Effect of Reynoutria Japonica and its Extract on Sirt1 in Cisplatin-Induced Renal Injury

Shangmei Cao
Luohe Medical College
Xiaojing Liu
Luohe Medical College
Xinxin sun
Luohe Medical College
Xixia Chen
Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine
Shuifu Tang (tsfchina2020@126.com)
Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine
https://orcid.org/0000-0002-0108-1223

Research Article

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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.

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

Design: Fifty adult male SD rats were randomly divided into five groups: blank group, model group, Reynoutria japonica group, resveratrol group and lotensin group. Each group was given the corresponding drug. ACR was measured on the seventh day every week. Creatinine and urea nitrogen were measured on the fourth weekend. All rats were sacrificed on the fourth weekend to detect the relevant indicators in the kidney.

Results: At the fourth week, the ACR, Scr and BUN of the Resveratrol group were higher than those of the lotensin group and Reynoutria japonica group ($P<0.05$). The values of Scr and BUN were lower in the Reynoutria japonica group than in the lotensin group ($P<0.05$). The levels of SOD, NO, and sirt1 gene and protein expression in the model group and treatment group were lower than those in the blank group, and those in the model group were lower than those in the treatment group ($P<0.05$). The levels of SOD, NO, and sirt1 gene and protein expression in the Reynoutria japonica group were higher than the lotensin group ($P<0.05$).

Conclusions: The therapeutic effect of the Reynoutria japonica group was better than that of the lotensin 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].

Cisplatin 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 cisplatin 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 and can be detected in blood and urine. Recent studies suggest that the development of drug-induced kidney injury and may serve 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 DKI.

Lotensin 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 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. The pathogenesis of drug-induced kidney injury is still unknown. To date, there is no specific drug to prevent drug-induced kidney injury, and 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 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.

Methods

2.1 Drugs

Resveratrol:Sigma,CAS#v900386;Adriamycin:Haizheng Pfizer Pharmaceutical Co.,Ltd.,CAS#131101;Lotensin:Solarbio,CAS#YZ100768; Reynoutria japonica:

Guangzhou Kangmei Pharmaceutical Co.,Ltd.; Cisplatin:Solarbio,CAS#15663271; 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 patholo-gical 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.

2.2 The animals for the experiment

Fifty male 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 Animal Care and Animal 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℃, 12 hours of day/night cycle) and were free to eat standard feed and drinking water.

2.3 Modelling

Fifty adult male SD rats were randomly divided into two groups: a blank group (10 rats) and a model group (40 rats). The model group rats were injected with cisplatin (10 mg/kg) via the tail vein. Three days later, the proteinuria test strip was green or orange, which proved that proteinuria was positive. The successfully modelled rats were randomly divided into a blank group, model group, Reynoutria japonica group, resveratrol group and lotensin group, with ten rats in each group.

2.4 Gastric perfusion method and collection of specimens

The Reynoutria japonica group was given 2.7g/kg/d, which converted from human dose, resveratrol group was given 40 mg/kg/d.respectively, while the lotensin group was given 0.90 mg/kg. The blank and model groups were given double distilled water at the same time and gavaged twice daily for four weeks. Doses of cisplatin[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, random urine was collected with a metabolic cage for urinary protein excretion measurement. All rats were anaesthetized with 3% sodium pentobarbital (0.1–0.2 ml/100g) on the fourth weekend to obtain blood taken from the heart to measure serum creatinine and urea nitrogen and their kidneys. The kidneys of both sides were divided into two halves along the sagittal line on ice and preserved in three parts: ① the kidneys were frozen in liquid nitrogen after being infiltrated with RNA protective solution and stored in -80°C for real-time PCR experiment; ②for detection of oxidative stress index after tissue homogenization saved as above; ③ the kidneys were fixed in paraformaldehyde for immunohistochemistry and PAS staining.

2.5 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].

2.6 Detection of urinary albumin/urinary creatinine

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

2.7 Detection of serum creatinine and urea nitrogen

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

2.8 Detection of oxidative stress index and plasma vasoactive substances

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

2.9 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.

3. Statistical analysis

The entire analyses used SPSS version 24.0. All values are expressed as the means ± SD. Single factor analysis of variance for statistical analysis of data. $P<0.05$ was considered statistically significant.

Results

4.1 Comparison of ACR in the urine of rats in each group

Within 4 weeks of treatment, the ACR levels in the treatment group and the model group were higher than those in the blank group, while the ACR levels in the resveratrol group were higher than those in the Reynoutria japonica group and the lotensin group. The ACR value of the lotensin group was significantly lower than that of the Reynoutria japonica group at the first, second weeks. The ACR value of the Reynoutria japonica group was significantly lower than that of the lotensin group at the fourth weeks

(P<0.05). The ACR value of the Reynoutria japonica group was lower than that of the lotensin group at the third weeks, but the comparison between them had no significance (P>0.05). (Table 2, Fig. 1).

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

At the fourth week, the Scr and BUN levels in the Reynoutria japonica group were significantly lower than those in the lotensin group and the resveratrol group (P<0.05). The creatinine and urea nitrogen levels of the resveratrol group were higher than those of the lotensin group (P<0.05) (Table 3, Fig. 2).

4.3 Comparison of superoxide dismutase, malonic dialdehyde and nitric oxide in renal tissue of rats in each group

At the fourth week, the superoxide dismutase and nitric oxide levels in the Reynoutria japonica group were significantly higher than those in the lotensin group (P<0.05). The superoxide dismutase and nitric oxide levels of the resveratrol group were lower than those of the lotensin group and Reynoutria japonica group (P<0.05). In contrast, the malonic dialdehyde level of the Reynoutria japonica group was significantly lower than that of the lotensin group (P<0.05). The malonic dialdehyde content of the resveratrol group was higher than that of the lotensin group and Reynoutria japonica group (P<0.05) (Table 4, Fig. 3).

4.4 Sirt1 mRNA expression level on the fourth weekend

At the fourth week, the Sirt1 mRNA levels in the Reynoutria japonica group were significantly higher than those in the lotensin group (P<0.05). The Sirt1 mRNA levels of the resveratrol group were lower than those of the lotensin group and Reynoutria japonica group (P<0.05) (Table 5, Fig. 4).

4.5 Sirt1 protein expression level on the fourth weekend

At the fourth week, the Sirt1 protein levels in the Reynoutria japonica group were significantly higher than those in the lotensin group (P<0.05). The Sirt1 protein levels of the resveratrol group were lower than those of the lotensin group and Reynoutria japonica group (P<0.05) (Table 5, Fig. 4 and Fig. 5).

4.6 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 lotensin group, while the lotensin group was better than that of Resveratrol group (Fig. 6).

4.7 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, SOD and MDA (Table 6).

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 lotensin group was significantly lower than that of the model group ($P<0.05$), suggesting that the effect of lotensin is rapid and obvious. At the third week, one rat died in the lotensin group, which indicated that the therapeutic effect of Reynoutria japonica was slow, but the side effect was less than that of lotensin.

It can be seen from the observation of the oxidation indexes 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[17]. 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[18]. 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.

With the passage of treatment time, the therapeutic effect of Reynoutria japonica group was significantly better than that of resveratrol group and lotensin group. The MDA content in the model group was significantly higher than that in the normal group, which also suggested that oxidative stress played an important role in the rat model of drug-induced renal injury, resveratrol can up regulate the expression of SOD and SIRT1, which also suggests that resveratrol may achieve its antioxidant physiological function by mediating Sirt1-SOD-ROS pathway. However, the therapeutic effect of resveratrol on drug-induced renal injury is significantly lower than that of Reynoutria japonica and positive control drugs, the reason may be that the therapeutic effect of Reynoutria japonica on renal injury is multi-target, and its protective effect on nephropathy is more comprehensive. Its mechanism includes not only anti lipid peroxidation, but also other mechanisms that need to be further studied.

Abbreviations

ACR urinary albumin/urinary creatinine

MDA malonic dialdehyde

NO nitric oxide

SOD superoxide dismutase

Scr serum creatinine

BUN urea nitrogen

DKI drug-induced kidney injury

Declarations

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The Science and Technology Department of Henan Province.

Ethics approval and consent to participate

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

Declaration of Competing Interest

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

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Author Contributions

All authors read and approved the final version of the manuscript and all authors contributed the same to the article. The authors declare that all data were generated in-house and that no paper mill was used.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

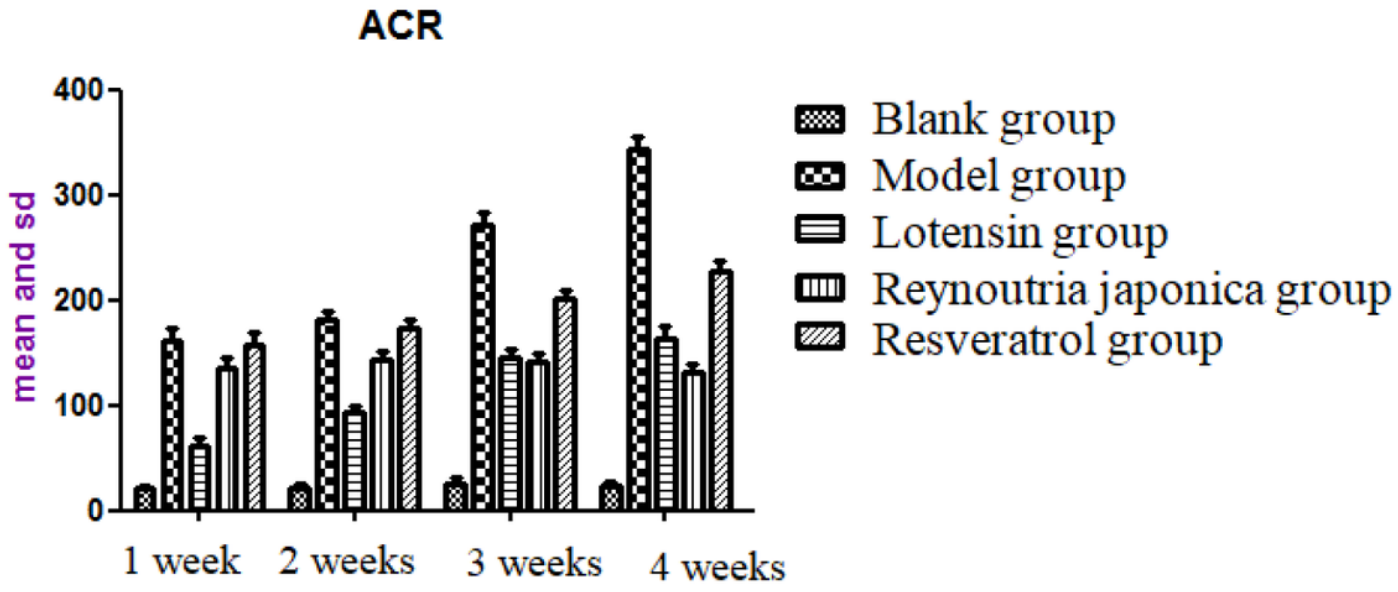

Figure 1

Changes in urinary albumin/urinary creatinine of each group

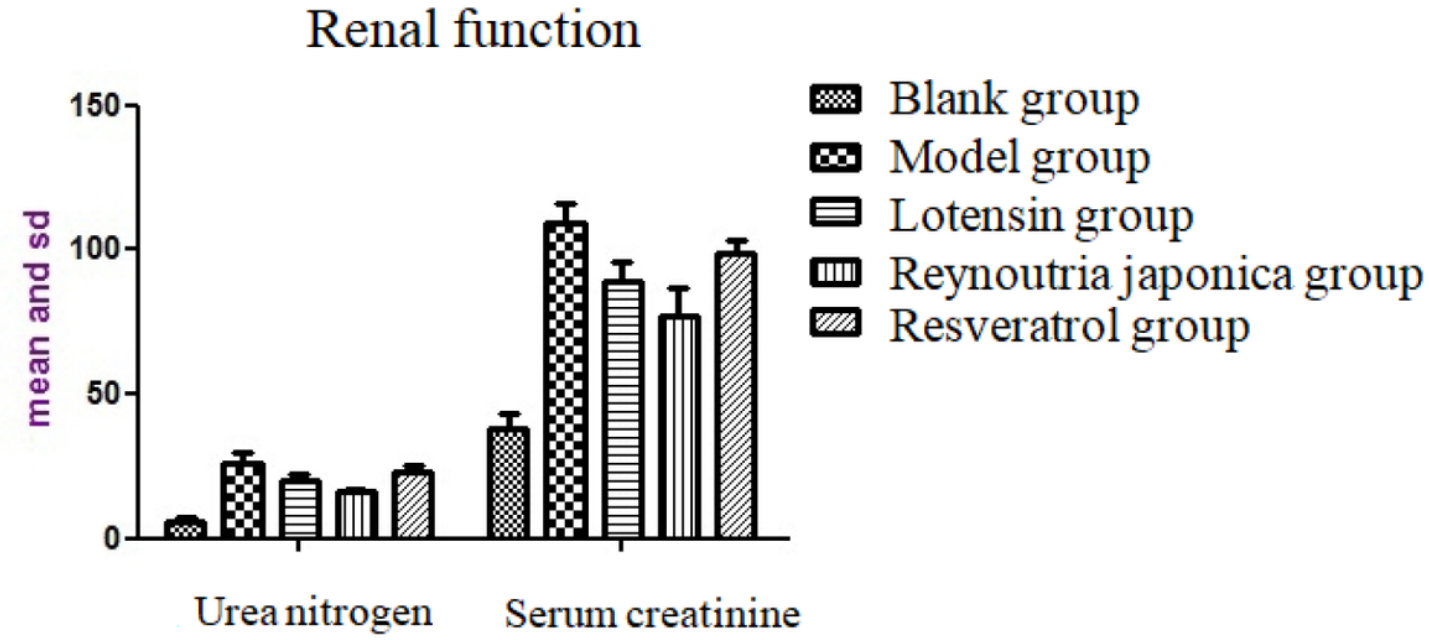

Figure 2

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

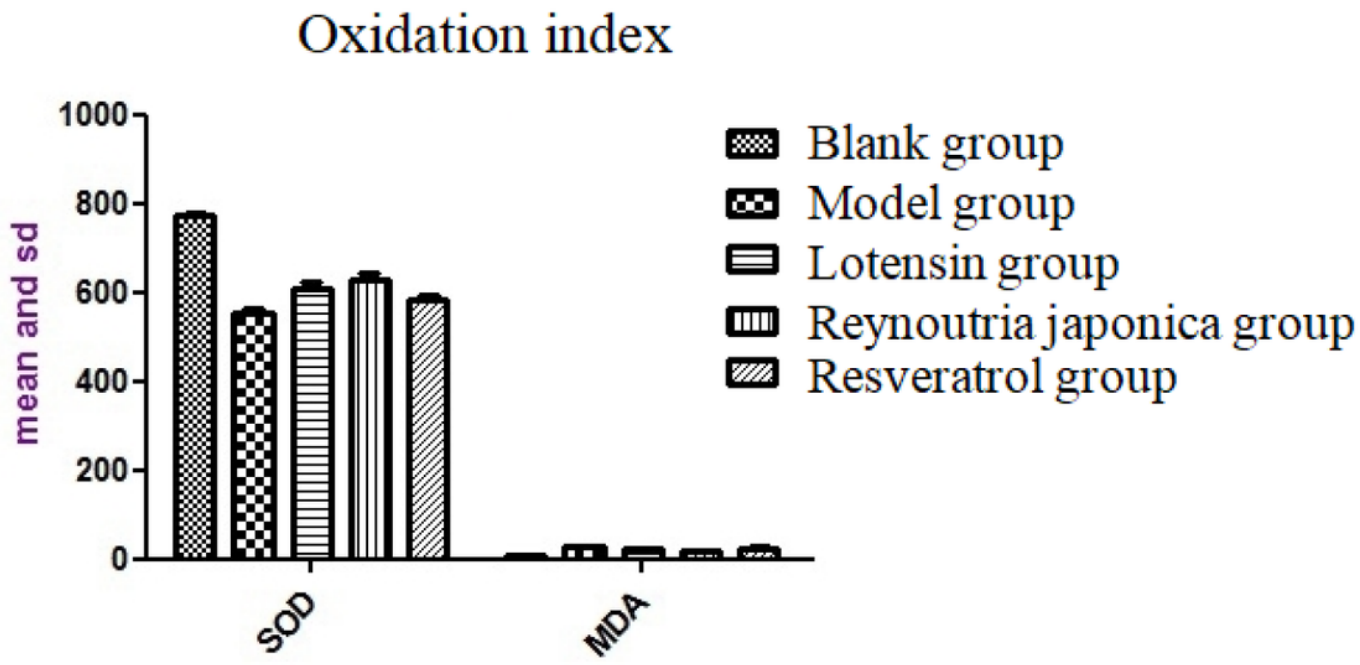

Figure 3

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

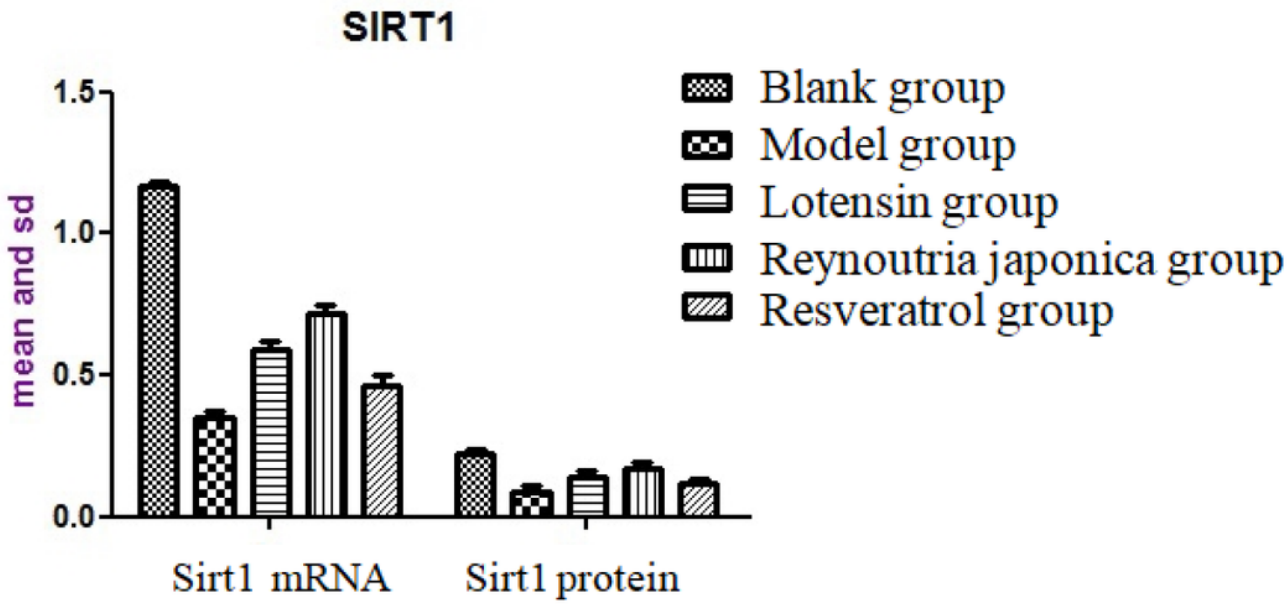

Figure 4

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

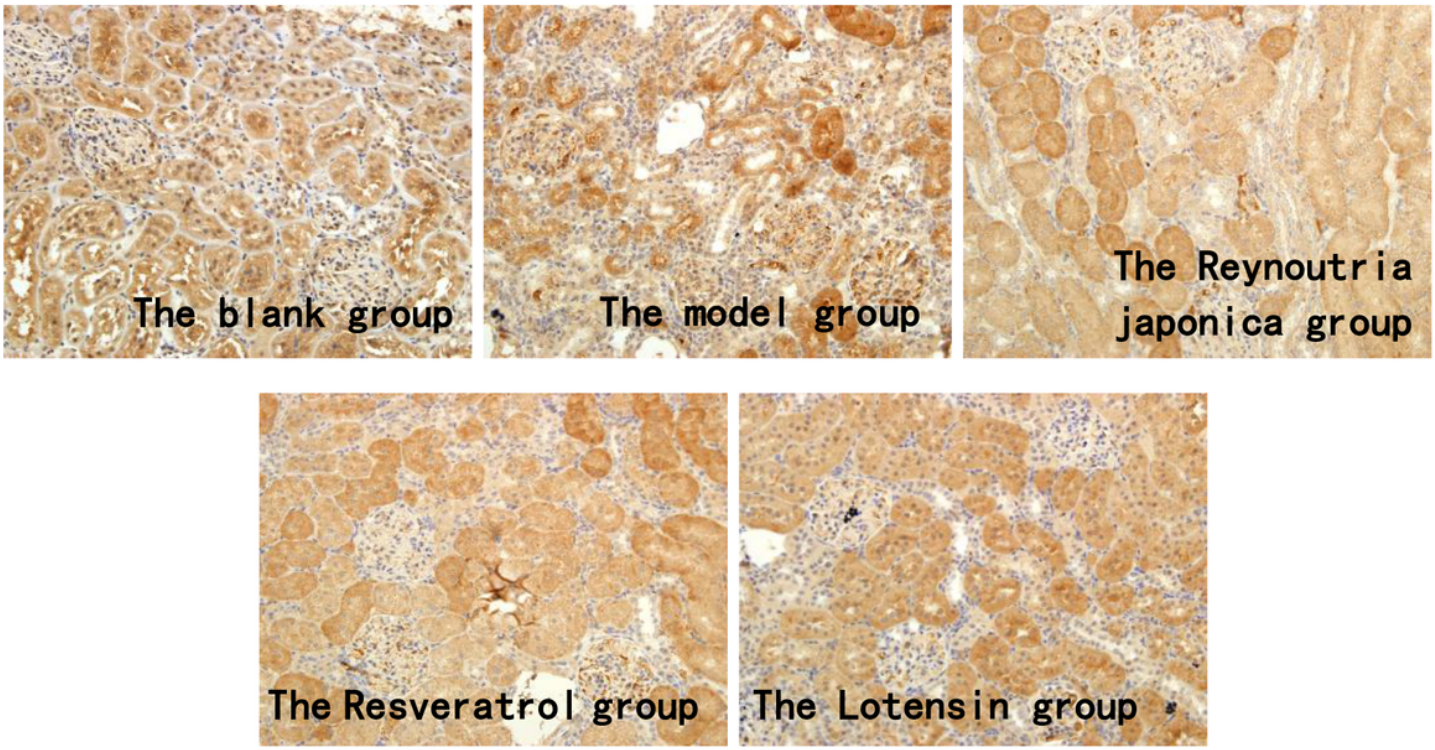

Figure 5

Immunohistochemistry (IHC) for sirt1 in experimental groups

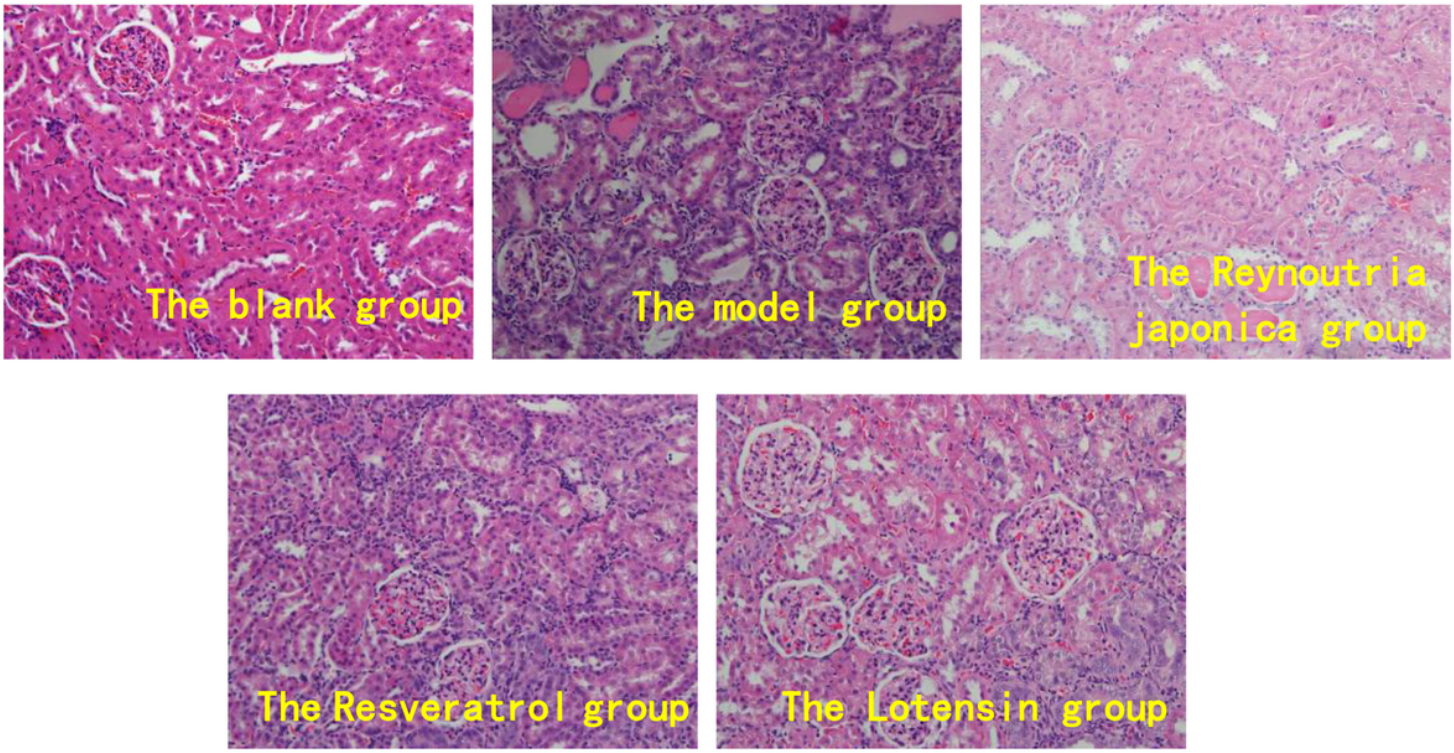

Figure 6

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

Supplementary Files

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- Table16.pdf