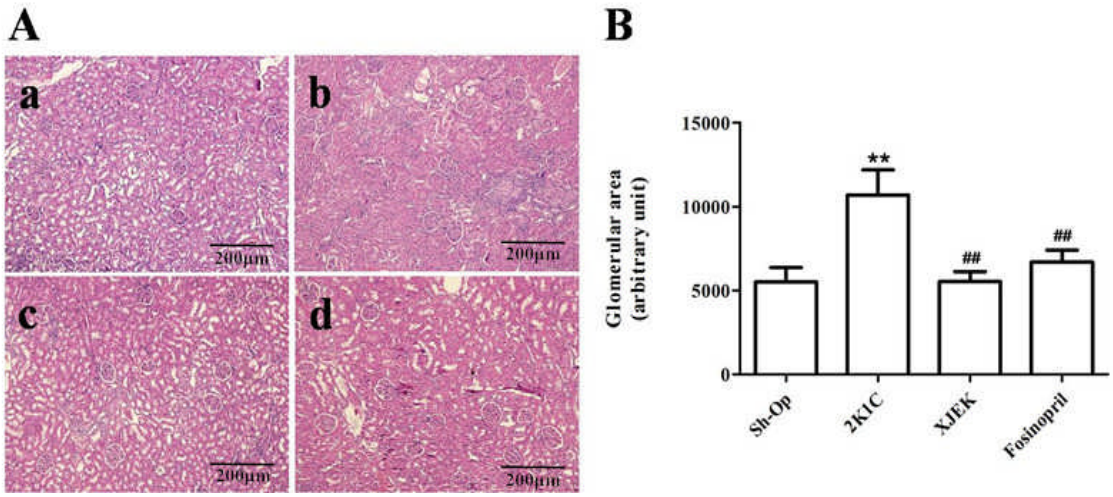

Figure 2: Effects of XJEK on hypertrophic glomeruli in 2K1C hypertensive rats. A. Representative images of histological sections of hypertrophic glomeruli (HE stain, ×100). a: Sh-Op rats; b: 2K1C rats; c: XJEK treatment rats; d: Fosinopril treatment rats. B. Statistic results. Data are expressed as the mean ± SD, n= 8 ~10. $^{**}P<0.01$ compared to Sh-Op group; $^{\#\#}P<$ 0.01 compared to 2K1C group.

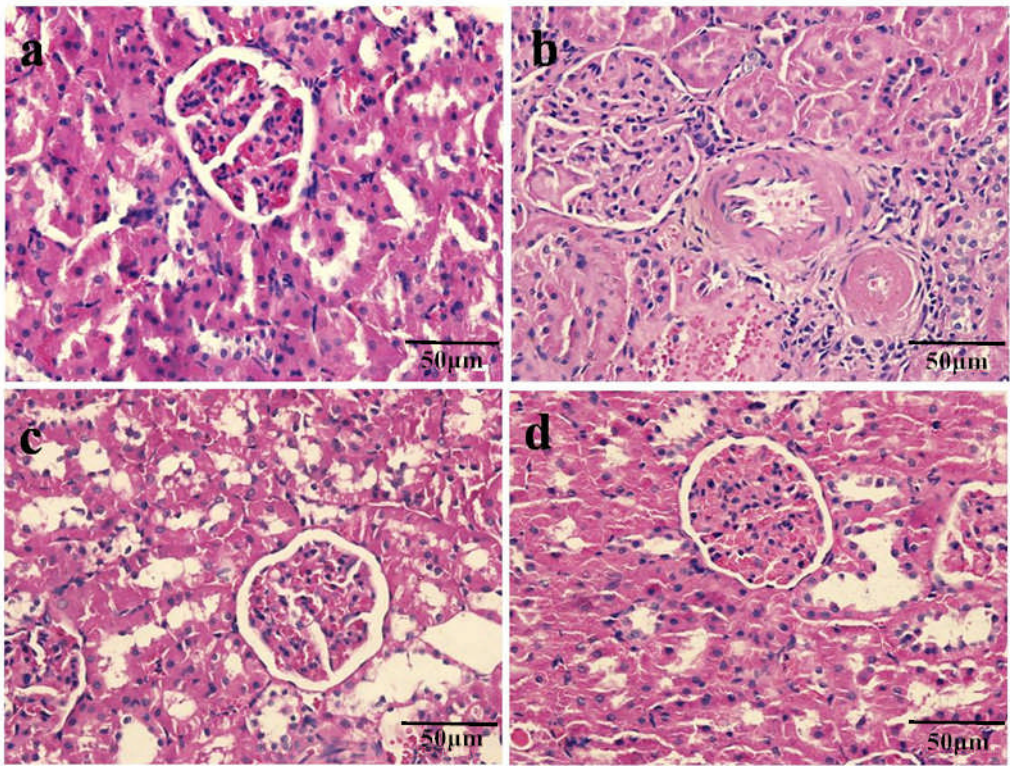

Figure 3: Effects of XJEK on glomerular sclerosis in 2K1C hypertensive rats. (HE stain, ×400). a: Sh-Op rats; b: 2K1C rats; c: XJEK treatment rats; d: Fosinopril treatment rats.

http://dx.doi.org/10.4314/ajtcam.v11i5.3

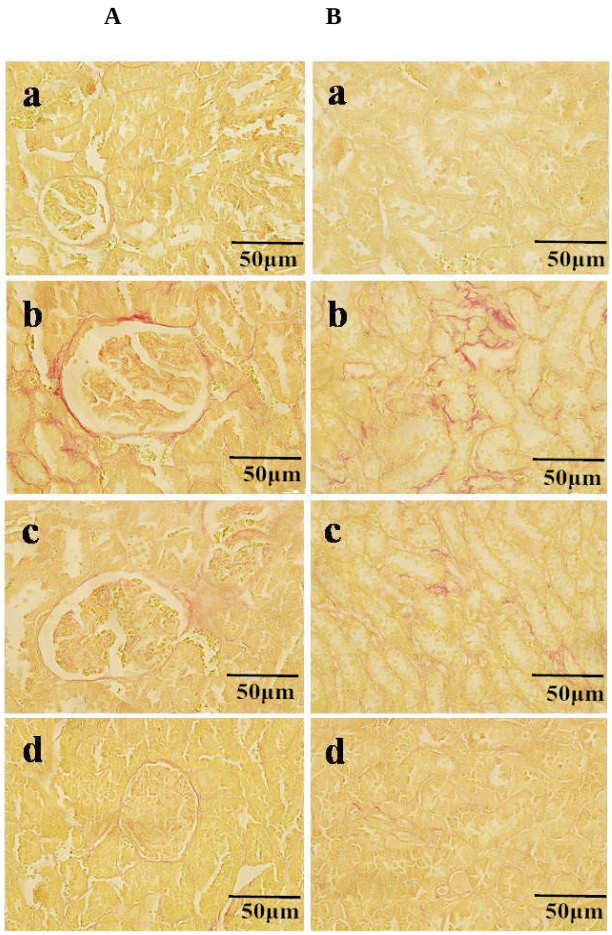

Figure 4: Effects of XJEK on renal fibrosis in 2K1C hypertensive rats. (VG stain, ×400). A. Representative images of histological sections of glomerular fibrosis; B. Representative images of histological sections of interstitial fibrosis a: Sh-Op rats; b: 2K1C rats; c: XJEK treatment rats; d: Fosinopril treatment rats.

Effect of XJEK on serum Ang II concentration

ELISA assay was used to detect serum Ang II concentration at the end of the 8th week and the results are shown in Figure 5. Compared to the Sh-Op group, serum Ang II concentration was increased by 174.61% in 2K1C hypertensive rats ($P< 0.01$). The 2K1C-induced increase of Ang II was attenuated by 31.22% and 31.49% in XJEK and Fosinopril treatment rats ($P<0.01$), respectively.

http://dx.doi.org/10.4314/ajtcam.v11i5.3

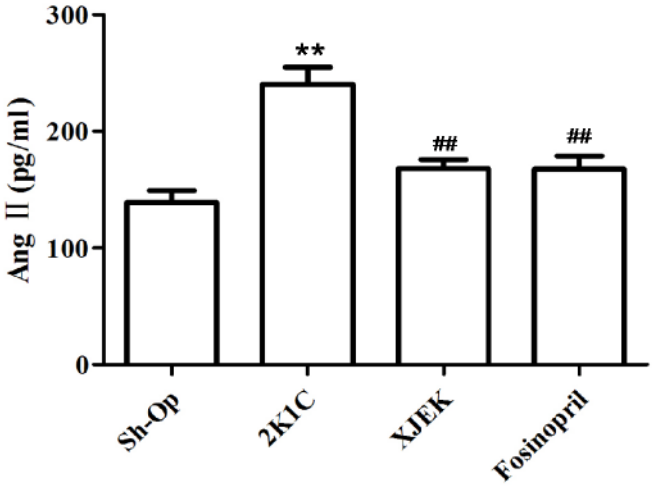

Figure 5: The serum Ang II concentration in different groups. Data are expressed as the mean ± SD, n = 8 ~10. $^{**}P$<0.01 compared to Sh-Op group; $^{\#\#}P$ < 0.01 compared to 2K1C group.

Effect of XJEK on serum TGFβ$_1$ concentration and TGFβ$_1$ protein expression in kidney tissue

To quantify the effect of antihypertensive therapy，ELISA analysis of serum TGF-β1 levels and immunohistochemical assay of TGFβ1 protein expression in kidney tissue were performed. Intra-renal TGF-β1 protein expression measured by immunohistochemistry was significantly increased in the 2K1C rats (Fig8). Similar to previous findings, 2K1C hypertensive rats in the present study showed increased levels of TGF-β1 in serum compared with Sh-op rats (Fig7). A decrease of intra-renal TGF-β1 protein expression and serum TGF-β1 abundance had been found in 2K1C hypertensive rats after antihypertensive therapy Figure 6. Interestingly，XJEK had the same function as Fosinopril in reducing TGF-β1 over-expression in tissue and content in serum.

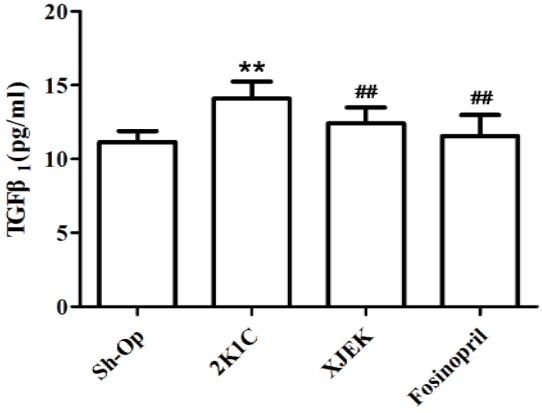

Figure 6: The serum TGFβ$_1$ concentration in different groups. Data are expressed as the mean ± SD, n = 8 ~10. $^{**}P$<0.01 compared to Sh-Op group; $^{\#\#}P$ < 0.01 compared to 2K1C group.

http://dx.doi.org/10.4314/ajtcam.v11i5.3

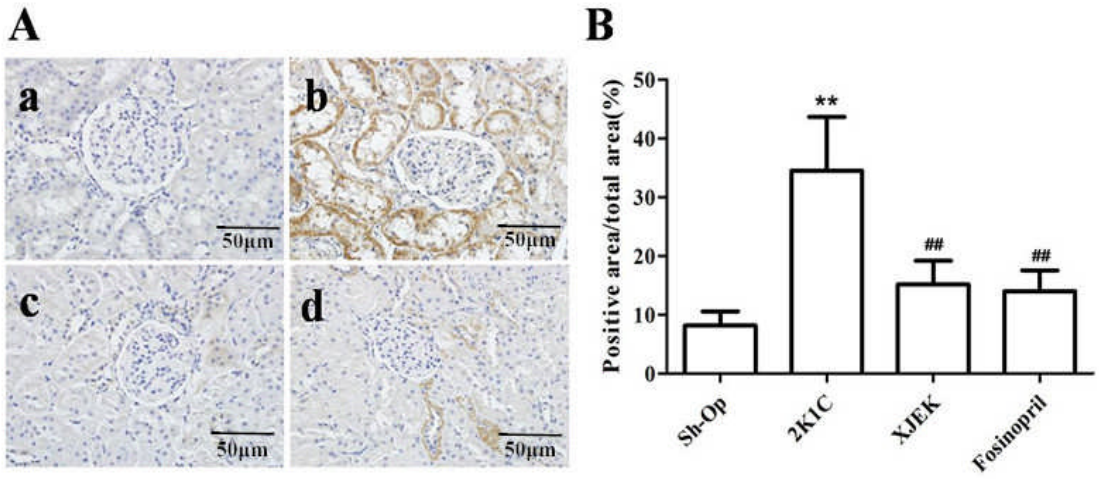

Figure 7: Effects of XJEK on intra-renal TGF-β_1 protein expression in 2K1C hypertensive rats. (A) Reprehensive images of TGFβ_1 protein expression in kidney tissue. (a, Sh-Op rats; b, 2K1C rats; c, XJEK treatment rats; d, Fosinopril treatment rats.) (B) Statistic results of TGFβ_1 protein expression in kidney tissue. Data are expressed as the mean ± SD, n = 8 ~10. $^{**}P$<0.01 compared to Sh-Op group; $^{\#\#}P$ < 0.01 compared to 2K1C group.

Discussion

XJEK is an effective clinical prescription which clear curative effect has been proved by lots of clinical experiences (Wang et al a, b. 1988; 1996). Among the compounds of XJEK formula, *ginseng radix et rhizoma* and *astragali radix* can reinforce qi and nourish blood circulation, *ophiopogonis radix* and *polygonat odorat rhizoma* have the protective effect on heart, *angelicae sinensis radix* and *salviae miltiorrhizae radix et rhizoma* promote blood circulation. Previous studies have also indicated that XJEK can prevent hypertension and cardiovascular remodeling in 2K1C rats, which seems to be related to the attenuation of oxidative stress (OS) and moderate endothelial dysfunction (ED) (Yu et al. 2013). This study further demonstrates that XJEK formula has nephro-protective effects on 2K1C rats.

The effects of XJEK on hypertensive renal damage were investigated in 2K1C rats. Renal hypertrophy is one of the key pathological changes and is fundamental to hypertensive renal damage, because in the 2K1C hypertension model, systemic blood pressure is increased and it directly affects glomerular perfusion and pressure, and leads to urinary protein, glomerulosclerosis, and interstitial renal fibrosis, as a result of renal dysfunction. In addition, renal hypertrophy is an early stage predictor of renal damage, which suggests that glomerular hyperperfusion, hyper-filtration, and glomerular hypertension (Venkatachalam et al. 2010). In our study, clipping of the right renal artery in the 2K1C rats induced severe hypertension with mean blood pressure levels of 174.9 mmHg. A significant increase in the non-clipped weight (or LKW) and the non-clipped/body weight (or LKW/BW) also developed. SCR, BUN in serum and urinary protein in 24h urine were increased, which indicated that an obvious damage of the kidney function was developed. Histological examination revealed marked renal damage within the non-clipped kidney of the 2K1C rats. HE staining showed increased glomerular hypertrophy, global or segmental sclerosis and general interstitial inflammation, and VG staining detected increased collagen production in 2K1C rats. The hypertension model rats were given XJEK and Fosinopril for 4 weeks by gavage, both of which not only decreased BP effectively, but also significantly alleviated the increases of the non-clipped weight (or LKW) and the non-clipped/body weight (or LKW/BW). Simultaneously the rise in SCR, BUN concentration in serum and urinary protein concentration in 24 h urine were lower, and reversed renal pathology significantly, including the prevalence of glomerular sclerosis, hypertrophy, fibrosis and tubule interstitial fibrosis.

In damage to the non-clipped kidney of the 2K1C hypertension model, a lot of mechanisms are involved. The interaction of the cytokines Ang II and TGF-β_1 is of paramount importance in the regulation of the fibrotic pathways, which ultimately leads to organ malfunction and organ death (Wang et al. 2010; Jeffrey et al. 2011; Ding and Choi. 2014). Accumulating data indicate that TGF-β_1 is a key pro-fibrotic cytokine that contributes to tubule interstitial damage and renal fibrosis. Data from TGF-$\beta_1$transgenic mice have shown that TGF-β_1 over-expression can cause urinary protein and progressive glomerulosclerosis (Casalena et al. 2012). In addition, targeting TGF-β_1 directly through antibody application has shown to reduce the progression of clinical and experimental chronic renal disease (Park et al. 2013; Li et al. 2013). TGF-β_1 is a potent cytokine

http://dx.doi.org/10.4314/ajtcam.v11i5.3

that promotes cell proliferation and regulates the synthesis and degradation balance of the extra cellular matrix and is also implicated in fibrosis and hypertrophy (Guo et al. 2013). Our results show that antihypertensive therapy with XJEK and Fosinopril ameliorates hypertensive renal damage, and is consistent with decreasing intrarenal TGF-β_1 expression, circulating TGF-β_1 concentration in 2K1C rats. These findings suggest that XJEK and Fosinopril protect the kidney from hypertensive damage partly through reducing TGF-β_1 production, may lead to a correction of extra cellular matrix synthesis and degradation imbalance.

Ang II is a critical biological substance of the renin-angiotensin system, which plays a key role in renal diseases, including renal injury and progression to renal fibrosis (Zhang et al. 2012).The studies by Ruiz-Ortega et al (2006) showed the superiority of angiotensin-converting enzyme (ACE) inhibitors and AT_1 antagonists in halting the progression of renal disease, suggesting that Ang II plays a pivotal role in the patho-physiology of chronic renal disease (Li et al. 2012). The evidences show that Ang II activates renal cells to produce pro-fibrotic factors and extracellular matrix proteins (ECM) (Dussaule et al. 2011; Habibi et al. 2014). There is a closely interaction between the RAAS and the TGF-β systems (Xia et al. 2014; Savoia and Volpe. 2014). Principally, Ang II stimulates TGF-β expression in the kidney by various mechanisms and up-regulates receptors for TGF-β (Habibi et al. 2014). In our study, we observed that plasma AngII concentration was increased in 2K1C model rats and decreased in XJEK and Fosinopril treatment rats. Antihypertensive therapy with XJEK and Fosinopril could ameliorate hypertensive renal damage, which might be consistent with the decreased overproduction of Ang II. Such findings suggest that XJEK protects the kidney from hypertensive damage partly by affecting renin-angiotensin system and lowering TGF-β_1 concentration.

In summary, our data demonstrate that XJEK have a therapy effects in the 2K1C rat model of RVH, such as the rise in blood pressure and ameliorates the severity of kidney injury. The protective effect of XJEK is most likely on the RAAS and the TGF-β systems.

Competing interests: The authors report no conflict of interest.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81073088; No. 81373774) and Anhui Provincial Science Foundation for Young Talents under Grant (No.2012QRL267)".

References

1. Balla A, Erdélyi LS, Soltész-Katona E, Balla T, Várnai P, Hunyady L (2011). Demonstration of angiotensin II-induced Ras activation in the trans-Golgi network and endoplasmic reticulum using bioluminescence resonance energy transfer-based biosensors. J Biol Chem, **286**(7):5319-27.
2. Barnes JL, Gorin Y (2011). Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. Kidney Int, **79**(9):944-56.
3. Casalena G, Daehn I, Bottinger E (2012). Transforming growth factor-β, bioenergetics,and mitochondria in renal disease. Semin Nephrol, **32**(3):295-303.
4. Chugh G, Pokkunuri I, Asghar M (2013). Renal dopamine and angiotensin II receptor signaling in age-related hypertension. Am J Physiol Renal Physiol, **304**(1):F1-7.
5. Ding Y, Choi ME (2014). Regulation of autophagy by TGF-β: emerging role in kidney fibrosis. Semin Nephrol, **34**(1):62-71.
6. Dussaule JC, Guerrot D, Huby AC, Chadjichristos C, Shweke N, Boffa JJ,Chatziantoniou C (2011). The role of cell plasticity in progression and reversal of renal fibrosis. Int J Exp Pathol, **92**(3):151-7.
7. Fuster V, Kelly BB, Vedanthan R (2011). Global cardiovascular health: urgent need for an intersectoral approach. J Am CollCardiol.,**58**(12):1208-10.
8. García-Donaire JA, Ruilope LM (2011). Cardiovascular and Renal Links along the Cardiorenal Continuum. Int J Nephrol, 2011:975782.
9. Gao S, Huang LL, Wang XH, Yu TT, Du SM, Guo YW (2012). Effects of Xinjierkang on two kidney one clip-induced hypertension and target organ injury in rats. J Chin Med Mat., **35** (4)591–5.
10. Gao S, Wang XH, Huang LL, Yu TT, Du SM, Guo YW, Jia Y (2012). Effects of a compound Chinese medicine Xinjierkang on isoproterenol-induced ventricular remodeling in mice. J Chin Intergr Med., **10**(3):330–6.
11. Guo DF, Chenier I, Lavoie JL, Chan JS, Hemet P, Tremalay J, Chen XM, Wang DH, Inagami T (2006). Development of hypertension and

http://dx.doi.org/10.4314/ajtcam.v11i5.3

kidney hypertrophy in transgenic mice over expressing ARAP1 gene in the kidney. Hypertension, **48**(3):453-9.

12. Guo DF, Sun YL, Hemet P, Inagami T (2001). The angiotensin II type 1 receptor and receptor-associated proteins. Cell Res., **61**(6):1150-2.
13. Habibi J, Hayden MR, Ferrario CM, Sowers JR, Whaley-Connell AT (2014). Salt Loading Promotes Kidney Injury via Fibrosis in Young Female Ren2 Rats. Cardiorenal Med, **4**(1):43-52.
14. Helle F, Hultström M, Skogstrand T, Plam F, Iversen BM (2009). Angiotensin II-induced contraction is attenuated by nitric oxide in afferent arterioles from the nonclipped kidney in 2K1C. Am J Physiol Renal Physiol., **296**(1):F78-86.
15. Huang LL, Yu TT, Guo K, Lan CZ, Liu B, Song JZ, Liu YJ, Wang XH, Gao S(2013). Comparison of different inner diameter silver impact on 2K1C hypertension model. Chin J Pharmacol Ther, **18**(6)621-6.
16. Lawrentschuk N, Trottier G, Mayo K, Rendon RA (2012). Effects of partial nephrectomy on postoperative blood pressure. Korean J Urol, **53**(3):154-8.
17. Li R, Wang Y, Liu Y, Chen Q, Fu W, Wang H, Cai H, Peng W, Zhang X (2013). Curcumin inhibits transforming growth factor-β1-induced EMT via PPARγ pathway, not Smad pathway in renal tubular epithelial cells. PLoS One, **8**(3):e58848.
18. Li XC, Hopfer U, Zhuo JL (2012). Novel signaling mechanisms of intracellular angiotensin II-induced NHE3 expression and activation in mouse proximal tubule cells. Am J Physiol Renal Physiol, **303**(12):F1617-28.
19. Lijnen PJ, Petrov VV, Fagard RH (2003). Association between transforming growth factor-beta and hypertension. Am J Hypertens, **16**(7):604-11.
20. Morris MJ, Na ES, Johnson AK (2008). Salt craving: the psychobiology of pathogenic sodium intake. Physiol Behav, **94**(5):709-21.
21. Olivares-Reyes JA, Smith RD, Hunyady L, Shah BH, Catt KJ (2001). Agonist-induced signaling , desensitization, and internalization of a phosphorylation-deficient $AT1_A$ angiotensin receptor. J BiolChem, **276** (41): 37761-8.
22. Park J, Lee SY, Ooshima A, Yang KM, Kang JM, Kim YW, Kim SJ (2013). Glucosamine hydrochloride exerts a protective effect against unilateral ureteral obstruction-induced renal fibrosis by attenuating TGF-β signaling. J Mol Med(Berl), **91**(11):1273-84.
23. Ruiz-Ortega M, Rupérez M, Esteban V, Rodríguez-Vita J, Sánchez-López E (2006). Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. Nephrol Dial Transplant, **21**(1):16–20.
24. Savoia C, Volpe M (2014). Impact of the Direct Angiotensin II Type 2 Receptor Stimulation on Renal Function: Toward a Sex-Specific Therapeutic Approach for Hypertension. Hypertension, pii: HYPERTENSIONAHA.114.03199.
25. Schiffer M, Bitzer M, Roberts IS, Kopp JB, ten Dijke P, Mundel P, Böttinger EP. (2001). Apoptosis in podocytes induced by TGF-beta and Smad7. J Clin Invest, **108**(6):807-16.
26. Turkstra E, Braam B, Koomans HA (2000). Impaired renal blood flow autoregulation intwo-kidney, one-clip hypertensive rats is caused by enhanced activity of nitricoxide. *J Am SocNephrol*, **11**(5):847-55.
27. Venkatachalam MA, Griffin KA, Lan R, Geng H, Saikumar P, Bidani AK (2010). Acute kidney injury: a springboard for progression in chronic kidney disease. Am J Physiol Renal Physiol, **298**(5):F1078-94.
28. Wang D, Zhuang Y, Tian Y, Thomas GN, Ying M, Tomlinson B (2012). Study of the effects of total flavonoids of Astragalus on atherosclerosis formation and potential mechanisms. Oxid Med Cell Longev. doi: 10.1155/2012/282383..
29. Wang J, Cao EZ (1996). XinJiEr Kang in 31 cases of myocarditis diseases. *Journal of Tianjin College of* Traditional Chinese Medicine, (2):8-10.
30. Wang J , Cao EZ , Li LZ (1988). XinJiErKang in coronary heart disease. Nanjing Zhong Yi Da XueXue Bao.,**14**(4):201-212.
31. Wang Y, Bai L, He X (1998). Scavenging action of shengmaiyin decoction on hydroxyl radical. Zhongguo Zhong Yao Za Zhi, **23**(1): 45-47.
32. Wang XH, Huang LL, Yu TT, Zhu JH, Shen B, Zhang Y, Wang HZ, Gao S (2013). Effects of oligomeric grape seed proanthocyanidins on heart, arota, kidney in DOCA-salt mice: role of oxidative stress. Phytother Res, **27**(6):896-76.
33. Wang S, Wilkes MC, Leof EB, Hirschberg R (2010). Noncanonical TGF-beta pathways, mTORC1 and Abl, in renal interstitial fibrogenesis. Am J Physiol Renal Physiol, **298**(1):F142-9.
34. Xia Y, Jin X, Yan J, Entman ML, Wang Y (2014). CXCR6 Plays a Critical Role in Angiotensin II-Induced Renal Injury and Fibrosis. Arterioscler Thromb Vasc Biol, pii: ATVBAHA.113.303172.
35. Yu TT, Guo K, Chen HC, Lan CZ, Wang J, Huang LL, Wang XH, Zhang Z, Gao S (2013). Effects of traditional Chinese medicine Xin-Ji-Er-Kang formula on 2K1C hypertensive rats: role of oxidative stress and endothelial dysfunction. BMC Complement Altern Med.
36. Zhang W, Wang W, Yu H, Zhang Y, Dai Y, Ning C, Tao L, Sun H, Kellems RE, Blackburn MR, Xia Y (2012). Interleukin 6 underlies angiotensin II-induced hypertension and chronic renal damage. Hypertension, **59**(1):136-44.
37. Zuo L, Wang M (2010). Chinese Association of Blood Purification Management of Chinese Hospital Association.Current burden and

http://dx.doi.org/10.4314/ajtcam.v11i5.3

probable increasing incidence of ESRD in China. Clin Nephrol., **74**(1):S20-2.

Supplementary data

Analysis of the orthogonal experiment result on XJEK extract

Factor / Level / Test number	Decoction number	Water amount A（multiple）	Extraction time B（h）	The amount of the extract（g）	Medicinal materials（g）	Extraction rate（%）
1	once	10	2	30.6	100.33	40.10%
	twice	8	1	9.63		
2	once	10	2	30.72	100.97	36.17%
	twice	8	0.5	5.8		
3	once	10	1	29.3	100.84	35.20%
	twice	8	0.5	6.2		
4	once	10	2	30.63	100.18	38.26%
	twice	6	1	7.7		
5	once	10	2	30.41	100.45	35.32%
	twice	6	0.5	5.07		
6	once	10	1	28.87	99.88	34.07%
	twice	6	0.5	5.16		
7	once	8	2	25.37	100.23	33.43%
	twice	6	1	8.14		
8	once	8	2	25.66	100.62	30.79%
	twice	6	0.5	5.32		
9	once	8	1	24.32	100.40	29.57%
	twice	6	0.5	5.37		

T value	Extraction rate（%）	
	A	B
T1	111.49	111.57
T2	107.67	102.28
T3	93.56	98.86
t1	37.16	37.19
t2	35.89	34.09
t3	31.19	32.95
Range R	5.98	4.23
The better level	A1	B1
Primary and secondary factors	BA	

Structural grouping	Water amount A（multiple）	Extraction time（h）	Extraction rate（%）
A1B1	10-8	2-1	40.1