Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Anwulignan Attenuates Remote Renal Injury Induced by Intestinal Ischemia/Reperfusion in Rats

Shu Jing, ChunRong Yu[1], ZiQi Song[2], HuiJiao Lin[3], Jia Wei Liu[3], Chunmei Wang[3], He Li[3], Dan Wang[2], Jianguang Chen[3]

Affiliated Hospital of Beihua University, Jilin City, Jilin, [1]Center for Drug Evaluation, NMPA, Beijing, [2]College of Basic Medicine, Beihua University, Jilin City, Jilin, [3]College of Pharmacy, Jilin City, Jilin, China

Submitted: 15-Dec-2021 Revised: 17-Feb-2022 Accepted: 01-Aug-2022 Published: 23-Nov-2022

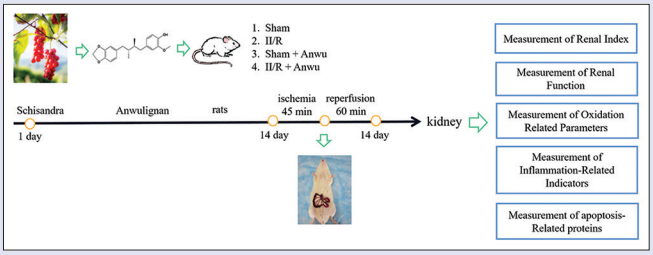
ABSTRACT

Background and Aim: Intestinal ischemia/reperfusion (II/R) can cause injury of remote organs, including acute renal injury. All of these, in turn, increase the rate of disability and mortality. Anwulignan (Anwu) is an active monomer from *Schisandra sphenanthera*, which has been used for thousands of years in China as a medical herb. In our previous study, we found that Anwu can improve the intestinal function after ischemia/reperfusion. Therefore, we were curious to know if Anwu can have a protective effect on remote organ injury after II/R. The purpose of the present study was to examine the effect of Anwu on the remote renal injury induced by II/R in rats and investigate its mechanism. **Materials and Methods:** Forty Sprague Dawley (SD) rats were divided into four groups, and they were sham operation (Sham), sham + Anwu, II/R, and II/R + Anwu groups. After reperfusion or sham operation, blood and kidneys were collected from the rats for the detection of relative biochemical parameters. **Results:** Renal indexes were not significantly different among all groups. In the II/R group the following were the findings: blood urea nitrogen and creatinine levels were increased, kidney injury score was increased, renal superoxide dismutase was reduced, the activities of reduced glutathione and catalase were decreased, the renal malondialdehyde content was increased, there was an improvement in the levels of renal proinflammatory cytokines, interleukin-6, tumor necrosis factor-α, and interleukin-1β, the expressions of renal oxidative stress-related p-Nrf2 and heme oxygenase (decycling) 1 (HO-1) were decreased, and the Kelch-like ECH-associated protein 1 (Keap1) expression was increased. The expression levels of the apoptotic protein, cleaved caspase-3, were raised, while the B-cell lymphoma 2 (Bcl-2)/Bcl-2-associated x protein (Bax) ratio was reduced. However, the administration of Anwu before the surgery significantly reversed the above changes. **Conclusion:** Anwu has a protective effect against the II/R-induced remote renal injury in rats, which may be related to its regulation of antioxidant, anti-inflammatory, and antiapoptotic pathways.

Key words: Antioxidant, Anwulignan (Anwu), intestinal injury ischemia/reperfusion, keap1/Nrf2/HO-1, remote renal injury

SUMMARY

- The fruit of Schisandra sphenanthera (Schisandra) is a traditional Chinese medicine, Which has anti-tumor, anti-fatigue, anti-aging, anti-gastric ulcer and other effects. Anwulignan (Anwu) is an active monomer from Schisandra sphenanthera.
- Forty Sprague Dawley(SD) rats were divided into four groups. The effect of Anwu on II/R rats was studied by the detection of kidney index, kidney function, antioxidant, anti-inflammatory and antiapoptotic related factors.
- In this study that Anwu could alleviate the renal injury after II/R in rats and reduce the level of BUN and CRE in the blood.
- Anwu has a protective effect against the remote renal injury after II/R in rats, which may be associated with its regulation of antioxidant, anti-inflammatory, and antiapoptotic pathways.



Abbreviations used:
AKI**:** acute kidney injury; Anwu: Anwulignan; Bax: Bcl-2-associated x protein; Bcl-2: B-cell lymphoma 2; BUN: blood urea nitrogen; CAT: catalase; CRE: creatinine; GSH: glutathione; HO-1: heme oxygenase (decycling) 1; II/R: intestinal ischemia/reperfusion; IL-1β: interleukin-1β; IL-6: interleukin-6; Keap1: Kelch-like ECH-associated protein 1; MDA: malondialdehyde; Nrf2: nuclear factor (erythroid-derived 2)-like 2; SMA: superior mesenteric artery; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-α.

Correspondence:

Dr. Dan Wang,
College of Basic Medicine, Beihua University, Jilin City, Jilin - 132013, China.
E-mail: wangdan626@126.com
DOI: 10.4103/pm.pm_578_21

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Intestinal ischemia is a surgical emergency that is common in patients with volvulus, enteritis, hypotension associated with trauma, and those undergoing an intestinal transplant. The restoration of blood flow to the ischemic bowel is one of the main purposes of therapy. However, it is this reperfusion that can also cause so-called intestinal ischemia/reperfusion (II/R) injury, which can result in high mortality of up to 80%.[1-3] Intestinal mucosa is rich in blood vessels and endothelial cells, and is prone to local and systemic inflammatory reactions. Even a short period of ischemia may activate a variety of inflammatory mediators, leading to the generation and release of oxygen free radicals, which not only aggravates local tissue or organ injury, but also induces the injury of remote organs and even leads to death.[4,5] Acute renal injury is a common remote organ injury following II/R and one of the main reasons for high mortality. Today, a few therapies have been proven to be effective in II/R-induced remote organ injuries.

Cite this article as: Jing S, Yu CR, Song ZQ, Lin HJ, Li JW, Wang C, *et al*. Anwulignan Attenuates Remote Renal Injury Induced by Intestinal Ischemia/Reperfusion in Rats. Phcog Mag 2022;18:1153-9.

The fruit of *Schisandra sphenanthera* Rehd. et Wils (*Schisandra*) has been used as a pharmaceutical and nutritional supplement in the USA, South Korea, and Japan for a long time.[6-8] Anwulignan is one of the monomer compositions in *Schisandra*.[9] Studies have reported that Anwu has antitumor, anti-fatigue, antiaging, anti-gastric ulcer, and anti-hepatic injury effects, indicating that it would be an ideal active ingredient for drug and health food development.[10-13] However, until now, there are not any relevant reports available on its effect on the kidney injury caused by II/R. It was found in our previous study that pretreatment with Anwu could significantly increase the mesenteric blood microcirculatory flow velocity, increase the intestinal motility, and alleviate the intestinal mucosal injury in rats with II/R, and the above effects were considered to be related to its strong antioxidation and anti-inflammation.[14] In addition, Anwu showed protective effect against mice renal injury initiated by d-Gal induced oxidative stress, suggesting that Anwu may offer potential protection against the acute kidney injury (AKI) caused by II/R.[15] Therefore, a kind of rat II/R model was established in this study to examine the effect of Anwu and explore the underlying mechanisms, which is expected to give a cue for the research and development of Anwu as a medicine and a food supplement in the treatment of II/R-induced remote organ injuries.

MATERIALS AND METHODS

Chemicals and reagents

Anwu (purity >99%) was obtained from Si chuan Weikeqi Biological Technology Co. Ltd, Chengdu, China. Blood urea nitrogen (BUN) and creatinine (CRE), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and hematoxylin–eosin (HE) staining and Hoechst cell apoptosis detection kits were obtained from Jiancheng Bioengineering Research Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were obtained from Enzyme-Linked biotechnology Co., Ltd. (Shanghai, China). N,N,N',N'-Tetramethylethylenediamine (TEMED), sodium dodecyl sulfate (SDS) sample loading buffer (5×), ammonium persulfate, HCl-Tris, glycine, 30% acrylamide, and BCA kit were from Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China), and all antibodies were obtained from ABclonal Biotech Co., Ltd. (Wuhan, China).

Animal handling

Male Sprague Dawley (SD) rats of age 9 ± 1 weeks and weight 230 ± 20 g from Yisi Laboratory Animal Technology Co.Ltd. (Changchun, China) were raised in an environment with a temperature of 20°C–22°C. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (China). All the animal experiments were approved by the Ethical Committee of Beihua University (No. 20200026).

SD rats were divided into Sham group (sodium carboxymethyl cellulose gavage and then sham operation), II/R group (sodium carboxymethyl cellulose gavage and then II/R), Sham + Anwu group (Anwu 7 mg/kg gavage and then sham operation), and II/R + Anwu group (Anwu 7 mg/kg gavage and then II/R). Rats in each group were administered sodium carboxymethyl cellulose or Anwu once daily by gavage before II/R or sham operation, continuously for 14 days. All rats were anesthetized by intraperitoneally injecting pentobarbital sodium (50 mg/kg) and then operated.[14] Briefly, a rat II/R model was reproduced by isolating the rats' superior mesenteric artery, clamping the artery for 45 min, and removing the clamp for 1 h, while in the Sham group, the superior mesenteric artery was isolated only, with the same time course. After the above procedure, the blood was collected via the abdominal aorta, and rats were sacrificed by injecting intraperitoneally pentobarbital sodium (200 mg/kg). Then, the kidneys were taken for histological and biological examinations.

Measurement of renal index

Renal index was calculated using the formula:

renal index: kidney weight (g)/body weight (g) × 100%.

Measurement of renal function

BUN and CRE levels in the blood of rats were measured by an automatic biochemistry analyzer.

Histopathologic observation of the renal tissue

With the standard protocol of HE staining, 2 μm paraffin-embedded kidney sections were prepared and the sections were observed. Paller scoring was performed by two professional pathologists for histopathologic evaluation.[16] In brief, by 400 magnification, 10 consecutive fields at the renal cortical medullary junction and 10 renal tubules for each field were randomly selected, and then the scores on these 100 renal tubules were calculated. The scoring criteria were as follows: morphological change of renal tubular epithelial cells was scored as 1 point, brush border damage as 1 point, drop necrotic cells (not tubular cast or cell fragments) in the lumen of renal tubules as 1 point, obvious deformation and widening of cast as 1 point, drop of brush border as 2 points, and cellular cast as 2 points.[17]

Measurement of oxidation-related parameters

The rat renal tissue was homogenized for the preparation of renal tissue, and the supernatant of homogenate was obtained by centrifuging at 4°C and 3500 g for 10 min. Oxidation-related parameters (MDA, CAT, SOD, and GSH) were determined using the methods provided by the kit manufacturers.

Western blot analysis

The proteins in the renal tissue were extracted with the method provided by the kit manufacturer, and BCA protein assay was used to determine the protein concentration in the renal tissue. Then, 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis was performed on 30 μg of the protein from each sample at 100 V for 1.5 h, and then the protein transfer onto polyvinylidene fluoride (PVDF) membranes was carried out at 200 mA for 1.5 h. Then, the membranes were incubated with rabbit anti-mouse antibodies at 4°C overnight, in which the following primary antibody dilutions (1:1000) were used: p-Nrf2 (cat: AP1133), cleaved caspase-3 (cat: A0214), Bcl-2-associated x protein (Bax; cat: A0207), B-cell lymphoma 2 (Bcl-2; cat: A0208), nuclear factor (erythroid-derived 2)-like 2 (Nrf2; cat: A0674), heme oxygenase (decycling) 1 (HO-1; cat: A1346), and Kelch-like ECH-associated protein 1 (Keap1; cat: A1820).

Immunohistochemistry

Cleaved caspase-3 was used for the immunohistochemical staining of paraffin sections of the kidney tissue. Then, the sections were routinely pathologically prepared, that is, dewaxing, dehydration with ethanol gradient, and antigen retrieval with citrate antigen retrieval solution. The sections were incubated with cleaved caspase-3 antibody dilutions (1:100) at 4°C overnight and then blocked with the blocking buffer and incubated with the second antibody dilution (1:250) at 25°C for 30 min. 3,3′-Diaminobenzidine was used as the chromogenic agent, and hematoxylin as the stain. Finally, the sections were observed and the cumulative optical density was measured.

Measurement of inflammation-related indicators

Renal inflammation-related indicators (TNF-α, IL-6, and IL-1β) were measured according to the instructions given in the kit.

Statistical analysis

Mean ± standard deviation (SD) was used to represent the data, and GraphPad Prism 7.0 statistical software was used to analyze the data. Differences among the groups were analyzed by two-way analysis of variance (ANOVA), followed by Tukey *post hoc* test for renal function-related data and the least significant difference (LSD) *post hoc* test for other data, in which a value of $P < 0.05$ indicated a statistically significant difference.

RESULTS

Anwu improved renal pathological damages

Renal indexes and pathological damages were observed in this study. As shown in Figure 1a, the changes in the renal indexes within all groups were not significant ($P > 0.05$). As shown in Figure 1c, under a 200 × light microscope, the renal structures in the Sham group were intact and the renal tubules were arranged neatly, without either obvious necrosis of renal tubular epithelial cells or protein cast. In the II/R group, the renal tubules were unclear in structure with epithelial cell edema and obvious nuclear abscission (black arrow), and some renal tubules were narrowed or even occluded. Anwu significantly reduced tubular injury, with most tubular epithelial cells settled neatly (black star). As shown in Figure 1b, in comparison to Sham group, the Paller score was increased in the II/R group ($P < 0.01$), and in comparison to the II/R group, the Paller score was decreased in the II/R + Anwu group ($P < 0.01$). Therefore, we concluded that Anwu could protect against renal tissue injury, as shown in Figure 1.

Anwu improved the renal function

CRE and BUN are vital indicators of the severity of renal injury.[18,19] In comparison to the Sham group, the II/R group showed significantly elevated CRE and BUN levels ($P < 0.01$), while their levels decreased significantly in the II/R + Anwu group in comparison with the II/R group ($P < 0.05$ and $P < 0.01$, respectively) [Figure 2], suggesting that Anwu could attenuate the renal damage induced by II/R.

Anwu changed oxidation-related parameters in the renal tissue

SOD, CAT, and GSH are key antioxidants that play an important role in resisting the injuries caused by reactive oxygen species (ROS). MDA is the product of lipid peroxidation; its content marks the degree of an oxidative damage.[20,21] The results shown in Figure 3 indicate that in comparison to the Sham group, the renal SOD, CAT, and GSH activities decreased significantly in rats treated with II/R, while the MDA content increased significantly ($P < 0.05$ and $P < 0.01$, respectively). However, compared to the II/R group, these indicators were obviously reversed in the II/R + Anwu group ($P < 0.05$ and $P < 0.01$, respectively), suggesting that the antioxidant activity effect induced by Anwu might play an important role in the protective effect against renal injury.

Anwu decreased inflammation-related indicator contents in the renal tissue

IL-1β, IL-6, and TNF-α are the proinflammatory cytokines responsible for harmful stimuli such as II/R and oxidative stress.[22-24] Therefore, the level of these cytokines can indicate the tendency of oxidation and antioxidation balance. TNF-α, IL-6, and IL-1β levels in the renal tissue

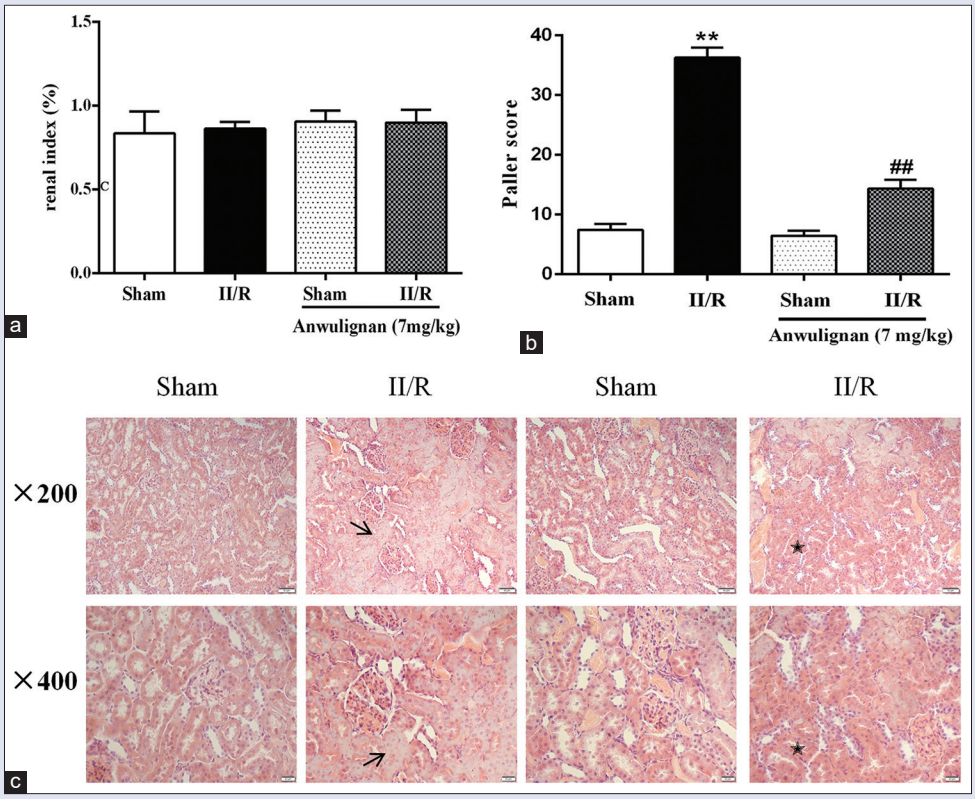


Figure 1: Effects of Anwulignan on the histopathologic changes of renal tissue in rats with II/R: (a) renal index; (b) Paller scores; (c) HE staining of renal tissue. Data are expressed as means ± SD, $n = 8$. $^{**}P < 0.01$ compared with the Sham group; $^{\#\#}P < 0.01$ compared with the II/R group. HE = hematoxylin–eosin, II/R = intestinal ischemia/reperfusion, SD = standard deviation

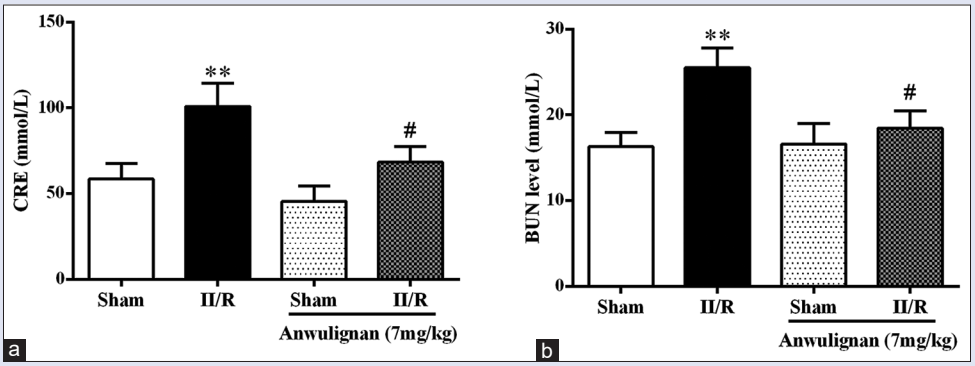


Figure 2: Effect of Anwulignan on serum CRE and BUN in rats with II/R: (a) serum CRE level; (b) serum BUN level. Data are expressed as means ± SD, $n = 8$. $^{**}P < 0.01$ compared with the Sham group; $^{\#}P < 0.05$ compared with the II/R group. BUN = blood urea nitrogen, CRE = creatinine, II/R = intestinal ischemia/reperfusion, SD = standard deviation

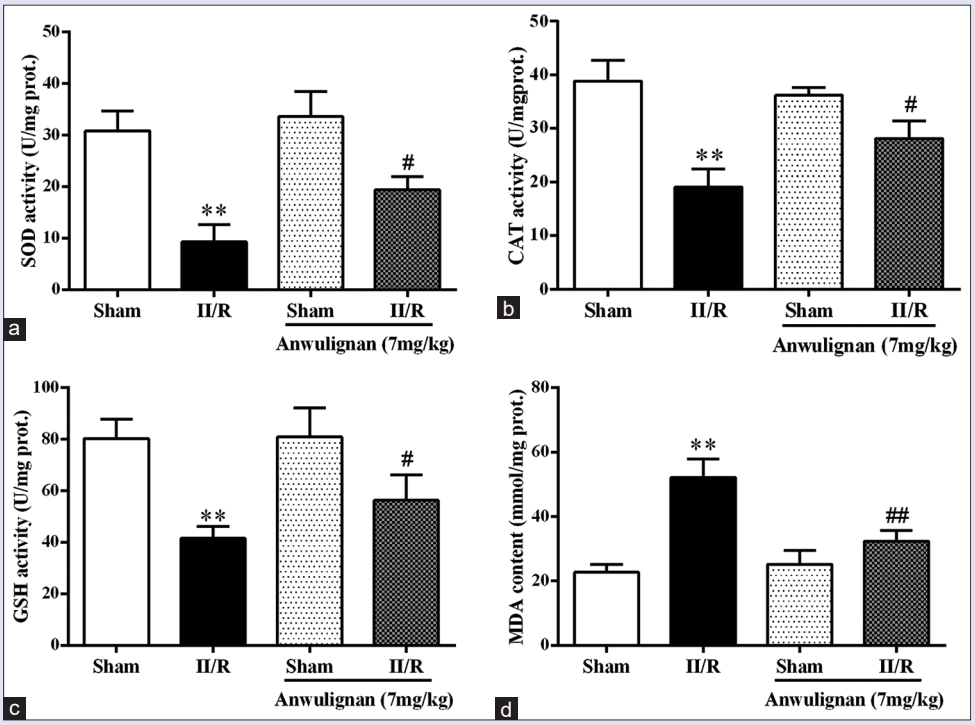


Figure 3: Effect of Anwulignan on the content and activities of SOD, CAT, GSH, and MDA in the renal tissue of rats: (a) SOD; (b) CAT; (c) GSH; (d) MDA. Data are expressed as means ± SD, $n = 8$. $^{**}P < 0.01$ compared with the Sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the II/R group. CAT = catalase, GSH = reduced glutathione, II/R = intestinal ischemia/reperfusion, MDA = malondialdehyde, SD = standard deviation, SOD = superoxide dismutase

increased significantly in the II/R-treated group in comparison to the Sham group; but in comparison to the II/R group, the levels of these cytokines decreased significantly in II/R + Anwu group ($P < 0.05$ and $P < 0.01$, respectively) [Figure 4], suggesting that Anwu might attenuate the renal damage partly through anti-inflammation.

Anwu regulated Keap1/Nrf2/HO-1 pathway

It is well known that the Keap1/Nrf2/HO-1 pathway plays a regulatory role in cellular redox balance in the body.[25-27] As shown in Figure 5, the expression level of Keap1 increased while the p-Nrf2/Nrf2 ratio and HO-1 decreased in the II/R group in comparison to the Sham group, but their expressions in the II/R + Anwu group showed the opposite trend in comparison to the II/R group ($P < 0.05$ and $P < 0.01$, respectively), suggesting that Anwu might improve the remote renal injury induced by II/R, by regulating the Keap1/Nrf2/HO-1 signaling pathway.

Anwu suppressed cell apoptosis in the renal tissue

In the study, we assessed typical apoptosis marker cleaved caspase-3 and other apoptosis-related proteins, including Bax and Bcl-2. As shown in Figure 6, the expression level of cleaved caspase-3 increased and Bcl-2/Bax ratio decreased significantly in the II/R group in comparison to the Sham group ($P < 0.05$ and $P < 0.01$, respectively); but in comparison to the II/R group, the expression level of cleaved caspase-3 decreased and the ratio of Bcl-2/Bax increased significantly in the II/R + Anwu group ($P < 0.05$ and $P < 0.01$, respectively), indicating that Anwu might alleviate the apoptosis of renal tissue.

DISCUSSION

The gut–kidney axis in many pathological conditions has attracted researchers' eyes recently.[28,29] Being a characteristic manifestation of remote organ injuries after II/R, AKI has been reported several times.[30] In the present study, a rat II/R injury model was reproduced successfully,[14,30] and consistent with the previous reports, the serum CRE and BUN levels decreased significantly and the pathological HE staining showed a significant renal tubular injury in the II/R group. It was found in this study that Anwu could alleviate the renal injury after II/R in rats and lower the level of BUN and CRE in the blood of rats, suggesting that Anwu could protect against the remote renal injury after II/R.

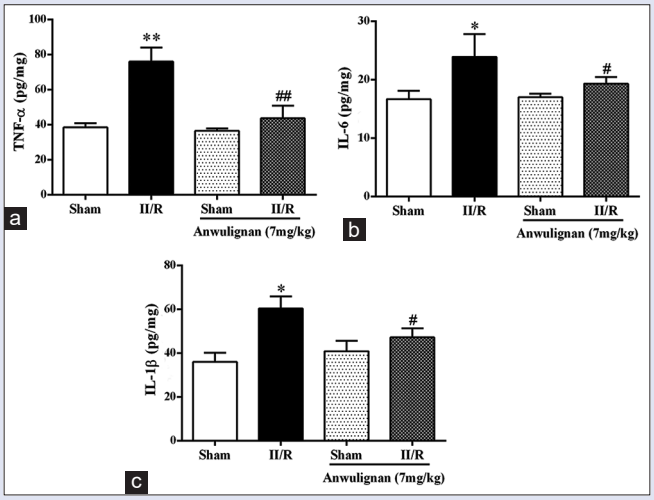


Figure 4: Effect of Anwulignan on the content of TNF-α, IL-6, and IL-1β in the renal tissue of rats: (a) TNF-α; (b) IL-6; (c) IL-1β. Data are expressed as means ± SD, $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the Sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the II/R group. II/R = intestinal ischemia/reperfusion, IL = interleukin, SD = standard deviation, TNF-α = tumor necrosis factor-α

Oxidative stress participates in the pathophysiological process of the remote renal injury caused by II/R. This is because the oxygen supply after reperfusion promotes the excessive production of ROS, and the amount of free radicals exceeds the scavenging capacity of antioxidant enzymes in the body, thus causing damage to cells.[31] Free radicals, catalyzed by SOD, are transformed into oxygen and hydrogen peroxide, and then the latter two, catalyzed by CAT and Glutathione peroxidase (GSH-Px), are converted into molecular oxygen and water. So, these three enzymes contribute to the body's antioxidant capacity to protect against excessive oxidative stress. MDA is a kind of lipid peroxide formed by the peroxidation reaction of lipids due to free radicals. MDA can cause polymerization of nucleic acid, protein, and other molecules, which is cytotoxic and affects the metabolic function of cells.[32] These four indexes were measured in this experiment to determine whether Anwu has an antioxidant effect. The results of this study showed that Anwu increased the activities of GSH, SOD, and CAT, but decreased the content of MDA, suggesting that Anwu may have an antioxidant effect in II/R. The Keap1/Nrf2/HO-1 pathway is involved in the process of redox.[33] In physiological conditions, Nrf2 (a basic leucine zipper transcription factor) can bind to Keap1 to sequester Nrf2 in the cytoplasm, and Nrf2 can defensively respond to extrinsic and intrinsic

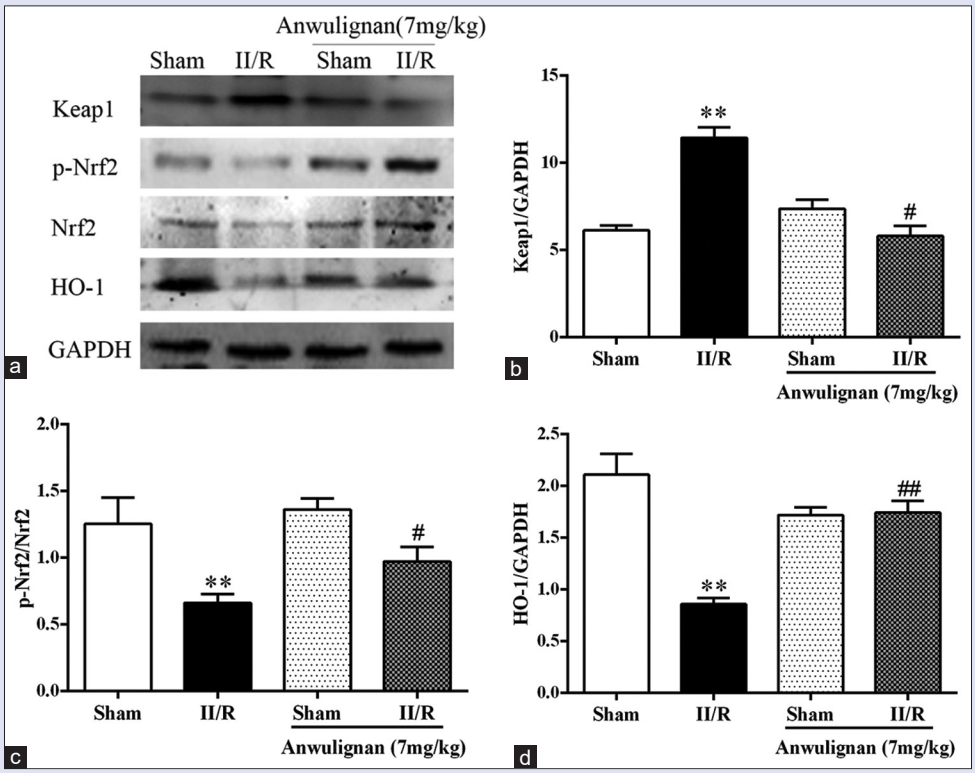


Figure 5: Effects of Anwulignan on the Keap1/Nrf2/HO-1 signaling pathway: (a) electrophoretogram of Nrf2, p-Nrf2, Keap1, and HO-1 proteins by western blot; (b) relative expression of Keap1; (c) relative expression of p-Nrf2; (d) relative expression of HO-1. Data are expressed as means ± SD, $n = 8$. $^{**}P < 0.01$ compared with the Sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the II/R group. HO-1 = heme oxygenase (decycling) 1, II/R = intestinal ischemia/reperfusion, Keap1 = Kelch-like ECH-associated protein 1, Nrf2 = nuclear factor (erythroid-derived 2)-like 2, SD = standard deviation

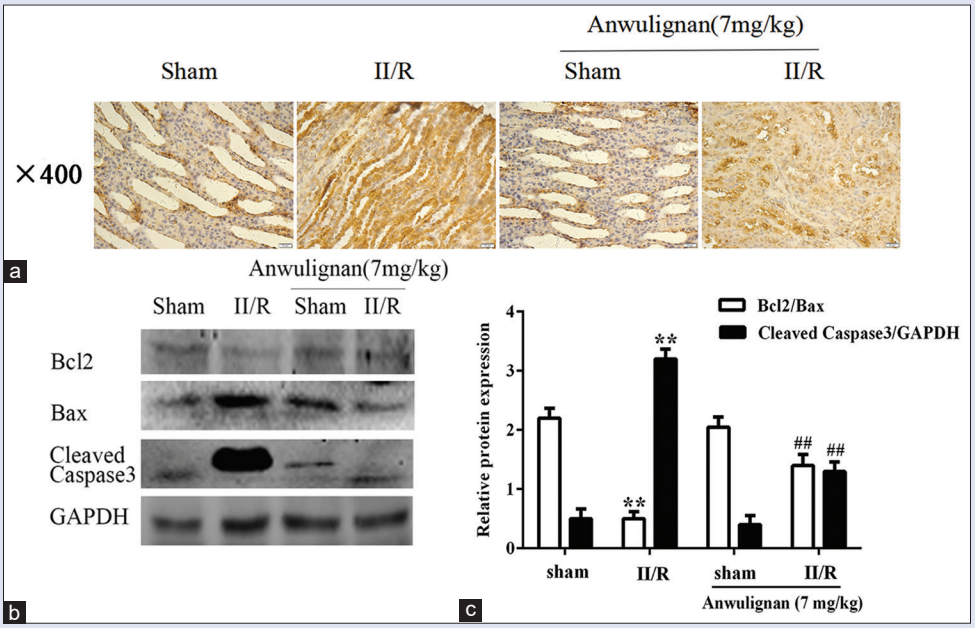


Figure 6: Effect of Anwulignan on the protein expressions of cleaved caspase-3, Bcl-2, and Bax: (a) expression of cleaved caspase-3 by immunohistochemistry; (b) electrophoretogram of cleaved caspase-3, Bax, and Bcl-2; (c) relative expressions of Bax/Bcl-2 and cleaved caspase-3. Data are expressed as means ± SD, $n = 8$. $^{**}P < 0.01$ compared with the Sham group, $^{\#}P < 0.05$ $^{\#\#}P < 0.01$ compared with the II/R group. Bax = Bcl-2-associated x protein, Bcl-2 = B-cell lymphoma 2, II/R = intestinal ischemia/reperfusion, SD = standard deviation

stressors in the body.[34] Nrf2 may be separated from Keap1 and being phosphorylated once the cells are suffering from oxidative stress. SOD, CAT, and HO-1 are regulated by Nrf2. Therefore, Nrf2, together with these enzymes, defends against the cytotoxic effect of oxidative stress. It was found in this study that Anwu could decrease the expression of Keap1, but increase the expression of p-Nrf2 and HO-1 in the renal tissue of rats. In our previous study, we found that Anwu had similar effects on the ischemia–reperfused intestinal tissue in rats and the liver, brain, and spleen in d-galactose–treated rat,[12-14,35] indicating that Anwu can attenuate the renal injury after II/R via the Keap1/Nrf2/HO-1 pathway and this activation may be the common mechanism of the antioxidant effect of Anwu.

In addition, ischemia/reperfusion can promote inflammation, which in turn exacerbates the damage of organs. Inflammatory mediators are not only the key link of ischemia/reperfusion, but also the most important injury-causing factor.[36] The results revealed that II/R increased the levels of IL-6, IL-1β, and TNF-α in the renal tissue in rats and Anwu could reduce the content of these inflammatory factors, indicating that Anwu may play an anti-inflammatory role in II/R.

Intestinal ischemia, hypoxia, and local inflammation can activate the expression of apoptotic genes. After reperfusion, excessive ROS is produced throughout the body and accumulated in renal tubular epithelial cells, resulting in lipid peroxidation of the cell membrane, DNA destruction, and other damages and also inducing cell apoptosis.[33] Cleaved caspase-3 is a very important proteolytic enzyme in cell apoptosis, which plays a core role in many pathways of apoptosis signal transduction and is also the last effector of all apoptotic pathways.[37] Being very important apoptotic regulators, Bcl-2 and Bax are often used to evaluate the extent of apoptosis. As Bcl-2 is an antiapoptotic regulator and Bax is a proapoptotic regulator, the ratio of Bcl-2/Bax can reflect the level of cell apoptosis.[38] Anwu decreased the expression of cleaved caspase-3 and increased the ratio of Bcl-2/Bax in II/R rats in this study, suggesting that Anwu might inhibit cell apoptosis in the renal tissue in II/R rats. However, the duration of these signals is not clear in the present study, and therefore, more apoptotic indicators need to be further explored.

CONCLUSION

In conclusion, this study shows that Anwu improves the remote renal injury after II/R in rats, which may be associated with its regulation of antioxidant, anti-inflammatory, and antiapoptotic pathways.

In summary, Anwu has a protective effect against the II/R-induced remote renal injury in rats.

Acknowledgements

We wish to thank Professor Chengyi Zhang from the College of Pharmacy for the critical comments on the manuscript and Xintian Fan for his English editing.

Financial support and sponsorship

This research was funded by Jilin Provincial Department of Science and Technology (grant no. 20200201521JC), Jilin Provincial Health and Family Planning Commission (grant no. 2021JC084), Jilin Provincial Department of Education (grant no. JJKH20210065KJ), Jilin City Science and Technology Bureau (20200404054), and innovation and entrepreneurship training program for college students in Jilin Province (202110201020).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Yang S, Guo J, Ni Q, Chen J, Guo X, Xue G, *et al.* Enteral nutrition improves clinical outcome and reduces costs of acute mesenteric is chaemia after recanalisation in the intensive care unit. Clin Nutr 2019;38:398-406.
2. Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of is chemia-reperfusion-induced intestinal injury: Progress and promise for translational research. Am J Physiol Gastrointest

Liver Physiol 2015;308:G63-75.

3. Mamode N, Pickford I, Leiberman P. Failure to improve outcome in acute mesenteric ischaemia: Seven-year review. Eur J Surg 1999;165:203-8.
4. Hu S, Du MH, Luo HM, Wang H, Lv Y, Ma L, *et al.* Electroacupuncture at zusanli (ST36) prevents intestinal barrier and remote organ dysfunction following gut ischemia through activating the cholinergic anti-inflammatory-dependent mechanism. Evid Based Complement Alternat Med 2013;2013:592127.
5. Arda-Pirincci P, Bolkent S. The role of epidermal growth factor in prevention of oxidative injury and apoptosis induced by intestinal ischemia/reperfusion in rats. Acta Histochem 2014;116:167-75.
6. Szopa A, Ekiert R, Ekiert H. Current knowledge of Schisandra chinensis (Turcz.) baill. (Chinese magnolia vine) as a medicinal plant species: A review on the bioactive components, pharmacological properties, analytical and biotechnological studies. Phytochem Rev 2017;16:195-218.
7. Nowak A, Zakłos-Szyda M, Błasiak J, Nowak A, Zhang Z, Zhang B. Potential of Schisandra chinensis (turcz.) baill. In human health and nutrition: A review of current knowledge and therapeutic perspectives. Nutrients 2019;11:333.
8. Panossian A, Wikman G. Pharmacology of Schisandra chinensis Bail.: An overview of Russian research and uses in medicine. J Ethnopharmacol 2008;118:183-212.
9. Li Z, He X, Liu F, Wang J, Feng J. Areview of polysaccharides from Schisandra chinensis and *Schisandra sphenanthera*: Properties, functions and applications. Carbohydr Polym 2018;184:178-90.
10. Zhang X, Jing S, Lin H, Sun W, Jiang W, Yu C, *et al.* Anti-fatigue effect of anwulignan via the NRF2 and PGC-1α signaling pathway in mice. Food Funct 2019;10:7755-66.
11. Xie X, Wang X, Shi X, Zhang Y, Laster KV, Liu K, *et al.* Anwulignan is a novel JAK1 inhibitor that suppresses non-small cell lung cancer growth. J Cell Mol Med 2021;25:2645-54.
12. Gao J, Yu Z, Jing S, Jiang W, Liu C, Yu C, *et al.* Protective effect of Anwulignan against D-galactose-induced hepatic injury through activating p38 MAPK-Nrf2-HO-1 pathway in mice. Clin Interv Aging 2018;13:1859-69.
13. Li X, Gao J, Yu Z, Jiang W, Sun W, Yu C, *et al.* Regulatory effect of Anwulignan on the immune function through its antioxidation and anti-apoptosis in D-galactose-induced aging mice. Clin Interv Aging 2020;15:97-110.
14. Lin H, Zhang X, Wang D, Liu J, Yuan L, Liu J, *et al.* Anwulignan ameliorates the intestinal ischemia/reperfusion. J Pharmacol Exp Ther 2021;378:222-34.
15. Liu C, Lin H, Jiawei L, Wang Y, Wang C, Sun J, *et al.* Anwulignan alleviates d-galactose induced renal damage by regulating Nrf2/ARE signaling pathway in mice. Food Sci Biotechnol 2021;30:1097-105.
16. Brunner PM, Silverberg JI, Guttman-Yassky E, Paller AS, Kabashima K, Amagai M, *et al.* Councilors of the International Eczema Council. Increasing comorbidities suggest that atopic dermatitis is a systemic disorder. J Invest Dermatol 2017;137:18-25.
17. Paller A, Jaworski JC, Simpson EL, Boguniewicz M, Russell JJ, Block JK, *et al.* Major comorbidities of atopic dermatitis: Beyond allergic disorders. Am J Clin Dermatol 2018;19:821-38.
18. Li MZ, Zhao Y, Wang HR, Talukder M, Li JL. Lycopene preventing DEHP-induced renal cell damage is targeted by aryl hydrocarbon receptor. J Agric Food Chem 2021;69:12853-61.
19. Xie W, Huang YY, Chen HG, Zhou X. Study on the efficacy and mechanism of Lycium barbarum polysaccharide against lead-induced renal injury in mice. Nutrients 2021;13:2072.
20. Guo H, Yin H, Zuo Z, Yang Z, Yang Y, Wei L, *et al.* Oxidative stress-mediated apoptosis and autophagy involved in Ni-induced nephrotoxicity in the mice. Ecotoxicol Environ Saf 2021;228:112954.
21. Erdemli Z, Erdemli ME, Gul M, Altinoz E, Gul S, Kocaman G, *et al.* Ameliorative effects of crocin on the inflammation and oxidative stress-induced kidney damages by experimental periodontitis in rat. Iran J Basic Med Sci 2021;24:825-32.
22. Un H, Ugan RA, Gurbuz MA, Bayir Y, Kahramanlar A, Kaya G, *et al.* Phloretin and phloridzin guard against cisplatin-induced nephrotoxicity in mice through inhibiting oxidative stress and inflammation. Life Sci 2021;266:118869.
23. Inan M, Bakar E, Cerkezkayabekir A, Sanal F, Ulucam E, Subaşı C, *et al.* Mesenchymal stem cells increase antioxidant capacity in intestinal ischemia/reperfusion damage. J Pediatr Surg 2017;52:1196-206.
24. Farmer DG, Shen XD, Amersi F, Anselmo D, Ma JP, Ke B, *et al.* CD62 blockade with P-selectin glycoprotein ligand-immunoglobulin fusion protein reduces ischemia-reperfusion injury after rat intestinal transplantation. Transplantation 2005;79:44-51.
25. Shin JW, Chun KS, Kim DH, Kim SJ, Kim SH, Cho NC, *et al.* Curcumin induces stabilization of Nrf2 protein through Keap1 cysteine modification. Biochem Pharmacol 2020;173:113820.
26. Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. Free Radic Biol Med 2009;47:1304-9.
27. Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 2004;10:549-57.
28. Zhang J, Ankawi G, Sun J, Digvijay K, Yin Y, Rosner MH, *et al.* Gut-kidney crosstalk in septic acute kidney injury. Crit Care 2018;22:117.
29. Peerapornratana S, Manrique-Caballero CL, Gómez H, Kellum JA. Acute kidneyinjury from sepsis: Current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int 2019;96:1083-99.
30. Lai HJ, Zhan YQ, Qiu YX, Ling YH, Zhang XY, Chang ZN, *et al.* HMGB1 signaling-regulated endoplasmic reticulum stress mediates intestinal ischemia/reperfusion-induced acute renal damage. Surgery 2021;170:239-48.
31. Kiliç K, Hanci V, Selek S, Sözmen M, Kiliç N, Citil M, *et al.* The effects of dexmedetomidine on mesenteric arterial occlusion-associated gut ischemia and reperfusion-induced gut and kidney injury in rabbits. J Surg Res 2012;178:223-32.
32. Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, *et al.* Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. J Physiol Pharmacol 2014;65:613-22.
33. Gendy A, Soubh A, Al-Mokaddem A, Kotb El-Sayed M. Dimethyl fumarate protects against intestinal is chemia/reperfusion lesion: Participation of Nrf2/HO-1, GSK-3 β and Wnt/β -catenin pathway. Biomed Pharmacother 2021;134:111130.
34. Marques GM, Rasslan R, Belon AR, Carvalho JG, Felice Neto R, Rasslan S, *et al.* Pentoxifylline associated to hypertonic saline solution attenuates inflammatory process and apoptosis after intestinal ischemia/reperfusion in rats. Acta Cir Bras 2014;29:735-41.
35. Yu Z, Jing S, Gao J, Liu C, Li N, Yu C, *et al.* Anwulignan improves d -galactose-induced learning and memory impairment via regulating P38 MAPK-Nrf2-HO-1 pathway in mice. Nat Prod Commun 2019;14. doi: 10.1177/1934578X19846316.
36. Lo HC, Yang RB, Lee CH. Guanylyl cyclase-G modulates jejunal apoptosis and inflammation in mice with intestinal ischemia and reperfusion. PLoS One 2014;9:e101314.
37. Chen R, Zhang YY, Lan JN, Liu HM, Li W, Wu Y, *et al.* Ischemic postconditioning alleviates intestinal ischemia-reperfusion injury by enhancing autophagy and suppressing oxidative stress through the Akt/GSK-3 β/Nrf2 Pathway in mice. Oxid Med Cell Longev 2020;2020:6954764.
38. Yoo ES, Choo GS, Kim SH, Woo JS, Kim HJ, Park YS, *et al.* Antitumor and apoptosis-inducing effects of piperine on human melanoma cells. Anticancer Res 2019;39:1883-92.