



Astragaloside IV protects against ischemia/reperfusion (I/R)-induced kidney injury based on the Keap1-Nrf2/ARE signaling pathway

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Background: This study sought to investigate the protective effects of Astragaloside IV (AS-IV) on ischemia-reperfusion (I/R) renal injury based on the Keap1-Nrf2/ARE signaling pathway.

Methods: A total of 36 male Sprague-Dawley rats (aged: 8 weeks; weighing: 200–220 g) were randomly divided into the following 6 groups (n=6 per group): (I) the control group; (II) the sham operation group; (III) the kidney I/R injury group (the I/R group); (IV) the kidney I/R injury group treated with 2 mg/kg of AS-IV (the low-dose group); (V) the kidney I/R injury group treated with 5 mg/kg of AS-IV (the mid-dose group); and (VI) the kidney I/R injury group treated with 10 mg/kg of AS-IV (the high-dose group). Serum creatinine (CRE), serum urea, malondialdehyde (MDA), and superoxide dismutase (SOD) were examined using enzyme-linked immunoassay kits. Cell apoptosis and the pathological damage to the renal tissue were determined by terminal deoxynucleotidyl transferase dUTP nick end labeling, hematoxylin and eosin, and periodic acid-Schiff (PAS) staining. The immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR), and western blot methods were used to determine the expression of Nrf2, HO-1, Bcl-2 and Bax in the kidney tissue.

Results: In the I/R rat model, the serum CRE level was increased. In the acute kidney injury (AKI) and chronic kidney disease (CKD) models, AS-IV treatment significantly decreased serum CRE levels in a dose-dependent manner. AS-IV treatment also reduced the injury of renal tubular epithelial cells, increased the expression levels of Nrf2 and HO-1, decreased the rate of apoptosis, downregulated the level of MDA, and elevated the activity of SOD. In the CKD rat model, the AS-IV treatment groups had reduced renal tubular epithelial cell injury, increased expression levels of Nrf2 and HO-1, decreased MDA levels, and increased SOD activity compared to the I/R group.

Conclusions: AS-IV induced the expression of Nrf2, enhanced the activity of antioxidant enzymes, reduced apoptosis, protected against renal I/R injury, and prevented AKI from transforming into CKD. These findings suggest that AS-IV is a promising drug for treating kidney injury.

Keywords: Astragaloside IV; acute kidney injury (AKI); chronic kidney injury; Keap1-Nrf2/ARE; oxidative stress

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Introduction

Acute kidney injury (AKI) commonly appears in clinical treatment, and has high morbidity and mortality (1). Studies have shown that severe AKI accounts for more than half of in-hospital deaths, and approximately 300,000 people die from AKI every year in the United States (2,3). The main feature of AKI is the rapid loss of renal excretion, which results in increased serum creatinine (CRE), urea, and decreased urine output, which is usually considered the diagnostic standard for AKI (4,5). Recently, epidemiological studies have shown that patients suffering from AKI are at high risk of developing chronic kidney disease (CKD) (2). CKD, which is characterized by chronic kidney structure and dysfunction, greatly increases the risk of several diseases, including cardiovascular disease, and thus has attracted public health attention worldwide (6).

Many studies have explored the possible role of Chinese medicine in the treatment of kidney disease and its complications (7). It has been reported that Bupleurum polysaccharides inhibit kidney inflammation and renal fibrosis (8), and alkaloids extracted from *Corydalis yanhusuo* to exert a protective effect in AKI by inhibiting apoptosis and inflammation (9). These studies suggest that traditional Chinese medicine could provide therapeutic drugs for the treatment of kidney disease. Astragaloside IV (AS-IV) purified from astragalus, which is a traditional Chinese medicine, has attracted wide interest in recent years, AS-IV has various pharmacological activities, including protective effects on cerebral injury and central nervous system (CNS), cardiovascular disease, respiratory system, kidney, endocrine system, organic immune system, liver and cancer due to its wide range of pharmacological actions,

Such as antioxidant, cardioprotective effects, anti-inflammatory, antiviral, anti-bacterial, anti-fibrosis, anti-diabetes and immunoregulation effects (10). Studies have shown that AS-IV attenuates the pathological process of DKA (diabetic ketoacidosis) mice by regulating the JNK/Nrf2 signaling pathway (11). Ding *et al.* reported that AS-IV alleviates rat pulpitis by exerting anti-inflammation and promoting differentiation effects (12). AS-IV has also been shown to have an anti-tumor effect in hepatocellular carcinoma (13) and to improve diabetic nephropathy (14).

Nrf2 plays a vital role in both the Keap1-Nrf2 signaling pathway and oxidative stress, which are closely related to kidney diseases (15,16). Recently, oxidative stress has been considered an important regulatory factor in AKI and CKD (17,18). The regulation of the Nrf2/HO-1 pathway has been shown to have a protective effect in an AKI rat model (19). Based on the above evidence, we sought to investigate whether AS-IV exerts any protective effects and explore its potential mechanism in an I/R-induced AKI rat model. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://tau.amegroups.com/article/view/10.21037/tau-22-505/rc>).

Methods

Animal groups

A total of 36 male Sprague-Dawley rats (aged: 8 weeks; weighing: 200–220 g), purchased from Laboratory Animal Center of Southern Medical University, were randomly divided into the following 6 groups (n=6 per group): (I) the control group; (II) the sham operation group; (III) the kidney I/R injury group (the I/R group); (IV) the kidney I/R injury group treated with 2 mg/kg of AS-IV (the low-dose group); (V) the kidney I/R injury group treated with 5 mg/kg of AS-IV (the mid-dose group); and (VI) the kidney I/R injury group treated with 10 mg/kg of AS-IV (the high-dose group). All the animals were housed in a temperature (72±3 °F) and air- (50%±20% relative humidity) controlled room at an SPF animal facility, with a 12-h light/dark cycle, and were fed a standard diet and tap water. Animal experiments were performed under a project license (No. 2019001) granted by the ethics committee of Southern Medical University (People's Hospital of Huadu District), in compliance with national guidelines for the care and use of animals. A protocol was prepared before the study without registration.

Establishment of I/R model in rats

AS-IV was intragastrically administered to the rats at doses of 10, 5, or 2 mg/kg 1 hour before the establishment of the I/R model, while equivalent amounts of physiological

saline were administered to those in the I/R group. The I/R rat model was established as described in a previous study (20). In brief, the rats were anesthetized, and unilateral renal ischemia was then conducted by applying microvascular forceps to the left renal pedicle for 45 min, followed by right renal nephrectomy. The sham operation group received an abdominal incision but did not undergo unilateral renal ischemia treatment. The rats (n=3 each group) were then sacrificed to establish the AKI and CKD models 24 hours and 14 days after the establishment of the I/R model, respectively. Serum and kidney tissues were collected for subsequent analyses.

Renal function tests

To analyze renal function, the rats serum was isolated from the whole blood after the end of the experiment, serum CRE and urea were measured using enzyme-linked immunoassay kits (Jiancheng Biotech, Nanjing, China). The operation was performed according to the manufacturer's instructions. The microplate reader (MB530; HEALES) was used to measure the A values of each group at the wavelengths of 546 nm (CRE), 640 nm (urea). The respective concentrations of CRE and urea were calculated by the kit instruction formula.

Oxidative stress analysis

The renal tissues of rats were isolated and then ground, centrifuged at 8,000 g 4 °C for 10 min, and the supernatant was obtained. Corresponding kits (Elabscience Biotechnology, Wuhan, China) were used to determine the production of malondialdehyde (MDA) and superoxide dismutase (SOD) in the kidney tissues. The operation was performed according to the manufacturer's instructions. After 30 min of 37 °C water bath, the absorbance of each tube was measured at 450 and 560 nm by microplate reader (MB530; HEALES).

Histomorphological staining

The kidney tissue was obtained and fixed with 4% paraformaldehyde at room temperature for more than 24 h, and then embedded and sliced into thin slices with a slicer, slices were baked at 62 °C for 2–6 h. After dewaxing with xylene, the tissue was stained with hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS). After drying, the slices were mounted and assessed under microscopic photographs

(BA210T; MOTIC, Xiamen, China).

TUNEL assays

According to the reagent instructions (Beyotime Biotechnology, Shanghai, China), the tissue sections were incubated with proteinase K working solution, followed by the prepared terminal transferase dUTP nick end labeling (TUNEL) reaction mixture at 37 °C for 1 h in a dark box. The sections were then observed in a later analysis by microscopic examination.

IHC staining

The slides were treated with 3% hydrogen peroxide at room temperature for 10 min, blocked with 10% BSA for 40 min, and then incubated with anti-Nrf2 (PA5-27882) or anti-HO-1 (PA5-77833) antibodies (Thermo Fisher Scientific, Waltham, MA, USA) at 4 °C overnight. The slides were washed with PBS and incubated with horseradish peroxidase for 40 min, and then stained with diaminobenzidine for 10 min at room temperature. After dehydration, the slides were mounted and observed under a microscope.

QRT-PCR

The RNAi Plus kit (9108, Otsu, Takakawa, Shiga, Japan) was used to extract the total ribonucleic acid (RNA) from the kidney tissue, and the concentration of RNA was determined after purification. Complementary deoxyribonucleic acid (DNA) was obtained using the Takara RT-PCR kit (RR047A, Takara), and messenger RNA (mRNA) levels were quantified using the Takara SYBR q-PCR kit (RR820A, Takara). We performed 40 cycles of RT-PCR reactions using the ABI 7500 system (Thermo Fisher Scientific). GAPDH was used as the internal reference, and the expression of the mRNA levels was normalized to GAPDH. Primer sequences for RT-PCR (*Table 1*).

Western blot

The kidney tissue was cut into small fragments with scissors, and RIPA lysis (P0013, Beyotime Biotechnology) was used to lyse the tissue on ice, after which the tissue was ground with a grinder to make it fully lysed. The supernatant was obtained after high-speed refrigeration centrifugation, and the protein concentration was determined by a BCA kit (CW0130S, CWBIO, Beijing,

Table 1 Primer sequences for RT-PCR

ID	Primer sequences (5'-3')
Nrf-2	Forward: 5'-ATTTGTAGATGACCATGAGTCGC-3'
	Reverse: 5'-CTGCCAAACTTGCTCCATGT-3'
HO-1	Forward: 5'-ACGCATATACCCGCTACCTG-3'
	Reverse: 5'-TCTCCAGAGTGTTCATGCCA-3'
Bcl-2	Forward: 5'-GCCTTCTTTGAGTTCGGTGG-3'
	Reverse: 5'-CTCAGTCATCCACAGAGCGA-3'
Bax	Forward: 5'-CGAATTGGCGATGAACTGGA-3'
	Reverse: 5'-CCAGTTGAAGTTGCCGTCTG-3'
GAPDH	Forward: 5'-CCTCGTCTCATAGACAAGATGGT-3'
	Reverse: 5'-GGGTAGATCATACTGGAACATG-3'

RT-PCR, reverse transcription-polymerase chain reaction.

China). Electrophoresis was carried out through 8–15% SDS-PAGE, and the sample was transferred to PVDF membranes, then blocked at room temperature for 1 h, and subsequently incubated with the primary antibody at 4 °C in incubators overnight. The membranes were washed with PBS and then incubated in the secondary antibody at room temperature for 1 h. The membranes were examined using a chemiluminescence system (Beyotime Biotechnology). The following primary antibodies were used: Nrf2 (PA5-27882), HO-1 (PA5-77833), Bcl-2 (PA5-27094), Bax (MA5-14003), and GAPDH (MA5-15738-D680, Thermo Fisher Scientific).

Statistical analysis

The statistical analysis was performed using GraphPad Prism 7. The data are presented as the mean ± standard deviation (SD). Statistical comparisons were carried out by a 1-way analysis of variance, followed by a Tukey's test. A P value <0.05 was considered statistically significant.

Results

AS-IV protected renal function and alleviated the renal tubular pathological damage caused by I/R

We first assessed renal function and the tubular pathological changes in the I/R-induced AKI rat model. The structure of AS-IV is shown in *Figure 1A*. As *Figure 1B,1C* show, the serum CRE and urea levels in the I/R group were markedly

elevated, indicating the successful establishment of the I/R model. AS-IV significantly decreased serum CRE and urea levels in a dose-dependent manner. Consistent with the above results, the H&E and PAS staining showed seriously damaged renal tubules tissues, disordered tubular epithelial cells, and increased inflammatory cell infiltration in the I/R group, while AS-IV inhibited these changes to some extent (see *Figure 1D,1E*). Our results showed the protective effect on the I/R rat model.

AS-IV treatment relieved I/R-induced apoptosis and regulated the Nrf2 /HO-1 pathway in rat kidney tissues

Cell viability is closely related to tissue function. To explore whether AS-IV exerted a protective effect on cell viability in the I/R-induced AKI rat model, we determined cell apoptosis using TUNEL assays. The results showed that renal cell apoptosis was significantly more alleviated in the AS-IV treatment groups, especially the high-dose group, than the I/R group (see *Figure 2A*). Next, we sought to examine whether AS-IV exerted a protective effect by regulating the Nrf2/HO-1 signaling pathway. The immunohistochemical (IHC) assay results showed that the expression of Nrf2 and HO-1 in the I/R group was decreased, but later increased after AS-IV treatment (see *Figure 2B,2C*). The qRT-PCR results further confirmed that the expression of the anti-apoptosis factor (Bcl-2) was downregulated and the expression of the pro-apoptosis factor (Bax) was upregulated in the I/R group, while AS-IV treatment suppressed these trends (see *Figure 2D,2E*), and the AS-IV treatment reversed the decreased expression of Nrf2 and HO-1 caused by I/R (see *Figure 2F,2G*). These results demonstrated that the AS-IV treatment decreased I/R-induced cell apoptosis in the kidneys. Consistent with these results, the western blot results also showed that the AS-IV treatment reversed the decreased expression of Nrf2 and HO-1 caused by I/R (see *Figure 2H-2J*), the anti-apoptosis factor (Bcl-2) was downregulated and the expression of the pro-apoptosis factor (Bax) was upregulated in the I/R group (*Figure 2K,2L*). Our data indicated that AS-IV relieved cell apoptosis, and this effect was partially related to the regulation of the Nrf2/HO-1 pathway in the I/R rat model.

AS-IV treatment alleviated lipid peroxidation in I/R-induced rat kidney damage

Lipid peroxidation alters the structural integrity of the cell

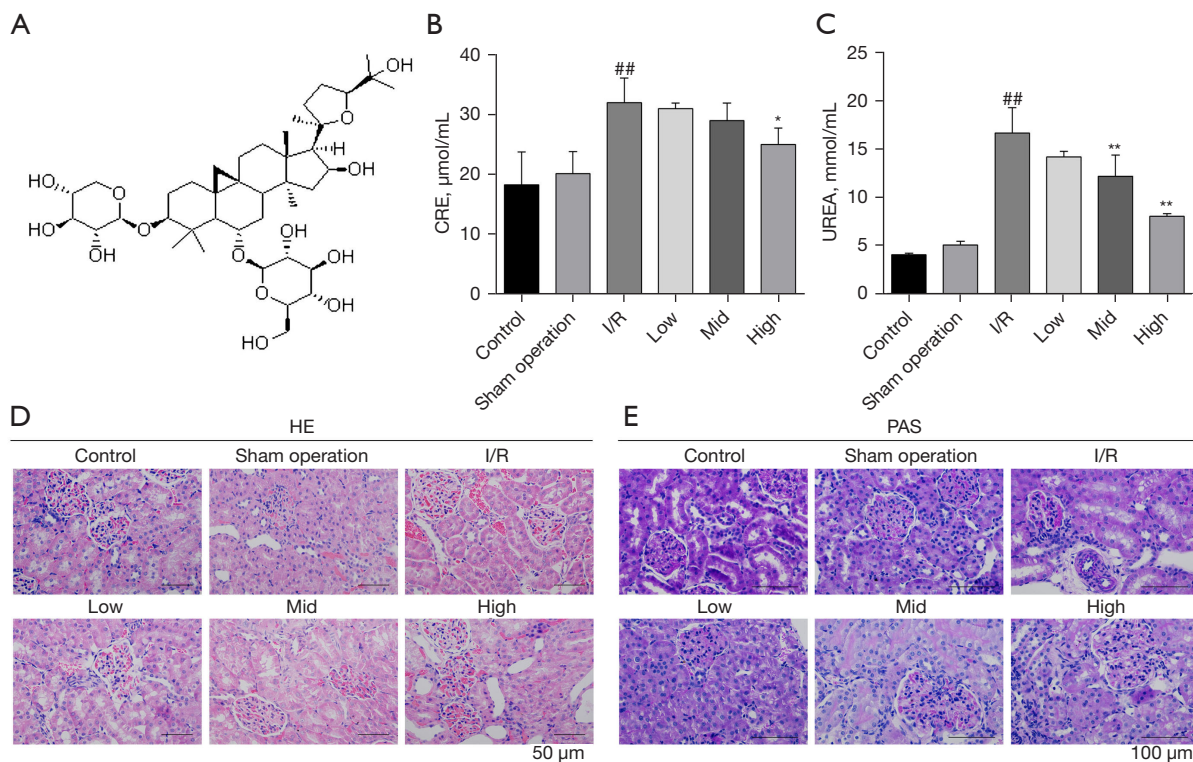


Figure 1 AS-IV protected renal function and alleviated renal tubular pathological damage caused by I/R. (A) Molecular structure of AS-IV. (B) Determination of CRE levels in AKI rat models. (C) Determination of urea levels in AKI rat models. (D) H&E staining of kidney tissue in AKI rat models. Magnification $\times 400$. (E) PAS staining of kidney tissue from AKI rats. Magnification $\times 400$. ^{##} $P < 0.01$, compared to the sham operation group; ^{*} $P < 0.05$, ^{**} $P < 0.01$, compared to the I/R group. CRE, creatinine; UREA, urea; AS-IV, Astragaloside IV; I/R, ischemia-reperfusion; AKI, acute kidney injury; PAS, periodic acid-Schiff.

membrane and causes an oxidative stress response, which in turn leads to cell death. We also investigated whether AS-IV regulated the lipid peroxidation response in AKI injury. We found that the increased MDA level and the decreased SOD activity in the I/R group were reversed after AS-IV treatment. Thus, AS-IV treatment had a protective effect to suffer from oxygen free radicals damage and alleviated I/R-induced kidney lipid peroxidation (see *Figure 3A,3B*).

AS-IV treatment protected renal function and protected against pathological damage in the I/R-induced CKD model

We sought to explore whether AS-IV continued to protect the kidneys if the establishment of the I/R model lasted for a longer time. To our surprise, even 14 days after the establishment of the I/R model, AS-IV exerted protective effects on kidney function and structure. Consistent with

previous results, we found that the serum CRE and urea levels were elevated in the I/R group and that these levels decreased after AS-IV treatment (see *Figure 4A,4B*). The H&E and PAS staining showed damaged renal tubules tissues with accumulated inflammatory cell infiltration and disordered epithelial cells in the I/R group, while the AS-IV treatment alleviated the pathological damage (see *Figure 4C,4D*). These results indicated that AS-IV treatment provided protection against the I/R-induced CKD.

I/R-induced CKD model

Next, we examined the renal cell apoptosis and the expression of the Nrf2/HO-1 pathway in the CKD model. Consistent with the results for the AKI model, renal cell apoptosis was also more alleviated in the AS-IV treatment group than the I/R group (see *Figure 5A*). The IHC results showed that the expression of Nrf2 and HO-1 in the I/R group were dramatically reduced, but increased after AS-IV

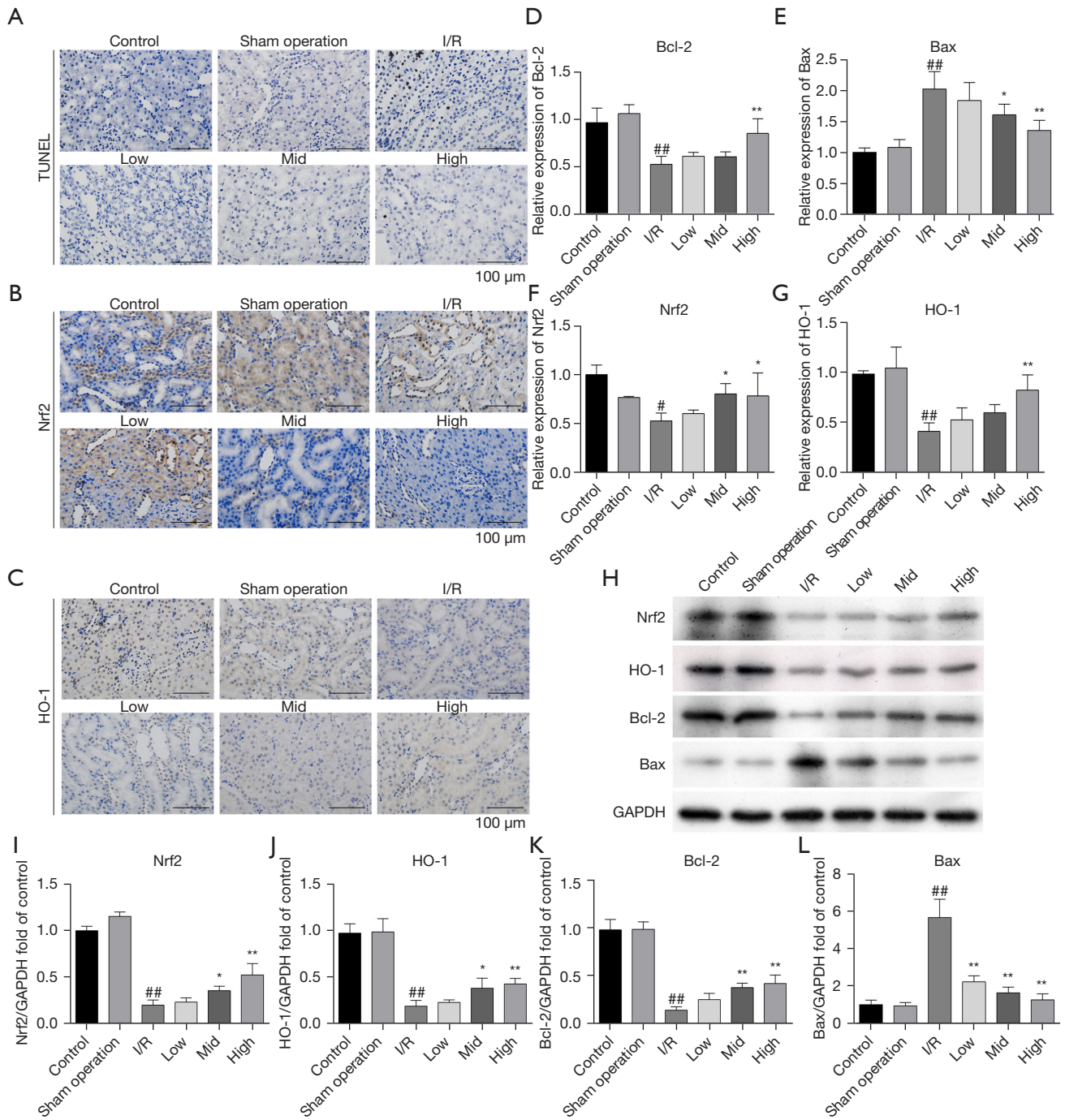


Figure 2 AS-IV treatment relieved I/R-induced apoptosis and regulated the Nrf2/HO-1 pathway in rat kidney tissues. (A) Apoptosis in the renal tissues of AKI rats as detected by TUNEL staining. Magnification $\times 400$. (B,C) Nrf2 and HO-1 were detected by IHC. Magnification $\times 400$. Bcl-2, Bax, Nrf2, and HO-1 expression were evaluated by qRT-PCR (D-G) and western blotting (H-L). # $P < 0.05$, ## $P < 0.01$ compared to the sham operation group; * $P < 0.05$, ** $P < 0.01$ compared to the I/R group. AS-IV, Astragaloside IV; I/R, ischemia-reperfusion; AKI, acute kidney injury; IHC, immunohistochemical; qRT-PCR, reverse transcription-polymerase chain reaction.

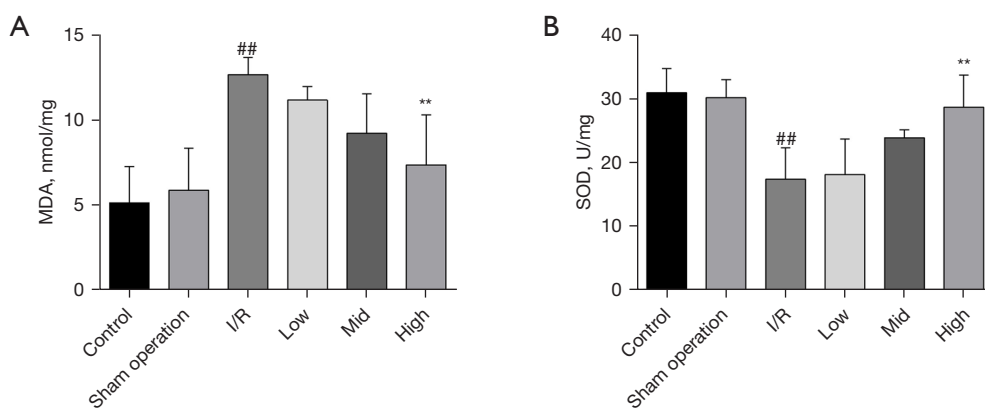


Figure 3 AS-IV treatment alleviated lipid peroxidation in I/R-induced rat kidney damage. (A) MDA levels as detected using an assay kit. (B) SOD activity as detected using an assay kit. ^{##}P<0.01 compared to the sham operation group; ^{**}P<0.01 compared to the I/R group. MDA, malondialdehyde; SOD, superoxide dismutase; AS-IV, Astragaloside IV; I/R, ischemia-reperfusion.

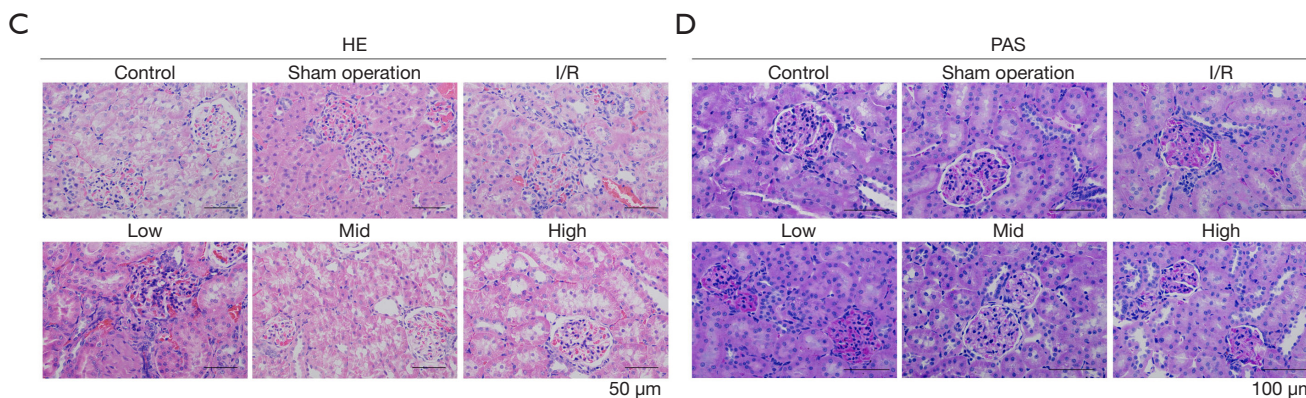
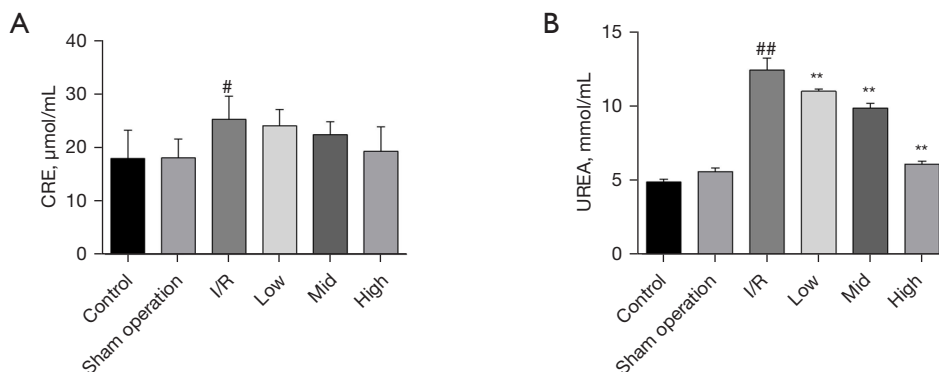


Figure 4 AS-IV treatment protected renal function and pathological damage in the I/R-induced CKD model. (A) Determination of CRE levels in CKD model rats. (B) Determination of UREA levels in the CKD rat models. (C) H&E staining of kidney tissue from the CKD rat models. Magnification $\times 400$. (D) PAS staining of kidney tissue from the CKD rat models. Magnification $\times 400$. [#]P<0.05, ^{##}P<0.01, compared to the sham operation group; ^{**}P<0.01, compared to the I/R group. CRE, creatinine; UREA, urea; AS-IV, Astragaloside IV; I/R, ischemia-reperfusion; CKD, chronic kidney disease.

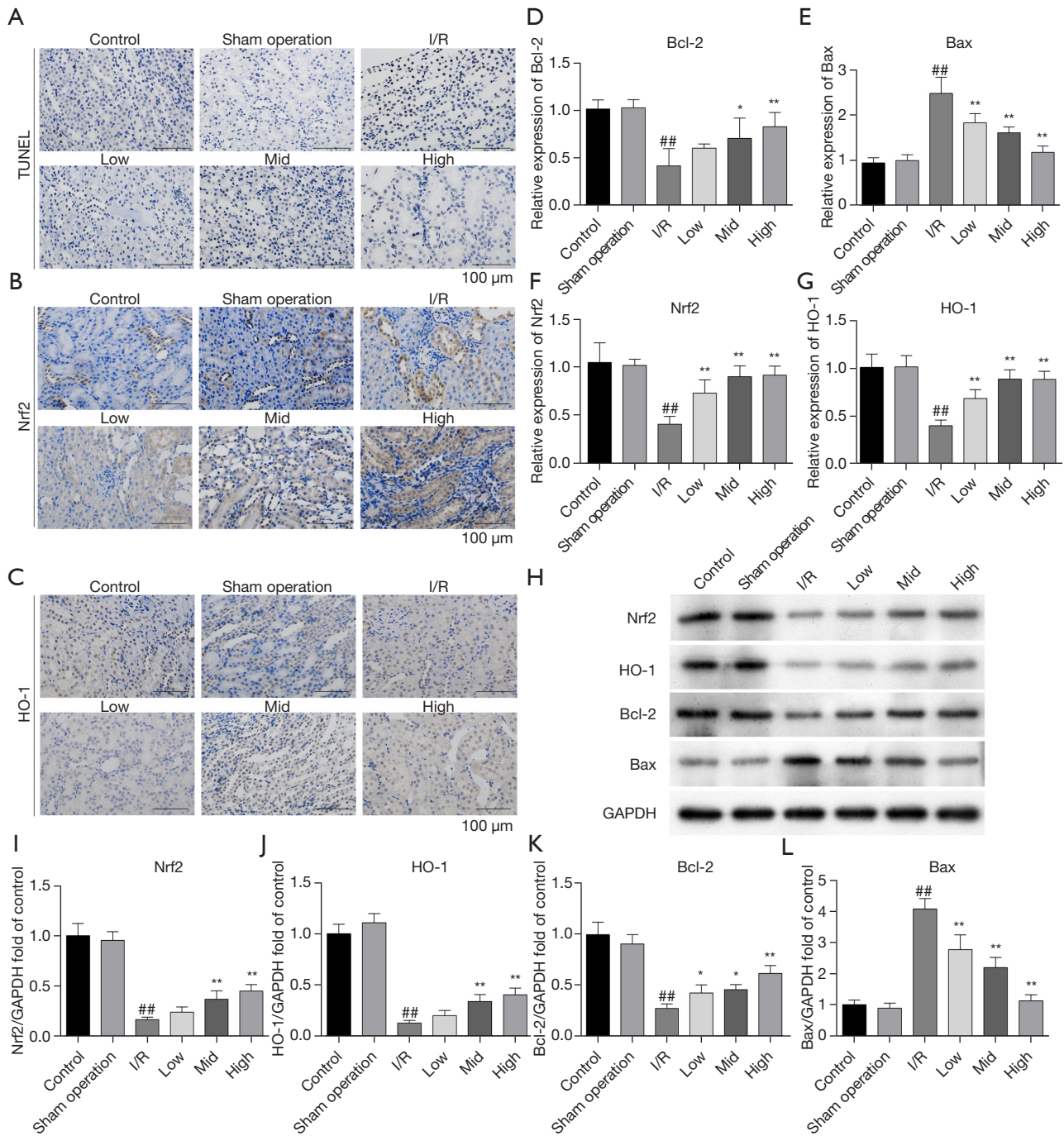


Figure 5 AS-IV relieved renal cell apoptosis and regulated the Nrf2/HO-1 pathway in the I/R-induced CKD model. (A) Apoptosis in the renal tissues of the CKD rats as determined by TUNEL staining. Magnification $\times 400$. (B,C) Nrf2 and HO-1 were detected by IHC. Magnification $\times 400$. Bcl-2, Bax, Nrf2, and HO-1 expression in kidney tissues was evaluated by qRT-PCR (D-G) and western blotting (H-L). ^{##} $P < 0.01$ compared to the sham operation group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ compared to the I/R group. AS-IV, Astragaloside IV; I/R, ischemia-reperfusion; CKD, chronic kidney disease; qRT-PCR, reverse transcription-polymerase chain reaction.

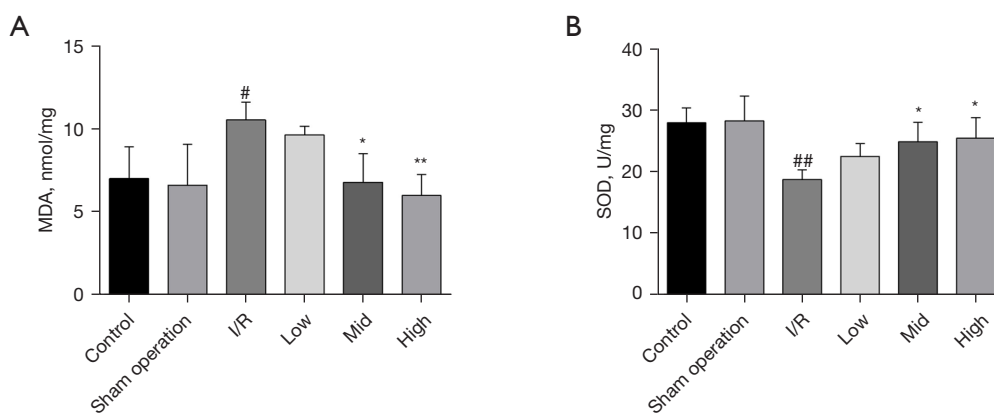


Figure 6 AS-IV attenuated renal lipid peroxidation in the I/R-induced CKD model. (A) MDA levels as detected using assay kits. (B) SOD activity as detected using assay kits. [#]P<0.05, ^{##}P<0.01 compared to the sham operation group; ^{*}P<0.05, ^{**}P<0.01 compared to I/R group. MDA, malondialdehyde; SOD, superoxide dismutase; AS-IV, Astragaloside IV; I/R, ischemia-reperfusion; CKD, chronic kidney disease.

treatment (see *Figure 5B,5C*). Additionally, the qRT-PCR results showed that the expression of the downregulated anti-apoptosis factor (Bcl-2) and the upregulated pro-apoptosis factor (Bax) in the I/R group were inhibited by the AS-IV treatment (see *Figure 5D,5E*), and the AS-IV treatment reversed the decreased expression of Nrf2 and HO-1 caused by I/R (see *Figure 5F,5G*), which indicates that AS-IV protects against I/R-induced apoptosis in CKD. Further, the expression of the Nrf2/HO-1 pathway in the CKD model was similar to that in the AKI model. The western blot results further confirmed the same results as those found for the IHC and q-PCR assays (see *Figure 5H-5L*). Our data indicated that AS-IV has a protective effect against apoptosis and oxidative stress in CKI.

AS-IV attenuated renal lipid peroxidation in the I/R-induced CKD model

Similarly, we examined the effect of AS-IV on lipid peroxidation in the CKD model. We found that 14 days after the establishment of I/R model, the rats in the I/R group had an elevated level of MDA and decreased SOD activity; however, AS-IV treatment improved these trends (see *Figure 6A,6B*), just as it did in the AKI model. Our data showed that AS-IV alleviated renal lipid peroxidation in the CKD model.

Discussion

Studies have shown that AS-IV leads to improvements in a variety of kidney diseases. Ju *et al.* reported that AS-IV

protected rats from diabetic nephropathy by regulating the apoptosis of the endoplasmic reticulum pathway (21). AS-IV has also been shown to alleviate cisplatin-caused kidney damage by regulating the autophagy pathway (22). Further, AS-IV relieves oxidative stress, which in turn decreases the tubulointerstitial damage induced by indoxyl sulfate (23). However, it was unclear whether AS-IV would have a protective effect on AKI and CKI.

According to a previous study, left renal ischemia for 45 minutes combined with right renal nephrectomy effectively establishes an I/R rat model (20). Our results showed that AS-IV exerted a protective effect against AKI and CKI partially by regulating the antiapoptotic and antioxidative stress pathway.

Apoptosis is part of programmed cell death, which aims to maintain a cell microenvironmental balance (24-26). Several studies have shown that apoptosis is involved in the pathogenesis of renal diseases (27). Apoptosis has two pathways (i.e., exogenous and endogenous) (28), and the endogenous pathway can be further divided into mitochondrial pathways and endoplasmic reticulum pathways (29-31). In our study, as the key factor in mitochondrial apoptosis, the decreased of Bcl-2 and the upregulation of Bax in the I/R-induced AKI and CKD models might indicate the occurrence of mitochondrial apoptosis. It may be that AS-IV suppresses cell apoptosis in kidney injury by regulating mitochondrial apoptosis.

Previous studies have shown that oxidative stress is related to the pathogenesis of AKI. Notably, Xia *et al.* reported that honokiol plays a protective role in AKI through the regulation of peroxidative stress (32). Progressive CKD

and its complications are closely associated with increased levels of oxidative stress (33). It is well known that decreased SOD and increased MDA are considered the biomarkers of oxidative stress. In our study, AS-IV increased SOD activity and decreased MDA content in the AKI and CKD models, which suggests that it has antioxidant effects in I/R-induced renal injury.

The activation of Nrf2 plays a vital role in the antioxidant system (34). Dai *et al.* found Scu led to up-regulation of HO-1 through activating the Nrf2 signalling pathway, protecting the kidneys from ischemia/reperfusion (I/R)-induced oxidative damage (35). Zhang *et al.* Studies demonstrated that UA can alleviate oxidative stress and promote autophagy by activating the p62-Keap1-Nrf2 signaling pathway, which could protect the kidneys from ischemia reperfusion injury (36). In the Nrf2/ARE/HO-1 signaling pathway, Nrf2 regulates the expression of HO-1 by binding to ARE (37). SAL effectively alleviated renal function and ameliorated epithelial tubular injury, oxidative stress, and inflammatory response via anti-apoptotic effects through activation of the Keap1-Nrf2-ARE signaling pathway in renal tubular cells (38). In our study, various experimental methods resulted in decreased expression levels of Nrf2 and HO-1 in the I/R-induced AKI and CKD models. It may be that I/R in the kidney decreases the expression of Nrf2 and its downstream factor HO-1, which in turn weakens their interactions and leads to antioxidant stress dysfunction. Interestingly, treatment with AS-IV was found to upregulate the expression of Nrf2 and HO-1, which suggests that AS-IV might partially exert its protective effects by regulating the Nrf2/HO-1 pathway. However, more research on this issue needs to be conducted in the future. Zhang *et al.* found that Irisin treatment alleviated renal injury, downregulated inflammatory response, improved mitochondrial function, and reduced ER stress and oxidative stress after renal I/R, which were associated with upregulation of GPX4 (39). Zheng *et al.* Studies demonstrated that Porous Se@SiO₂ nanospheres exhibited a protective effect in I/R-induced AKI by resisting oxidative stress and inflammation (20). Up to now, there are many literatures about Astragaloside IV, and Astragaloside IV is widely used in clinic with less side effect, and it has extensive anti-inflammatory and enhancing immune function, so its clinical application value is high.

In summary, we found that AS-IV protected kidney function in I/R-induced AKI and CKD rat models, and it might partially achieve this through the antiapoptotic and antioxidative response. Our findings indicate that AS-IV is a

promising drug for treating kidney injury.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at <https://tau.amegroups.com/article/view/10.21037/tau-22-505/rc>

Data Sharing Statement: Available at <https://tau.amegroups.com/article/view/10.21037/tau-22-505/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tau.amegroups.com/article/view/10.21037/tau-22-505/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. Animal experiments were performed under a project license (No. 2019001) granted by the ethics committee of Southern Medical University (People’s Hospital of Huadu District), in compliance with national guidelines for the care and use of animals.

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