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Original Article

Inhibition of adriamycin-induced nephropathy in rats by herbs based kangshenoral solution

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Abstract: The Chronic kidney disease (CKD) is characterized by the progressive loss in renal function over a period. The progression of CKD will finally result the End Stage Renal Disease (ESRD) Symptoms which needs permanent renal replacement therapies. Therefore, control the progression of CKD is necessary. In this study, based on the theory of Traditional Chinese Medicine and the Traditional Chinese Herbology, we developed the Kangshen Oral Solution based ona combination of different herbs for extraction. By utilizing adriamycin (ARD)-induced chronic renal failure in rats as the CKD model, our results demonstrated thatadministration of the Kangshen Oral Solution reduced the kidney disease induced weight loss in rats. The Kangshen Oral Solution could also relieve the proteinuria and kidney index induced by ARD which indicated the partially restoration of the kidney function. The improved kidney function was further supported by biochemical tests for blood total protein level, albumin level as well as cholesterol, triglycerides and Creatinine. Moreover, the histology examination also confirmed the ARD induced pathological changes in kidney was relieved by Kangshen Oral Solution. Taken together, these findings suggested Kangshen Oral solution could reduce ARD-induced nephropathy in rats model and may be employed as an alternative treatment for CKD patients.

Keywords: Chronic kidney disease (CKD), Chinese traditional medicine, herbology, adriamycin-induced nephropathy

Introduction

The Chronic kidney disease (CKD), also known as chronic renal disease, is characterized by the progressive loss in renal function over a period of months or years [1]. Although dialysis or kidney transplantation is only needed for about 1% patients with CKD, the treatment of CKD remains the most expensive of chronic diseases and significantly reduces lifespan [1]. Current studies viewed the causes of CKD are mainly due to diabetes mellitus, hypertension, and glomerulonephritis. These factors had been thought to contribute about 75% of the total CKD cases. However, there was still CKD case with unknown causes which are world widely distributed [2]. Moreover, as a chronic disease, CKD confers the patients with increased risks for cardiovascular diseases [3]. As a result, the cardiovascular diseases become the top cause of death in CKD patients rather than renal failure [3, 4]. Therefore, control the progression of CKD is necessary to avoid the End Stage Renal Disease (ESRD) Symptoms or other CKD related diseases.

The progression of CKD can be divided to 5 different stages with stage 1 as the most slightly diminished renal function and stage 5 as the ESRD [1]. The only treatments for ESRD are permanent renal replacement therapies, such as hemodialysis or kidney transplantation. On the other hand, current therapy for earlier stage of CKD mainly relied on the management of primary diseases such as controlling of the high blood pressure [5]. The angiotensin converting

enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (ARBs) had been used as the standard of care for CKD patients and had been shown to slowdown the progression of CKD [5, 6]. However, even with the ARBs and ACEIs treatment, progressively losing of kidney function had been observed in some patients [7], which indicated that current CKD therapies have their limitations and are not enough.

Although the treatment options for CKD remain limited, application of complementary and alternative medicines (CAMS) for CKD has increased drastically over the past decade [8]. In China, Traditional Chinese medicine has been widely used for the treatment of CKD related nephropathy. Several studies reported that Chinese herbal can be used as an alternative therapy to improve kidney function of patients with diabetic nephropathy [9-11].

In this study, we developed the Kangshen (means healthy kidney in Chinese) Oral Solution. By utilizing ARD-induced chronic renal failure in rats as the CKD model [12, 13], we investigated the potentialtherapeutic effect of Kangshen Oral Solution for ARD induced nephropathy. Our resulted demonstrated administration of Kangshen Oral solution could reduce kidney failure induced weight loss in rats. Furthermore, the reduction of proteinuria and kidney index demonstrated the partially restoration of the kidney function and was supported by biochemical tests. Moreover, the histology examination also confirmed the ARD induced pathological changes in kidney could be relieved by Kangshen Oral Solution. In conclusion, our findings suggested that all nature herbs extraction based Kangshen Oral Solution may be a novel therapy for CKD patients.

Materials and methods

Ethics statement

The animal procedures used in this study were approved by the 152nd Center Hospital of The People's Liberation Army in accordance with the *Guidelines for Experimental Animals* which was issued by Ministry of Science and Technology (Beijing, China). All handling procedures were performed according to recommendations proposed by the 152nd Center Hospital of The People's Liberation Army, and all efforts had been made to minimize suffering. Animals were housed in a temperature-controlled room with proper darkness-light cycles, fed with a regular diet, and maintained under the care of the Experimental Animal Center of the 152nd Center Hospital of The People's Liberation Army.

Preparation of kangshen oral solution

Based on the theory of the Traditional Chinese Medicine and the herbs combination principle of Traditional Chinese Herbology, there are totally 11 different herbs from Chinese traditional medicine had been used as the raw materials for Kangshen Oral solution. They were Ginseng (root of *Panax trifolius*), Astragalus (*Astragalus onobrychis*), Atractylodes (*Atractylodes macrocephala*), Poria (*Wolfiporia extensa*), Epimedium (*Epimedium sagittatum*), Yam (*Liliales Dioscorea*), Earthworm, flower of *Carthamustinctorius, Rosa laevigata,* seeds of *Euryale ferox, root of Glycyrrhizainflatable.* All raw herbs mentioned above were subjected to the traditional preparation as recommended by the Traditional Chinese Herbology before further processing.

All those herbs were boiled in water for 40 min twice to get the crude herbs extract. Then extract was filtered to remove solid impurities and vaporized under 80°C until the relative density of extract reaching 1.15 to 1.30. After the temperature of the extract down to RT, the 95% ethanol was added to the extract with a final volume of 60% for 24 hours precipitation. Then the supernatant were extracted via rotary evaporation under reduced pressure until no ethanol can be smelled. The simple syrup was added to the remaining supernatant and the PH was modulated to the value of 5.0 to 7.0 to form the crude solution. After the solution was sterilized, it was sealed to bottom at the volume of 10 mL for each.

Adriamycin-induced chronic kidney disease model in rats

Male Wistar rats weighing 180-200 grams (n=72) were randomly divided into 6 groups with 12 rats in each group. Except one control group which was injected with PBS, tail vein injection of adriamycin (ADR) with a dose of 5 mg/kg was conducted for all other groups to induce the chronic kidney disease. One week after ADR induction, all rats were placed in met-

Table 1. Kangshen Oral Solution could reduce the body weight lossinduced by kidney damage

Groups	N	Weight (g)			
		Pre-treatment	One week post induction	One week after administration	Two weeks after administration
MOCK	12	236.4±10.5	259.2±9.8	274.7±15.7	290.5±17.9
AN	10	239.1±9.0	224.5±10.4	236.6±10.6	233.5±13.0
Prednisone	12	236.8±8.9	222.1±5.5	235.6±15.1	236.8±16.0
High Dose	12	237.8±9.2	230.0±8.6	247.9±7.8	269.1±9.3*
Medium Dose	12	236.8±9.9	223.2±7.8	237.8±10.9	262.4±10.3*
Low Dose	11	238.0±10.1	225.8±11.9	235.6±15.7	271.9±11.4*

AN: ADR induced Nephropathy group; Significant different had been marked as "*", which indicated that P<0.05.

Table 2. Total Urinary protein test from different groups

Group	N	Urinary protein (g/L)		
		1 week after AN induction	1 week after administration	2 weeks after administration
MOCK	12	4.761±2.872	4.633±2.162	5.283±1.992
AN	10	10.682±3.944	20.517±4.926	22.260±4.905
Prednisone	12	10.212±2.341	15.528±4.005	16.562±4.583
High Dose	12	9.411±3.443	14.299±3.212*	16.514±3.904*
Medium Dose	12	11.451±3.821	15.079±5.456	19.876±4.636
Low Dose	11	10.993±4.589	15.973±4.998	19.886±4.868

AN: ADR induced Nephropathy group; Significant different had been marked as "*", which indicated that P<0.05.

abolic cages to collect 24-hour urine. The 24-hour urine total protein concentration of collected urine was determined by the Coomassie brilliant blue protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according manufacturer's instruction and measured with an ultraviolet spectrophotometer (UV-2000, Unico Instrument, Shanghai, China). One day later, the rats was administrated with Kangshen Oral solution (20 ml /kg for high dose, 10 ml solution/kg for medium dose and 5 ml solution/kg for low dose) and Prednisone (5 mg/kg). The rats administrated with distilled water was included in here as negative controls. Administration of Kangshen solution was lasted for two weeks, urine collection was conducted once a week. Blood sample was collected from orbital sinus of rats at the end of administration. The total protein level (TP), albumin level (ALB), cholesterol (CHO), triglycerides (TG), Creatinine (Cr) and Urea nitrogen (BUN) were conducted with corresponding kits (Nanjing Jiancheng Bioengineering Institute) according manufacturer's instruction.

Collection of kidney tissue sample and calculation of the kidney index

After CKD induction and end of Kangshen Oral Solution administration, the rats were euthanized for specimen collection for kidney. The percentage of the two kidneys' total weighs to the whole body weighs of rats had been considered as the kidney index.

For the histology analysis, after calculation of the kidney index, the kidneys were removed and fixed in 10% formaldehyde. Slides with paraffin-embedded sections were stained by hematoxylin-eosin staining for pathological analysis.

Statistical analysis

Data are presented as means ± standard deviation (SD). Comparisons between different groups were subjected to the Student's t test. A two tailed *P*-value of less than 0.05 was considered significant. Statistical analysis was performed by SPSS 19.0 software.

Results

The kangshenoral solution reduced the kidney disease induced weight loss in rats model

To determine the overall effect of Kangshen oral solution as the treatment for CKD, we first examined the weight loss of different rats group since the weight loss had been considered as a symptom for chronic kidney disease [14]. As demonstrated in **Table 1**, no significant difference had been observed before the induction

Table 3. The changes of Renal index, albumin and total protein level in each groups

Groups	N	Renal index	Albumin level (g/L)	Total Protein (g/L)
MOCK	12	6.454±0.412	33.2±4.6	55.4±5.3
AN	10	8.392±1.050	20.2±3.0	49.0±4.6
Prednisone	11	7.063±0.405*	24.6±4.5	61.8±3.6*
High Dose	12	6.993±0.520*	27.6±7.9	59.5±4.8*
Medium Dose	12	7.271±0.405*	30.4±4.8*	60.2±2.8*
Low Dose	11	7.166±0.386*	26.7±7.9	55.3±4.9

AN: ADR induced Nephropathy group; Significant different had been marked as "*", which indicated that P<0.05.

Table 4. The changes of biochemical indicators in each groups

Groups	N	Triglycerides (mmol/L)	Cholesterol (mmol/L)	Creatinine (μmol/L)	Urea nitrogen (mg/L)
MOCK	12	0.886±0.412	1.649±0.804	56.3±7.2	181.1±12.2
AN	10	1.902±0.574	3.898±0.509	87.2±7.1	281.6±22.2
Prednisone	12	1.620±0.401	3.043±0.530*	71.1±8.7	249.5±15.2
High Dose	12	1.304±0.692*	2.550±0.705*	60.6±8.8*	201.0±26.3*
Medium Dose	12	1.432±1.015	2.597±0.860*	61.0±10.9*	208.4±31.6*
Low Dose	11	1.322±0.774	2.943±0.840*	66.6±8.0*	218.0±13.2*

AN: ADR induced Nephropathy group; Significant different had been marked as "*", which indicated that P<0.05.

of CKD. With the inducing the kidney damage by ADR, a 10% weighs reduction was shown, suggested a successful establishment of the chronic kidney disease model. In the Prednisone treated groups, the average weight were 235.6 and 236.8 after one week and two weeks administration, which was very similar to the AN group. However, by the administration of Kangshen Oral solution, reduced weight loss had been observed in all three dosing groups which were 235.6 g, 237.8 g and 247.9 g for low, medium and high dose groups, suggested an improvement of kidney function in rats (**Table 1**). After two weeks administration, the average weights were 271.9 g, 262.4 g and 269.1 g for low, medium and high dose groups, which were much higher than 233.5 g in ADR induced Nephropathy (AN) group. Moreover, Kangshen Oral solution demonstrated improved weight loss than rats group given prednisone (**Table 1**). Prednisone is a common drug or combined with other drug such as ketoconazole for patients with kidney disease, especially for Nephrotic Syndrome as a typical kidney disorder marked by excess protein in urine [15].

Thekangshen oral solution could partially restore the kidney function in rats with ARD induced CK

To further confirm the improvement of the kidney function by Kangshen Oral Solution, we conducted the biomedical tests for both urine sample and blood sample of rats from different groups. The urinary protein level is a key indicator for the kidney damage as the serum protein could be reabsorbed from urine. The presence of excess protein in urine indicates either an insufficiency of absorption or impaired filtration of kidney. In the normal MOCK group, the urinary protein levels for different time points were very stable and around 4.6-5.2 g/L (**Table 2**). However, with the induction of CKD, the protein level increased dramatically to 10.6 and 20.5 g/L after one week and two weeks ADR induction, respectively (**Table 2**). However, after one week administrated of Kangshen, the high dose group could reduce the urinary protein to 14.2 g/L compared with 15.52 of Prednis one group. Moreover, with the two weeks administration of the Kangshen, the high dose shown the best improvement which reduced the urinary protein to 16.514 in three different dose groups and demonstrated the similar effect as Prednisone treated rats (**Table 2**).

To determine the improvement of the kidney damage, we set the Kidney Index as a comparison between different groups. It looks that kidney damage would cause enlarged kidney volume and result increased Kidney Index from 6.454 to 8.392 (**Table 3**). With the administration of Kangshen, we deed see reduced Kidney Index to 6.993, 7.271, 7.166, in high dose, medium dose and low dose groups, respectively, while Prednisone could reduce the Renal Index to 7.063 (**Table 3**). On the other hand, by compared the blood albumin and total protein

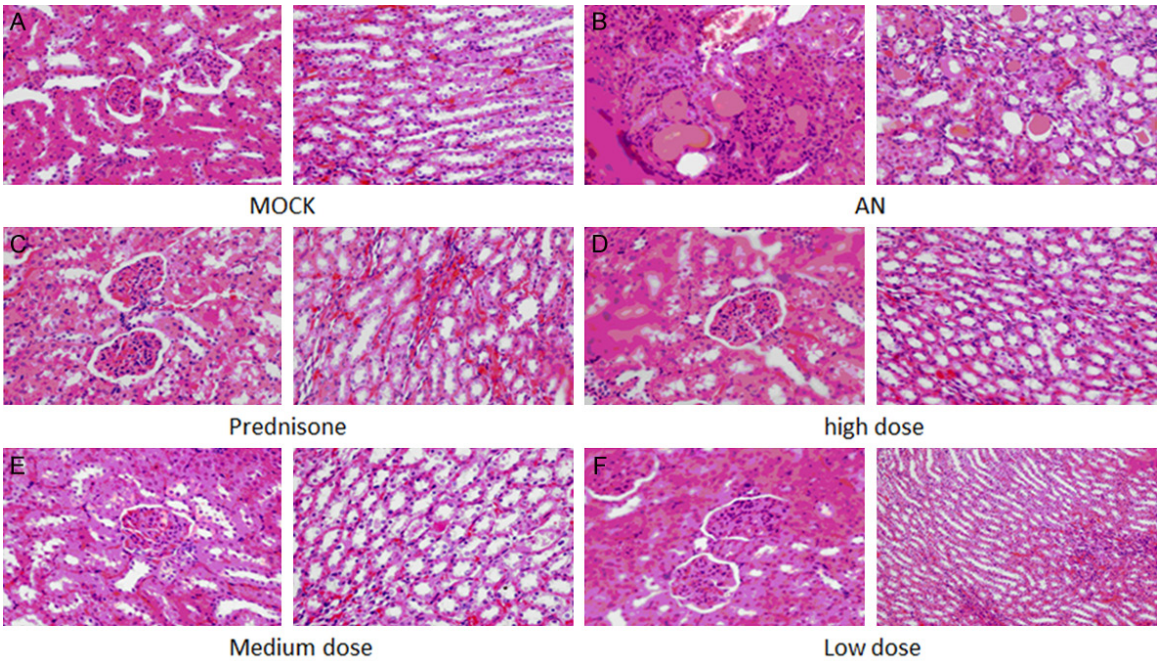

Figure 1. Kangshen Oral Solution could reduce pathological changes in kidney in rats with ARD induced CKD. After CKD induction and the end of Kangshen Oral Solutionadministration(two weeks), the rats were euthanized for kidney specimen collection. The kidney samples were fixed in 10% formaldehyde for HEstaining. A: MOCK PBS control group; B: ADR induced Nephropathy (AN) group; C: Prednisone treated group; D: High dose administration of Kangshen Oral Solution; E: Meium dose administration of Kangshen Oral Solution; F: Low dose administration of Kangshen Oral Solution.

level between different groups, application of medium dose Kangshen solution demonstrated the strongest increasing of both. It was interesting that high dose did not demonstrated better improvement for albumin level than medium dose groups, while the total protein level between those two groups were similar (**Table 3**). It was also notable that rats administrated with medium dose and high dose of Kangshen, as well as Prednisone, could result the higher level even than MOCK group (**Table 3**).

Moreover, the general biochemical tests for cholesterol (CHO), triglycerides (TG), Creatinine (Cr) and Urea nitrogen (BUN) had been conducted as well to measure the renal function. As demonstrated in **Table 4**, the significant difference results for tests mentioned above between the MOCK group and AN group suggested a successful establishment of the CKD model while application of Prednisone deed show some improvement. For the Kangshen oral solution administrated groups, although all three groups demonstrated better reading for those tests than Prednisone group, except Cr level, the high dose groups shown the best improvement for the TG, BUN and CHO level while the medium dose had a better performs than high dose group only in blood Cr level. Taken together, these data suggest the renal function was partial restored by Kangshen Oral solution and the high dose administration appeared to demonstrated best improvement of renal function.

Histology examination demonstrated reduced pathological changes in kidney by Kangshen Oral Solution in rats with ARD induced CKD

As the improved renal function had been demonstrated by biochemical tests, we would like to confirm our observation in the cellular level by histology examination of renal tissue. Compared with the MOCK group with normal morphology in kidney sample, CKD induction caused significant morphology changing which could be characterized by disappearing of glomerular, congestion of renal interstitial, abnormal tubules resulted from tubular epithelial cell swelling as well as atrophy of most kidney epithelial cells. Hyaline casts had been also observed in collecting conduct of the renal medulla in this groups (**Figure 1A** and **1B**). In

Table 5. Kangshen Oral solution reduced the pathology changing of rat kidney (n=12)

Groups	-	+	++	+++
MOCK	12	0	0	0
AN	0	0	2	10
Prednisone	0	2	10	0
High Dose	0	9	3	0
Medium Dose	0	6	6	0
Low Dose	0	4	8	0

The grading for pathological changing in kidney. (-): The morphology of glomerular, tubules, interstitial and collecting duct all look normally. (+): The morphology of glomerular looks normal. There was a little congestion could be seen for renal interstitial and renal tubular cells. Slight atrophy could be seen in the some of the renal collecting duct cells. (++): Some glomerulars are disappearing. The congestion could be seen for renal interstitial and renal tubular cells. Atrophy or swelling could be seen in the some of the renal collecting duct cells. Some Hyaline casts could beobserved in collecting conduct of the renal medulla. (+++): Most glomerulars are disappearing. The congestion was seen for renal interstitial and tubules. Atrophy could be seen in the renal collecting duct cells. Lots of Hyaline casts could beobserved in collecting conduct of the renal medulla.

the Prednisone group, only dampen hyaline casts had been observed and suggested there was a little reduction in the kidney damage (**Figure 1C**). However, in high dose Kangshen Oral Solution administrated groups, normal glomerular with little morphological changing had been demonstrated with a little congestion of renal interstitial. Moreover, tubular epithelial cell swelling was also reduced significantly, suggesting the minimum damage was caused by CKD induction (**Figure 1D**). On the other hand, as the dose of Kangshen solution was reduced, we deed see increased pathological changing in the kidney samples (**Figure 1E** and **1F**), which means reduced damage was due to the administration of Kangshen Solution. Moreover, a semi-quantification of the pathological changing for the HE stained kidney samples from different rats and different groups had been conducted as well (**Table 5**). The quantification results further supported our histology observation. Taken together, those data further confirmed than Kangshen Oral solution could slow down the CKD induction and protected progression of CKD.

Discussion

Chinese Herbology is the theory of traditional Chinese herbal therapy, and it accounts for the majority of the treatment in Traditional Chinese Medicine. Although there are still lots of argument for the rationale of the basic theory of Traditional Chinese Medicine itself, herb based therapy had been widely used in different parts of the world and had been proved to be effective, especially in chronic disease such as asthma. As a typical example, a herbal extract mixture from traditional Chinese medicine, had been shown to relieve the asthma through inhibiting type I hypersensitivity reaction via the suppression of histamine release [16, 17]. On the other hand, in traditional Indian system of health care, Boswellia had also been confirmed to relieve the asthma in patients with different ages in clinical trial [18]. In the western world, Pycnogenol, another a standardized extract from French maritime pine bark demonstrated the benefits in asthma patients and improve their lung function [19-21]. Thus, for a chronic disease like CKD, herbal extract could be an alternative way to slow down the progression and traditional Chinese herbal medications are frequently used as adjuvant along with western pharmacotherapy for treatment of CKD in China as well as other Asian countries [22].

In recent years, several study provided *in vitro* and animal data confirmed the biological activity and therapeutic effects of several traditional herbs in CKD [22]. *Rheum officinale*andAstragalus are two of those herbs which had potential therapeutic effects [23, 24]. In this study, we tested the potential of the extract mixturewhich named as Kangshen Oral Solution from 11 different herbs which used in traditional Chinese medicine on ARD induced CKD in rat model. Based on our data, the high dose administration had shown the best performance for reducing the pathological changing in histology, as well as improving the kidney function by. Our study also demonstrated that Kangshen Oral solution administrated rat demonstrated better improvement in their renal function when compared with Prednisone treated rats, suggest that Kangshen solution is superior in slow down the progression of CKD than Prednisone. Moreover, as longtime application of Prednisone has many sides effect, such as weight gain, acne, increased sweating and dry skin, all nature herbs based Kangshen Oral Solution may have less side effect. However, the biologically active component in Kangshen Oral Solution may need further investigation. Taken together, our results provided a novel alternative therapy for treating CKD.

In conclusion, in this study, our findings demonstrated that ARD-induced nephropathy in rats as a CKD model could be relieved by the administration of the Kangshen Oral Solution. In detail, The Kangshen could reduce e weighs loss, proteinuria as well as the kidney index in ARD treated rats. The improved kidney function was further supported by biochemical tests and histology examination for the kidney samples. Taken together, these findings suggested Traditional Chinese Medicine and Herbology based Kangshen Oral solution could reduce ARD-induced nephropathy in rats model and may be employed as an novel alternative treatment for CKD patients.

Disclosure of conflict of interest

None.

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