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Effect of Reynoutria Japonic and Resveratrol on Sirt1/SOD/MDA of Adriamycin-Induced Renal Injury: A Randomised Trial

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Research

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Abstract

Background: Mechanisms of drug-induced kidney injury include mitochondrial dysfunction and oxidative stress. Resveratrol is a natural activator of sirt1 that is related to oxidative stress.

Objectives: To explore the mechanism of treating drug-induced kidney injury with Reynoutria japonica and its extract (Resveratrol).

Methods: Fifty adult male SD rats were randomly divided into five groups: blank group, model group, Reynoutria japonica group, resveratrol group and Benazepril group. Except the blank group, each group used a one-time tail vein injection of 7.5mg/kg adriamycin to make the rat model of drug-induced renal injury. After three days, the proteinuria test strip showed green, which was positive for proteinuria. Each group was given the corresponding drug. ACR was measured on the seventh day every week. All rats were anaesthetized death on the fourth weekend to obtain blood and kidneys.

Results: At the fourth week, the MDA levels of blank group and Reynoutria japonica group were significantly lower than those of benazepril hydrochloride group (P<0.05), and the MDA levels of resveratrol group and model group were significantly higher than those of benazepril hydrochloride group (P<0.05). The Sirt1 mRNA levels of the blank group and the Reynoutria japonica group were significantly higher than those in the benazepril hydrochloride group (P<0.05), and the Sirt1 mRNA levels of resveratrol group and model group were significantly lower than those of the benazepril hydrochloride group (P<0.05), and the Sirt1 mRNA levels of resveratrol group and model group were significantly lower than those of the benazepril hydrochloride group (P<0.05). The comparison results between groups of the Sirt1 protein expression were the same as those of the Sirt1 mRNA expression (P<0.05).

Conclusions: The therapeutic effect of the Reynoutria japonica group was better than that of the Benazepril group and resveratrol group.

Introduction

In recent years, the number of patients with renal damage caused by antineoplastic drugs, targeted drugs, antipsychotics and painkillers has increased year by year. In the future, drug-induced renal injury will become an increasingly serious economic and major public health problem. As early as the 1960s, people had a clear understanding of the renal damage caused by drugs. All renal structural and functional damage caused by drugs, chemicals or biotoxins, with clinical manifestations, can be called drug-induced kidney injury or toxic nephropathy.^[1–2]

Adriamycin is a widely used anticancer drug. Dose-related nephrotoxicity greatly limits its clinical application. It was previously thought that the mechanism of drug-induced kidney injury induced by adriamycin may include inflammatory mediators, oxidative stress, necrosis, apoptosis, autophagy, etc.^[3–6]

Sirtuin1 (abbreviated as sirt1) is an anti-ageing gene. It is mainly expressed in renal tubular epithelial cells. Recent studies suggest that the development of sirt1 may serve it as a new marker for the diagnosis and prognosis of drug-induced kidney injury.^[7–9]These studies suggest that sirt1 may be an effective target for the prevention of drug-induced kidney injury.

Benazepril can reduce renal injury by dilating blood vessels, reducing renal load and reducing renal hyperfiltration as a common kidney disease drug.^[10]

Traditional Chinese medicine believes that blood stasis, damp heat and toxin are closely related to the occurrence and development of drug-induced renal injury. Therefore, the active use of methods such as promoting blood circulation and removing blood stasis, promoting dampness and resolving phlegm and detoxification is of great positive significance for the prevention and treatment of drug-induced renal injury. Reynoutria japonica is a traditional Chinese medicine, it is the root of Reynoutria japonica Houtt., it is a genus of Polygonaceae plants. In China, it is taken directly by boiling water with its roots. Reynoutria japonica has the effects of clearing heat and detoxification, diuresis, promoting blood circulation and removing blood stasis, it has a good effect on chronic renal insufficiency. According to the above TCM theories, we believe that the role of Reynoutria japonica in promoting blood circulation and removing blood stasis may play an important role in the prevention and treatment of drug-induced renal injury.

Resveratrol, an extract of Reynoutria japonica (*Reynoutria japonica Houtt.*), has antiaging and antifungal effects^[11] and inhibits the proliferation of vascular endothelial cells.^[12] Resveratrol is a natural sirt1 activator. To date, there is little study on the pathogenesis and prevention mechanism of drug-induced kidney injury by Reynoutria japonica and resveratrol through the sirt1 pathway.

Recent studies^[13] have increased the understanding of the subcellular mechanisms of drug-induced kidney injury, including mitochondrial dysfunction, oxidative stress, direct toxicity to renal tubules and cell apoptosis. At present, there is no specific drug to prevent drug-induced kidney injury, and thus it is necessary to explore prevention strategies for drug-induced kidney injury.

Materials And Methods

Drugs

Resveratrol:Sigma,CAS#v900386;Adriamycin:Haizheng Pfizer Pharmaceutical

Co.,Ltd.,CAS#131101;Benazepril:Solarbio,CAS#YZ100768; Reynoutria japonica:

Guangzhou Kangmei Pharmaceutical Co.,Ltd.; MDA Reagent:Solarbio,CAS#BC0025100;SOD Reagent:Solarbio,CAS#BC0175100; TRI Reagent®:Sigma,CAS#93289;SYBR® Premix Ex Taq TM II:Takara, CAS#RR-820A;dNTP mix:Sigma,CAS#D7295;96 well PCR plate:Axygen,CAS#96M2HSC; MicroAmp[™] Optical Adhesive Film:Thermo,CAS#4311971;DAB:Solarbio,CAS#S-W1010;light cycle^R 96 RT-PCR:Roche Diagnostics;EDTA:Solarbio,CAS#C1034;PV-600:Beijing Zhongshan Jinqiao Biotechnology Co.,Ltd.;SIRT1 antibody:Abcam,CAS #ab110304;GAPDH antibody:Abcam,CAS#ab9485;Pathological slicer:Thermo HM-340E(American);Microscope and camera system:OLYMPUS(Japan);Digital pathological image analysis system:MoticMed 6.0;Urinary albumin/creatinine Test: BECK-MAN COULTER iRICELL3000 Automatic urinalysis line;Urinary Creatinine/BUN Test:HITACHI 7600 automatic biochemical analyzer;Pentobarbital sodium:Sigm-a,CAS#P3761;4% Paraformaldehyde:Sigma,CAS#P6148.

The animals for the experiment

Fifty male Sprague Dawley (SD) rats (180–200 g) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China) on September 13, 2019 with the production licence number SCXK (Beijing 2016-0002), Certificate No. 1103241911005725. The experimental protocol was approved by the Ethics Committee of the Luohe Central Hospital, Certification No. 2019027. And this study was performed according to the National Institute of Health guidelines. All animals were housed in a standard feeding environment (room temperature 21-23°C, 12 hours of day/night cycle) and were free to eat standard feed and drinking water.

Modelling

After three days of adaptive feeding, fifty adult male SD rats were randomly divided into five groups: blank group, model group, Reynoutria japonic group, resveratrol group and benazepril hydrochloride group, with 10 rats in each group. The model group, Reynoutria japonic group, resveratrol group and benazepril hydrochloride group used a one-time tail vein injection of 7.5mg/kg adriamycin to make the rat model of drug-induced renal injury. After three days, the proteinuria test strip showed green, which was positive for proteinuria. For the rats that failed to make the model, the above methods can be repeated. In this experiment, the rats were successfully made.

Gastric perfusion method and collection of specimens

The Reynoutria japonica group was given 10g/kg/d, which converted from human dose, resveratrol group was given 40 mg/kg/d.respectively, while the Benazepril group was given 0.90 mg/kg/d. The blank and model groups were given double distilled water at the same time. All the drugs were gavaged twice daily for four weeks. Doses of doxorubicin ^[14] and resveratrol^[15] were based on previous literature. The rats were weighed on the first morning of each week to adjust the dosage. On the morning of the seventh day of each week, 24-hour urine was collected with a metabolic cage for urine volume and urinary protein excretion measurement. All rats were anaesthetized death with 3% sodium pentobarbital (0.110.2 ml/100g) on the fourth weekend to obtain blood and kidneys, the blood was taken from the heart to measure serum creatinine and urea nitrogen. The kidneys of both sides were divided into two halves along the sagittal line on ice and preserved in three parts: 10 the kidneys were frozen in liquid nitrogen after being infiltrated with RNA protective solution and stored in -80°C for real-time PCR experiment; 10 for detection of oxidative stress index after tissue homogenization saved as above; 10 the kidneys were fixed in paraformaldehyde for immunohistochemistry and PAS staining.

Immunohistochemistry and pathological section observation

Part of the right kidney was paraffin-embedded, dewaxed, dehydrated, soaked and washed. The negative control used PBS instead of primary and secondary antibodies. For observation, DAB was used for coloration, as described previously.^[16]

Pathological section observation

The kidneys were observed under a light microscope as described previously.^[16]

Western blot

The total pulmonary protein was extracted from 100 mg of frozen lung tissue. Then the total proteins either from cells or tissues by RIPA lysis buffer were applied for the western blot analysis, which was processed following our previous methods.^[17]

Detection of urinary albumin/urinary creatinine

Urinary albumin/urinary creatinine (mg/g) was detected by BECKMAN COULTER iRICELL3000 Automatic urinalysis line on November 12, 2019.

Detection of serum creatinine and urea nitrogen

Serum creatinine (µmol/L) and urea nitrogen (mmol/L) were detected by HITACHI 7600 automatic biochemical analyzer on November 28, 2019.

Detection of oxidative stress index

The levels of malonic dialdehyde (nmol/L) and superoxide dismutase (U.ml-1) in the kidney tissue were measured by mature commercial kits by colorimetry on November 18, 2019.

Quantitative real-time PCR

Real-time polymerase chain reaction analysis was performed as described previously.^[16]Oligonucleotide PCR primers for rat genes were as Table 1.

Oligonucleotide Oligonucleotide PCR primers				
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SIRT1	F: CAGTTCCAGCCGTCTCTGTG			
	R: TCCTTTGGATTCCTGCAACC			
GAPDH	F: AGAAGGCTGGGGCTCATTTG			
	R: AGGGGCCATCCACAGTCTTC			
Note: F:Forward; R: Reverse.				

Statistical analysis

The count data is expressed in frequency and percentage. Firstly, the *Shapiro Wilk* normality test and *Levene* variance homogeneity test are carried out. When the measurement data meet the normal distribution, values are expressed as the means \pm SD; If the normal distribution is not satisfied, use M ($P_{25} \sim P_{75}$) to describe. If it does not conform to the normal distribution, multiple groups of comparative data are selected, and *Kruskal Wallis* rank sum test is used. *Bonferroni* test is used for those with uniform variance, and *Dunnett T*₃ test is used for those with uneven variance. It conforms to the normal distribution and the variance is homogeneous. The sample mean between multiple groups is compared by one-way *ANOVA -Bonferroni* test. It was considered statistically significant (*P<0.05*). The data were statistically analyzed by SPSS version 24.0.

Results

General conditions

After injection of doxorubicin, the rats gradually appeared laziness, drowsiness and other phenomena. Compared with the blank group, the hair had no luster, and was accompanied by severe ascites, oliguria and weight loss in the later stage. The rats in resveratrol group and benazepril hydrochloride group were relatively poor, and the mortality was higher than that in Reynoutria japonica group.

Comparison of weight changes in each group of rats within four weeks

At the first week, the body weight levels in the benazepril hydrochloride group and the blank group were significantly higher than those of the model group (P<0.05), there were no significant differences between the Reynoutria japonica group, the resveratrol group and the model group (P>0.05). The body weight levels in the model group was significantly lower than that in the benazepril hydrochloride group (P<0.05), There were no significant differences in the blank group, the Reynoutria japonica group, the resveratrol group, the Reynoutria japonica group, the resveratrol group and the benazepril hydrochloride group (P<0.05). At the second week, the body weight levels in the benazepril hydrochloride group (P>0.05).

benazepril hydrochloride group, the Reynoutria japonica group and the blank group were significantly higher than those of the model group (*P*<0.05), there was no significant difference between the resveratrol group and the model group (*P*>0.05). The body weight levels in the model group and the resveratrol group were significantly lower than those in the benazepril hydrochloride group (P<0.05), There were no significant differences between the blank group, the Reynoutria japonica group and the benazepril hydrochloride group (P>0.05). At the third week, the body weight levels in the benazepril hydrochloride group and the blank group were significantly higher than those of the model group (P<0.05), There were no significant differences between the model group, the Reynoutria japonica group and the resveratrol group (P>0.05). The body weight level in the blank group was significantly higher than that of the benazepril hydrochloride group (P<0.05), the body weight level in the model group was significantly lower than that of the benazepril hydrochloride group (P<0.05), there were no significant differences between the benazepril hydrochloride group, the Reynoutria japonica group and the resveratrol group (P>0.05). At the fourth week, the body weight levels of the benazepril hydrochloride group, the Reynoutria japonica group and the blank group was significantly higher than those of the model group (P<0.05), there was no significant difference between the resveratrol group and the model group (P>0.05). The body weight level of the blank group was significantly higher than that in the benazepril hydrochloride group (P<0.05), the body weight levels of the resveratrol group and the model group were significantly lower than the benazepril hydrochloride group (P<0.05). there was no significant difference between the benazepril hydrochloride group and the Reynoutria japonica group (P>0.05) (Table 2, Figure legend 1).

Groups	1 week	2 weeks	3 weeks	4 weeks
Blank group	300.70±12.63*	316.60±8.85*	358.40±22.40∠*	421.50±12.55∠*
Model group	278.80±11.77∆	267.10±10.58∠	283.56±12.09	344.00±11.43∠
Benazepril group	294.90±5.95*	315.80±12.38*	312.56±18.77*	365.00±8.38*
Reynoutria japonica group	291.60±9.38	321.20±11.74*	305.80±26.55	369.90±5.95*
Resveratrol group	288.00±7.94	258.40±12.76∠	291.70±18.54	351.00±7.79∆
F	6.892	71.810	19.931	98.831
Р	<0.001	<0.001	<0.001	<0.001

T I I O

Note: $\triangle P < 0.01$, compared to the values of the Benazepril group, **P*<0.05, compared to the values of the model group.

Comparison of Urine volume changes in each group of rats within four weeks

At the first week, the urine volume values in the blank group and the benazepril hydrochloride group were significantly higher than that of the model group (P<0.05), and there were no significant differences between the resveratrol group, the Reynoutria japonica group and the model group (P>0.05). The urine volume value in the blank group was significantly higher than that of the benazepril hydrochloride group (P<0.05), the urine volume values in the resveratrol group, the Reynoutria japonica group and the model group were significantly lower than that of the benazepril hydrochloride group (P<0.05). At the second week, there were no significant differences between the model group and the other groups (P>0.05). The urine volume value of blank group was significantly higher than that of the benazepril hydrochloride group (P<0.05). There were no significant differences between the resveratrol group, the Reynoutria japonica group, the model group and the benazepril hydrochloride group (P>0.05). At the third week, the urine volume values of the blank group, the Reynoutria japonica group and the benazepril hydrochloride group were significantly higher than those of the model group (P<0.05), there was no significant difference between the resveratrol and the model group (P>0.05). The urine volume value of the blank group was significantly higher than that of the benazepril hydrochloride group (P<0.05), the urine volume value in the model group was significantly lower than that of the benazepril hydrochloride group (P<0.05). There was no significant difference between the resveratrol the Reynoutria japonica group and the benazepril hydrochloride group (P>0.05). At the fourth week, the urine volume values of the blank group, the Reynoutria japonica group and the benazepril hydrochloride group were significantly higher than those of the model group (P<0.05), there was no significant difference between the resveratrol group and the model group (P>0.05). The urine volume value of the blank group was significantly higher than that of the benazepril hydrochloride group (P<0.05), the urine volume value in the model group was significantly lower than that of the benazepril hydrochloride group (P<0.05). There was no significant difference between the resveratrol, the Reynoutria japonica group and the benazepril hydrochloride group (P>0.05) (Table 3, Figure legend 2).

-Changes in Urine volume of each group (mL,, <i>n=10</i>)						
Groups	1 week	2 weeks	3 weeks	4 weeks		
Blank group	14.06±1.41 <i>*</i> ∆	8.43±1.77	11.75±1.95⊿*	13.77±2.05△*		
Model group	6.09±1.39∠	6.31±1.61	4.68±1.73∠	5.17±1.01		
Benazepril group	8.77±1.46*	5.45±1.52	7.79±1.30*	7.59±0.90*		
Reynoutria japonica group	6.07±1.57∠	6.92±2.49	7.34±1.78*	7.54±1.28*		
Resveratrol group	6.18±1.67⊿	6.43±1.70	6.70±1.68	6.11±1.43		
F	52.749	3.502	22.083	55.890		
Р	<0.001	0.014	<0.001	<0.001		
Note: $\triangle P < 0.01$, compared to the values of the Benazepril group, * $P < 0.05$, compared to the values of						

the model group.

Comparison of urinary albumin to creatinine ratio (ACR) in the urine of rats in each group

At the first week, the ACR levels in the blank group, the Reynoutria japonica group and the benazepril hydrochloride group were significantly lower than those of model group (P<0.05), and there was no significant difference between the resveratrol group and the model group (P>0.05). The ACR level in the blank group was significantly lower than that in the benazepril hydrochloride group (P<0.05), the ACR levels in the Reynoutria japonica group, the resveratrol group and the model group were significantly higher than those of benazepril hydrochloride group (P<0.05). At the second week, the ACR levels in the blank group, the Reynoutria japonica group and the benazepril hydrochloride group were significantly lower than those of model group (*P<0.05*), and there was no significant difference between the resveratrol group and the model group (*P*>0.05). The ACR level in the blank group was significantly lower than that in the benazepril hydrochloride group (P<0.05), the ACR levels in the Reynoutria japonica group, the resveratrol group and the model group were significantly higher than those of benazepril hydrochloride group (P<0.05). At the third week, the ACR level in each group was significantly lower than those of the model group (*P<0.05*), The ACR level in the blank group was significantly lower than that in the benazepril hydrochloride group (P<0.05), the ACR levels in the resveratrol group and the model group were significantly higher than those of the benazepril hydrochloride group (P<0.05), and there was no significant difference between the Reynoutria japonica group and the benazepril hydrochloride group (P>0.05). At the fourth week, the ACR levels in each group was significantly lower than those of the model group (P<0.05), The ACR level in the blank group was significantly lower than that in the benazepril hydrochloride group (P<0.05), the ACR levels in the resveratrol group and the model group were significantly higher than those of the benazepril hydrochloride group (P<0.05), and there was no significant difference between the Reynoutria japonica group and the benazepril hydrochloride group (*P>0.05*) (Table 4, Figure legend 3).

-Changes in urinary albumin/urinary creatinine of each group (mg/g, <i>n=10</i>).					
Groups	1 week	2 weeks	is 3 weeks 4 v		
Blank group	21.33±3.70⊿*	22.40±3.95 ₂ *	25.84±5.95 <i>/</i> *	23.24±4.90 <i>∆*</i>	
Model group	161.83±12.76∠	181.95±9.25∠	271.31±11.87⊿	343.85±12.24∠	
Benazepril group	60.77±8.44*	92.78±6.95*	145.37±8.86*	163.78±12.69*	
Reynoutria japonica group	136.47±9.55∆*	143.72±8.19 <i>∆*</i>	142.04±6.80*	132.46±7.10*	
Resveratrol group	158.03±10.71∠	173.28±8.38∆	202.64±8.46∠*	227.04±10.28∠*	
F	473.843	771.151	1101.890	1400.662	
Р	<0.001	<0.001	<0.001	<0.001	
Note: $\triangle P < 0.01$, compared to the Benazepril group, * $P < 0.01$, compared to the model group.					

Table 4

Comparison of Scr and BUN in the blood of rats in each group

At the fourth week, the Scr levels of the blank group, the Reynoutria japonica group, the resveratrol group and the benazepril hydrochloride group were significantly higher than that of model group (P<0.05). The Scr levels of the blank group and the Reynoutria japonica group were significantly higher than those of the benazepril hydrochloride group (P<0.05). The Scr levels of the resveratrol group and the model group were significantly lower than those of the benazepril hydrochloride group (P<0.05). The comparison results between groups of the BUN values were the same as those of the Scr values (Table 5, Figure legend 4).

Table 5 Changes in urea nitrogen/serum creatinine of each group on the fourth weekend ($n=10$).					
Groups	Urea nitrogen (mmol/L)	Serum creatinine (µmol/L)			
Blank group	6.45±1.22 <u>∕</u> *	37.52±5.34 <u></u> ∕*			
Model group	26.80±3.80∠	109.29±7.45∠			
Benazepril group	19.84±1.73*	88.57±6.93*			
Reynoutria japonica group	15.54±1.35∠*	77.24±9.62 <i>∆*</i>			
Resveratrol group	22.60±1.79∠*	99.46±4.07∠*			
F	136.898	157.953			
Р	<0.001	<0.001			

Note: $\triangle P < 0.01$, compared to the values of the Benazepril group, **P*<0.05, compared to the values of the model group.

Comparison of SOD activity and MDA level in renal tissue of rats in each group

At the fourth week, the SOD activities of blank group, Reynoutria japonica group, resveratrol group and benazepril hydrochloride group were significantly higher than that of model group (P<0.05). The SOD activities of blank group and Reynoutria japonica group were significantly higher than that of benazepril hydrochloride group (P<0.05), and the SOD activities of resveratrol group and model group were significantly lower than that of benazepril hydrochloride group (P<0.05). The levels of MDA in blank group, Reynoutria japonica group, resveratrol group and benazepril hydrochloride group were significantly lower than that of benazepril group and benazepril hydrochloride group were significantly lower than those in the model group (P<0.05). The MDA levels of blank group and Reynoutria japonica group were significantly lower than those of benazepril hydrochloride group (P<0.05), and the MDA levels of blank group and model group were significantly lower than those of benazepril hydrochloride group (P<0.05), and the MDA levels of blank group and the MDA levels of resveratrol group and model group were significantly higher than those of benazepril hydrochloride group (P<0.05), and the MDA levels of resveratrol group and model group were significantly higher than those of benazepril hydrochloride group (P<0.05), and the MDA levels of resveratrol group and model group were significantly higher than those of benazepril hydrochloride group (P<0.05) (Table 6, Figure legend 5).

Changes in the oxidation index in the renal tissue of rats on the fourth weekend (, <i>n=10</i>).					
Groups	Superoxide dismutase (U.ml-1)	Malonic dialdehyde (nmol/L)			
Blank group	771.41±7.26∠*	7.56±0.09∠*			
Model group	552.73±12.05∠	28.07±1.43∠			
Benazepril group	606.74±17.05*	21.40±1.39*			
Reynoutria japonica group	628.58±15.28∠*	18.66±1.58∠*			
Resveratrol group	582.59±10.45∠*	25.14±1.79∠*			
F	420.403	307.702			
Р	<0.001	<0.001			

Table 6

Note: $\[Action P<0.01\]$, compared to the values of the Benazepril group, *P<0.05, compared to the values of the model group.

Sirt1 mRNA and protein expression level on the fourth weekend

At the fourth week, the Sirt1 mRNA levels in the blank group, the Reynoutria japonica group, the resveratrol group and the benazepril hydrochloride group were significantly higher than those in the model group (P<0.05). The Sirt1 mRNA levels of the blank group and the Reynoutria japonica group were significantly higher than those in the benazepril hydrochloride group (P<0.05), and the Sirt1 mRNA levels of resveratrol group and model group were significantly lower than those of the benazepril hydrochloride group (P<0.05). The comparison results between groups of the Sirt1 protein expression were the same as those of the Sirt1 mRNA expression (*P<0.05*) (Table 7, Figure legend 6 and Fig. 1 and Fig. 2).

Table 7 Changes in Sirt1 mRNA/Sirt1 protein in rat tussle expression of each group on the fourth weekend (, n=10).

Groups	Sirt1 mRNA	Sirt1 protein
Blank group	1.17±0.01⊿*	0.22±0.02∠*
Model group	0.35±0.02∠	0.09±0.02
Benazepril group	0.59±0.03*	0.14±0.02*
Reynoutria japonica group	0.72±0.03∠*	0.17±0.02∠*
Resveratrol group	0.46±0.04∠*	0.12±0.01∠*
F	169.451	111.848
Р	<0.001	<0.001

Note: $\triangle P < 0.01$, compared to the values of the Benazepril group, **P*<0.05, compared to the values of the model group.

Changes in each group of rat renal ultrastructures

The histological examination of the kidney under a light microscope showed that on the fourth weekend, the rats in the model group had the characteristics of fibrosis and hyperplasia, interstitial hyperaemia, glomerular haemorrhage, inflammatory cell infiltration, tubular protein, and tubular and glomerular atrophy. In the treatment group, there was slight glomerular haemorrhage and a small amount of inflammatory cell infiltration in interstitial tissue. The lesion degree of Reynoutria japonica group was better than that of Benazepril group, while the Benazepril group was better than that of Resveratrol group (Fig. 3).

Pearson linear correlation analysis was used to analyse the correlation between Sirt1, SOD and MDA

According to the correlation results table, there was a significant correlation between Sirt1, SOD and MDA. The correlation coefficients were between *0.8-1*, indicating that there was a strong positive correlation between Sirt1 and SOD, there was a strong negative correlation between Sirt1 and MDA, SOD and MDA (Table 8).

Table 8 Pearson linear correlation analysis was used to analyze the correlation between sirt1, SOD and MDA

	SOD	MDA	Sirt1 mRNA	Sirt1 protein
SOD	1			
MDA	950**	1		
Sirt1 mRNA	.976**	948**	1	
Sirt1 protein	.907**	937**	.911**	1
**. Correlation is significant at the 0.01 level (2-tailed).				

Discussion

The results showed that the urinary albumin/urinary creatinine of the treatment group and the model group were higher than that of the blank group, indicating that the model was successful. At the first week, the urinary albumin/urinary creatinine value of the Benazepril group was significantly lower than that of the model group (P<0.05), suggesting that the effect of Benazepril is rapid and obvious. At the third week, one rat died in the Benazepril group, which indicated that the therapeutic effect of Reynoutria japonica was slow, but the side effect was less than that of Benazepril.

It can be seen from the observation of the oxidation indices of each group that the level of superoxide dismutase in the kidney of each treatment group can be increased, and the level of malonic dialdehyde can be reduced.

MDA is an important product of lipid peroxidation in vivo, which can indirectly reflect the degree of lipid peroxidation, and is an important index to evaluate the level of oxidative stress. SOD can eliminate oxygen free radicals in vivo and alleviate the damage to tissue caused by free radicals, and superoxide can affect the activity of SOD. When tissue damage is serious, SOD function will decline or even become inactive.^[18]Therefore, SOD plays an important role in antioxidation. The results of this study suggest that the level of MDA in the treatment group was lower than that in the model group, and the level of SOD in the serum was higher.Studies have shown that the sirt1 protein has an antioxidative stress effect. Whether sirt1 can improve drug-induced renal injury by regulating the synthesis and activity of SOD and MDA has not been reported.^[19]The sirt1 gene and protein expression was higher than that of the control group, which indicated that oxidative stress played an important role in the model of drug-induced renal injury.

Some studies have shown that Sirt1-SOD-ROS is the signalling mechanism of antioxidant stress in the kidney.^[20]In our experiment, the SOD value in the model group was lower than that of the normal group,

and the MDA value was higher than that of the normal group at the same time, suggesting that the pathogenesis of DKI may be related to oxidative reactions. The better the treatment effect, the higher the content of sirt1 and the content of SOD, the lower of the MDA. The increase of SOD means the decrease of ROS level in vivo, the high correlation among SIRT1, SOD and MDA indicates that they can affect each other, sirt1 and SOD are positive regulatory relationships, suggesting that sirt1 could enhance SOD expression and inhibit oxidative stress.^[21]

The effect of traditional Chinese medicine on diseases is multi-target, there are many mechanisms for drug-induced renal injury, possible mechanisms include local tissue inflammation, tissue peroxidation, injury and other pathological changes, other components in Reynoutria japonica can also play a role in the treatment of nephropat-hy. In this experiment, resveratrol may only protect the kidney from oxidation and lipid peroxidation, therefore, the protection of kidney is not as comprehensive as Reynoutria japonica. This is also one of the reasons why the experimental results of resveratrol are not as good as those of Reynoutria japonica.

Conclusion

In our experiment, the content of MDA in DKI rats was significantly higher than that in normal group, which also suggested that oxidative stress played an important role in the pathogenesis of drug-induced renal injury. In the experiment, the tested drugs can up regulate the expression of SIRT1, and all indexes are better than the model group. It has a certain therapeutic effect, and it can improve the expression of SIRT1 gene, increase SOD activity and reduce MDA level, indicating that it may play a therapeutic role through oxidative stress. It also suggests that the tested drugs may regulate the anti lipid peroxidation of the body through mediating SIRT1, Increase the synthesis of SOD in cells, so as to reduce the synthesis of reactive oxygen species in cells, then inhibit the oxidative damage of rat kidney induced by adriamycin, and finally realize its antioxidant physiological function. The therapeutic effect of Reynoutria japonica group is significantly better than that of resveratrol group and benazepril hydrochloride group, indicating that the effect of Reynoutria japonica on kidney is multi-target and the protective effect on kidney disease is more comprehensive, including not only anti lipid peroxidation, but also other mechanisms, which need to be further studied.

Abbreviations

ACR urinary albumin/urinary creatinine BUN urea nitrogen DKI drug-induced kidney injury MDA malonic dialdehyde Scr serum creatinine SOD superoxide dismutase

Declarations

Ethics approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Luohe Central Hospital, Certification No. 2019027.

Consent to publish

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interest

All the authors declare that they have no known competing financial interests.

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Authors' Contributions

S. T. and S. C. designed the study. S. T. performed experiments, acquired and analyzed data, drafted and edited the manuscript; X. L. performed molecule dynamic simulation and docking. S. C., X. S. and X. C. assisted with experiments. S. C. and S. T.contributed to provide analytical instruments and review of the manuscript.

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Figures



Changes in weight of each group





Changes in Urine volume of each group



Changes in urinary albumin/urinary creatinine of each group





Changes in urea nitrogen/serum creatinine of each group on the fourth weekend



Oxidation index

Figure 5

Changes in the oxidation index in the renal tissue of rats on the fourth weekend



Changes in Sirt1 mRNA/Sirt1 protein in rat tussle expression of each group on the fourth weekend.



Immunohistochemistry (IHC) for sirt1 in experimental groups

SIRT1	_	-	-	-	-	-
GAPDH		ļ	-	J	•	ſ

SIRT1 protein expression was detected by Western blot. Note: from left to right: marker; Blank group; Reynoutria japonica group; Benazepril hydrochloride group; Resveratrol group; Model group.



Glomerular morphology changes in experimental groups (PAS stain, 400×magnification).