ORIGINAL ARTICLE

Evaluation of the methanol extract of BaiYangJie: Toxicology and protective effect against acute kidney injury

Jiaxin Li[1,2], Lingjuan Zhou[3], Jinhui Wang[2,4], Lixia Zhang[2,4], Bin Xia[1,2], Guang Li[2,4], Jiahui Ren[2,4], Jiacheng Li[5]

[1]College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin, [2]Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Yunnan Branch, [3]The ICU of People's Hospital of Xishuangbanna Dai Autonomous Prefecture, [4]Yunnan Key Laboratory of Southern Medicine Utilization, Jinghong, [5]College of Integrated Traditional Chinese and Western Clinical, Beijing University of Chinese Medicine DongFang College, Cangzhou, China

J. Adv. Pharm. Technol. Res.

ABSTRACT

BaiYangJie (BYJ) is a terrestrial perennial plant commonly used as a Dai medicine and has therapeutic effects on liver and kidney diseases. Cisplatin (CP), a chemotherapy drug, has good therapeutic effects but causes many side effects, including nephrotoxicity. This article investigated the toxicology of the methanol extract of BYJ (ME-BYJ) and its protective effect on CP-induced acute kidney injury (AKI) through pharmacological experiments. The results showed that the treated mice had no toxicological symptoms and no anatomical, physiological, or histological abnormalities. The BYJ-high-dose group showed significantly attenuated CP-induced AKI. It is concluded that ME-BYJ has the most significant protective effect on AKI at a dose of 8 g/kg and BYJ was not toxic.

Key words: Acute kidney injury, BaiYangJie, cisplatin, toxicology

INTRODUCTION

Acute kidney injury (AKI) is a common clinical disease characterized by acute and transient renal dysfunction. The incidence of AKI is increasing rapidly to epidemic proportions. According to the results of a joint survey conducted in multiple locations, including China, the incidence of AKI accounts for 3.2%–9.6% of ordinary inpatients, 14.8% of elderly inpatients, and 22% of intensive care unit patients. It has been estimated that 2 million people worldwide die from AKI every year.[1-3] Among them, AKI caused by cisplatin (CP) as a chemotherapy drug is more common in the process of disease treatment. Although CP has significant therapeutic effects, it induces several side effects, such as ototoxicity, nephrotoxicity, myelosuppression, and anaphylaxis.[4] Nephrotoxicity is one of the most common adverse reactions after chemotherapy. Therefore, searching for drugs that can reduce the toxic side effects of CP and inhibit the occurrence of AKI has become a focus of research.

There are many medicinal herbs in the Dai nationality that can detoxify, such as *Arundina graminifolia* is called Wenshanghai, which means "the medicine to eliminate hundreds of poisons," so it is also called BaiYangJie (BYJ), which is characterized by its abilities to "relieve the effects of poison before sickness results and treat illness."[5,6]

Address for correspondence:
Dr. Guang Li,
138 Xuanwei Avenue, Jinghong, Xishuangbanna Dai Autonomous Prefecture, Yunnan, China.
E-mail: lhbg311@hotmail.com

Submitted: 12-Oct-2023 **Revised:** 14-Apr-2024
Accepted: 02-May-2024 **Published:** 22-Jul-2024

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/JAPTR
	DOI: 10.4103/JAPTR.JAPTR_467_23

How to cite this article: Li J, Zhou L, Wang J, Zhang L, Xia B, Li G, *et al*. Evaluation of the methanol extract of BaiYangJie: Toxicology and protective effect against acute kidney injury. J Adv Pharm Technol Res 2024;15:185-93.

Clinically, it is mainly used to treat liver and kidney diseases, and for detoxification.[7] However, there were no reports about BYJ to treat AKI by CP induced. Therefore, this article explores the toxicology and protective effects against AKI of the methanol extract of BYJ (ME-BYJ), providing a scientific basis for exploring the development of new drugs for CP-induced kidney injury and the clinical efficacy of Dai medicine.

MATERIALS AND METHODS

Drug

BYJ was collected from Xishuangbanna Dai Autonomous Prefecture, Yunnan Province, and identified as a BYJ by researcher Li Guang.

Animals

KM mice (40 female mice and 40 male mice) weighing 16–18 g were purchased from SPF (BEIJING) BIOTECHNOLOGY CO., LTD. Mice were kept in the Animal Research Center of Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. License key: SYXK（滇）K2018-0003. Animal Ethical Clearance no 20220901014. The study was approved by the Institutional Ethical Committee on Animal Care.

Preparation of methanol extract of BaiYangJie

BYJ powder (200.0 g) was soaked in 10 volumes of methanol solvent and ultrasonicated twice, and the two filtrates were combined. The filtrate was evaporated and dried, and the yield was calculated (8.56%).

Identification of the chemical constituents of the methanol extract of BaiYangJie

BYJ powder (1.0 g) was placed into a 50 mL conical flask with 10 mL of methanol solvent, ultrasonicated for 30 min, centrifuged for 5 min at 10,000 *r*/min, filtered through a 0.22 μM microporous membrane, and placed into a sample bottle.

High-performance liquid chromatography-mass spectrometry

High-performance liquid chromatography conditions

ACQUITY UPLC HSS T3 chromatographic column (100 mm × 2.1 mm, 1.8 μm), column temperature 40°C, and injection volume was 5 μL. The detection time was 20 min. The specific gradient elution procedure is shown in Table 1.

Mass spectrometry conditions

Adopting positive and negative ion detection modes, the primary mass spectrometry (MS) scanning range was 100~1200 m/z, Collision Energy was 10 ± 0V and the secondary MS scanning range was 50~1200 m/z, Collision Energy was 40 ± 20 V.

Table 1: Gradient elution procedure

Time (min)	Flow rate ($mL \cdot min^{-1}$)	A (0.1% formic acid-water, %)	B (0.1% formic acid-acetonitrile, %)
0~2	0.3	95	5
6~7	0.3	70	30
10~11.5	0.3	40	60
13~13.5	0.3	20	80
16~17	0.3	10	90
17.5~20	0.3	0	100
20.5~23	0.3	95	5

Acute toxicity test

Twenty mice were used to evaluate the acute toxicity of BYJ. The mice were divided into two groups (half male and half female), with 10 in each group. On the 1st day of the experiment, the control group were given sodium carboxymethyl cellulose (CMC, Intragastrica, i.g) and treatment group were given the maximum dose of ME-BYJ (5.5 g dissolved in 40 mL of CMC, i.g) three times, 0.6 mL each time, with an interval of 6 h. The mice's appearance and behavior were observed and weighed once every 2 days. On the 14th day, organs such as the heart, liver, and lungs were taken and weighed, and the organ coefficients were calculated. The organs hematoxylin-eosin (H and E) stains were performed and the LD50 was calculated.

Pharmacodynamic study

CP with a dose of 13 mg/kg was intraperitoneally injected (i.p.) into mice to induce nephrotoxicity. Divided 60 mice into six groups, with 5 females and 5 males in each group. Groups I, II were given CMC for 10 days (i.g) and they were given physiological saline as Control group and CP as model group on the 7th day (i.p.). Groups III, IV, and V were given ME-BYJ for 10 days (2 g/kg, 4 g/kg, and 8 g/kg, i.g), they were given CP (i.p.) as groups low-dose group (BYJ-L), medium-dose group (BYJ-M), and high-dose group (BYJ-H) on the 7th day. Group VI was given ME-BYJ (4 g/kg) for 10 days (i.g) and received physiological saline (i.p.) on the 7th day as Control + BYJ group. The body weight was measured every day. On the 10th day, 2 h after administration, serum samples were collected to detect serum creatinine (CREA), blood urea nitrogen (BUN), and serum uric acid (URIC). The kidneys were weighed and photographed, and the relative kidney weights were calculated. The kidneys H and E, Masson stains were performed and superoxide dismutase (SOD), glutathione peroxidase(GSH-Px), and catalase (CAT)levels were measured.

Statistical analysis

Multiple groups were compared using one-way analysis of variance, and *t*-tests were used to compare the two groups. When $P < 0.05$, it was considered significant.

RESULTS

Chemical composition analysis and identification of methanol extract of BaiYangJie

Chemical composition analysis of methanol extract of BaiYangJie

The chemical composition analysis of ME-BYJ showed that BYJ contained flavonoids, terpenoids, phenols, alkaloids, coumarins, stilbenes, glycosides, tannins, and other substances [Figure 1a and b]. Some substances had certain anti-inflammatory and antioxidant activities, such as flavonoids and terpenoids.

Chemical composition identification of methanol extract of BaiYangJie

The identification of compounds took kaempferol and kaempferol-3-O-glucoside as examples, and the specific fragmentation pathway is shown in Figure 2a and b.

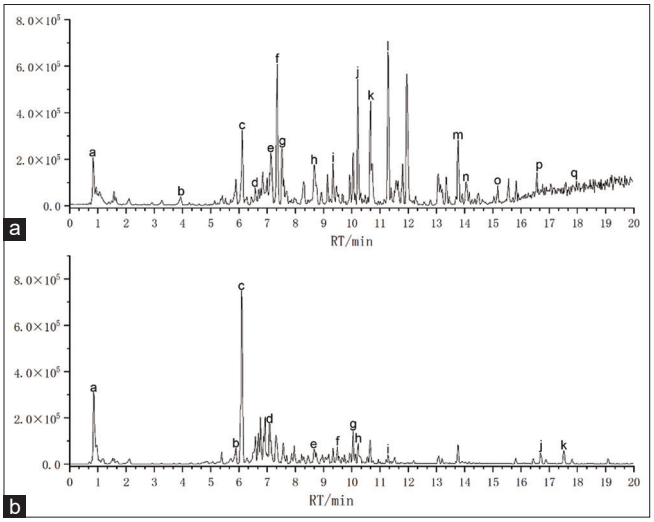

Figure 1: Base peak chromatogram of methanol extract of BaiYangJie in positive and negative ion modes. (a) a: BETAINE, b: 3-O-Caffeoylquinic acid methyl ester, c: Khelloside, d: Kaempferol, e: Thermoactinoamide_J, f: Astilbin, g: Gamma-sitosterol, h: Metoxuron, i: Daidzein, j: Batatasin III, k: Lauryldiethanolamine, l: Trans-pterostilbene, m: Gamma-Linolenic acid, n: Daniellic acid, o: d-estradiol, p: Laurocapram, q: Deoxykhivorin. (b) a: Xanthotoxin, b: benzoic acid, c: 1-O-β-D-glucopyranosyl sinapate, d: Kaempferol-3-O-glucoside, e: Parishin, f: Picroside II, g: 3-[(e)-2-(3-hydroxyphenyl) ethenyl]-5-methoxyphenol, h: Batatasin III i: Trans-pterostilbene, j: Linoleic acid, k: Palmitic acid

Acute toxicity test of methanol extract of BaiYangJie

Changes in the appearance and morphology of mice

After each administration, the appearance, behavior, secretions, and excretions of the mice were observed to be normal, with normal weight gain and no death.

Relative organ weight

Table 2 shows that there was no significant difference in the relative weight of organs between the control group and the treatment group.

Histopathological effects of methanol extract of BaiYangJie on mice in the acute toxicity test

Figure 3 shows that the control group and treatment group had no significant differences in histopathology, which showed that the ME-BYJ had no acute toxicity.

Protective effects of methanol extract of BaiYangJie against cisplatin-induced acute kidney injury

Morphological changes in the kidneys

Figure 4 shows that ME-BYJ could significantly inhibit the kidney ischemia phenomenon of the model group. The BYJ-H had the most obvious protective effect on the kidneys.

Effect of methanol extract of BaiYangJie on the body weight of cisplatin-induced mice

Figure 5 shows that the body weights of the Control and Control + BYJ groups were normal. The four groups treated with CP showed significant weight loss, which proved that the modeling was successful. However, ME-BYJ did not significantly restore weight.

Effect of methanol extract of BaiYangJie on the relative kidney weight in cisplatin-induced mice

Table 3 shows that ME-BYJ could significantly inhibit the relative kidney weight increase of the model group. The BYJ-H had the most obvious protective effect on the kidneys.

Table 2: Relative organ weight

Organ	Control group (female)	Treatment group (female)	Control group (male)	Treatment group (male)
Stomach	19.91±1.430	18.92±1.316	19.31±2.317	19.48±1.488
Heart	4.71±0.206	5.14±0.133	5.61±0.600	5.107±0.370
Liver	46.87±1.757	47.02±2.497	51.36±2.071	54.32±1.694
Kidneys	9.89±0.502	11.69±0.752	14.11±0.94	13.38±0.408
Spleen	4.87±0.250	4.01±0.438	4.95±0.794	4.51±0.556
Lung	5.85±0.408	5.98±0.158	5.58±0.429	4.92±0.234
Brain	14.09±0.463	14.50±0.325	13.94±1.339	13.75±0.645
Large intestine	22.99±0.663	23.66±0.441	22.17±1.174	21.06±0.621
Small intestine	39.22±3.138	38.64±3.844	32.59±4.086	33.24±2.007
Uterus	4.38±0.727	4.54±0.383	/	/
Testicles	/	/	5.72±0737	5.40±0.284

Organ/body weight (g/g ×1000) %. Mean±SEM values (n=5). SEM: Standard error of the mean

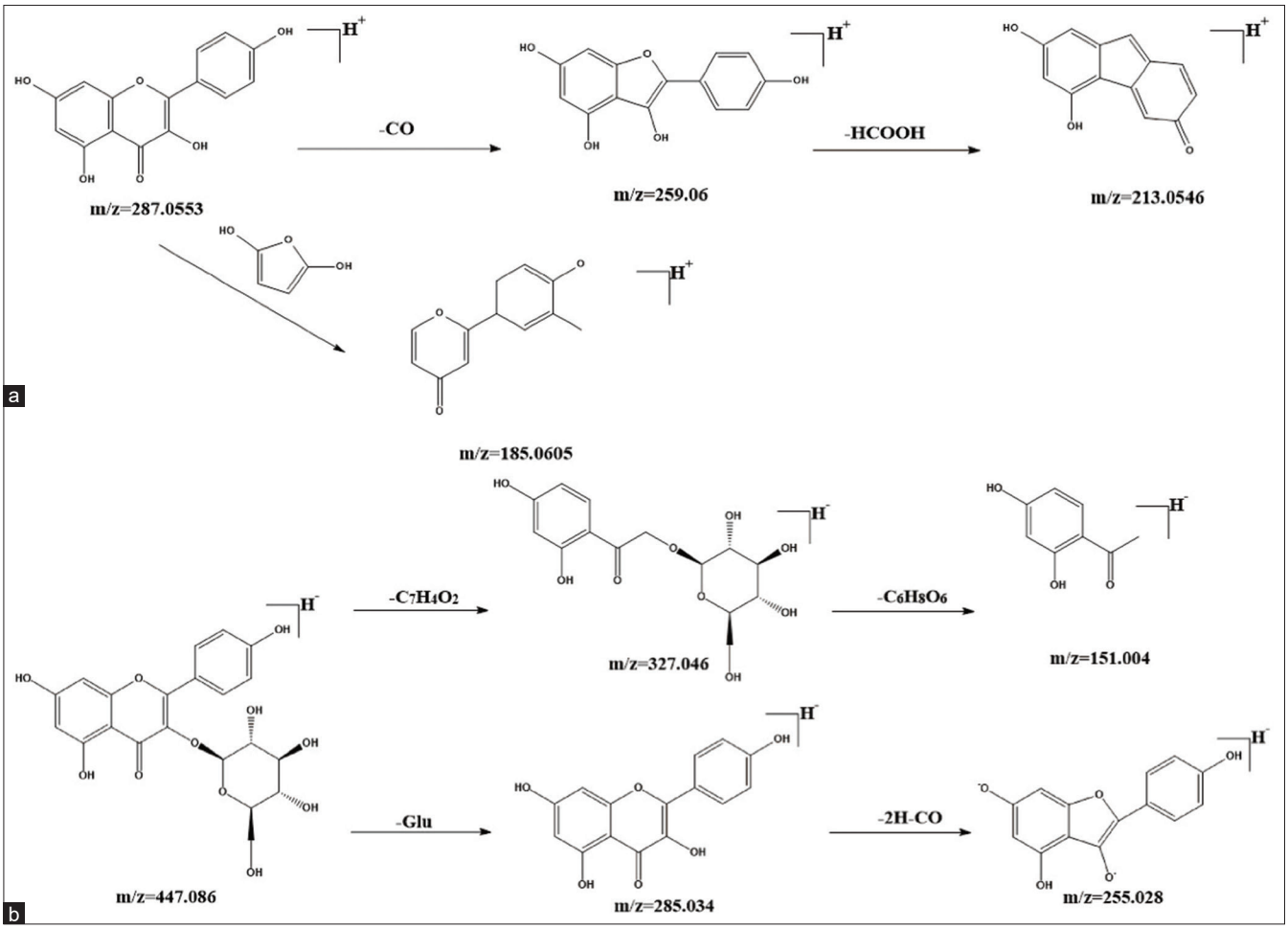

Figure 2: The fragmentation pathway of kaempferol and kaempferol-3-O-glucoside (a) is kaempferol, (b) is kaempferol-3-O-glucoside

Table 3: Relative kidney weight

Group	Relative kidney weight (%)	
	Female	Male
Control	8.28±0.307	12.19±0.428
Model	11.05±0.240$^{(\#\#\#\#)}$	14.92±0.499$^{(\#)}$
BYJ-L	10.55±0.270	12.76±0.806
BYJ-M	11.75±0.578	12.69±0.391
BYJ-H	10.30±0.261	11.44±1.031$^{(**)}$
Control + BYJ	9.36±0.351$^{(*)}$	12.08±0.578$^{(*)}$

Organ/body weight*1000(g/g)%, Mean ± SEM values (n=5), *$p < 0.05$, **$p < 0.01$ vs. Model and $^{\#}p < 0.05$, $^{\#\#\#\#}p < 0.0001$ vs. Control. BYJ: BaiYangJie, BYJ-L: BYJ-low-dose group, BYJ-M: BYJ-medium-dose group, BYJ-H: BYJ-high-dose group, SEM: Standard error of the mean

The effect of methanol extract of BaiYangJie on the values of inflammatory factors in the serum of cisplatin-induced mice

Figure 6a-d show that compared with that in the control group, the CREA and BUN values in the model group were significantly increased. All treatment groups had certain attenuating effects on the increase in the CREA and BUN values. Figure 6e and f show that compared with that in the control group, the URIC value in the model group was significantly increased. For female mice, the URIC value in the BYJ-L and BYJ-M dose groups was significantly increased, surpassing that in the model group. The BYJ-H group protective effect was obvious. For male mice, all treatment groups can significantly inhibit the increase of the URIC value of the model group. The BYJ-H group showed the most significant inhibitory effect on the increase of inflammatory factor values.

Effect of methanol extract of BaiYangJie on the kidney superoxide dismutase, catalase, and glutathione peroxidase values in cisplatin-treated mice

Figure 7a, b, e, and f show that compared with that in the control group, the kidney dismutase, catalase, and glutathione peroxidase values in cisplatin-treated mice values in the model group were significantly reduced, and all treatments increased the kidney SOD and GSH-Px values of the mice to varying degrees. Figures 7c and d show that compared with that of the control group, the kidney CAT value of the model group was significantly reduced. For female mice, the BYJ-H had an inhibitory effect on the decrease of CAT value in the model group. For male mice, all treatment groups can significantly inhibit the decrease of kidney CAT value of the model group. The BYJ-H group had a good inhibitory effect on the decrease of kidney SOD, CAT, and GSH-Px values.

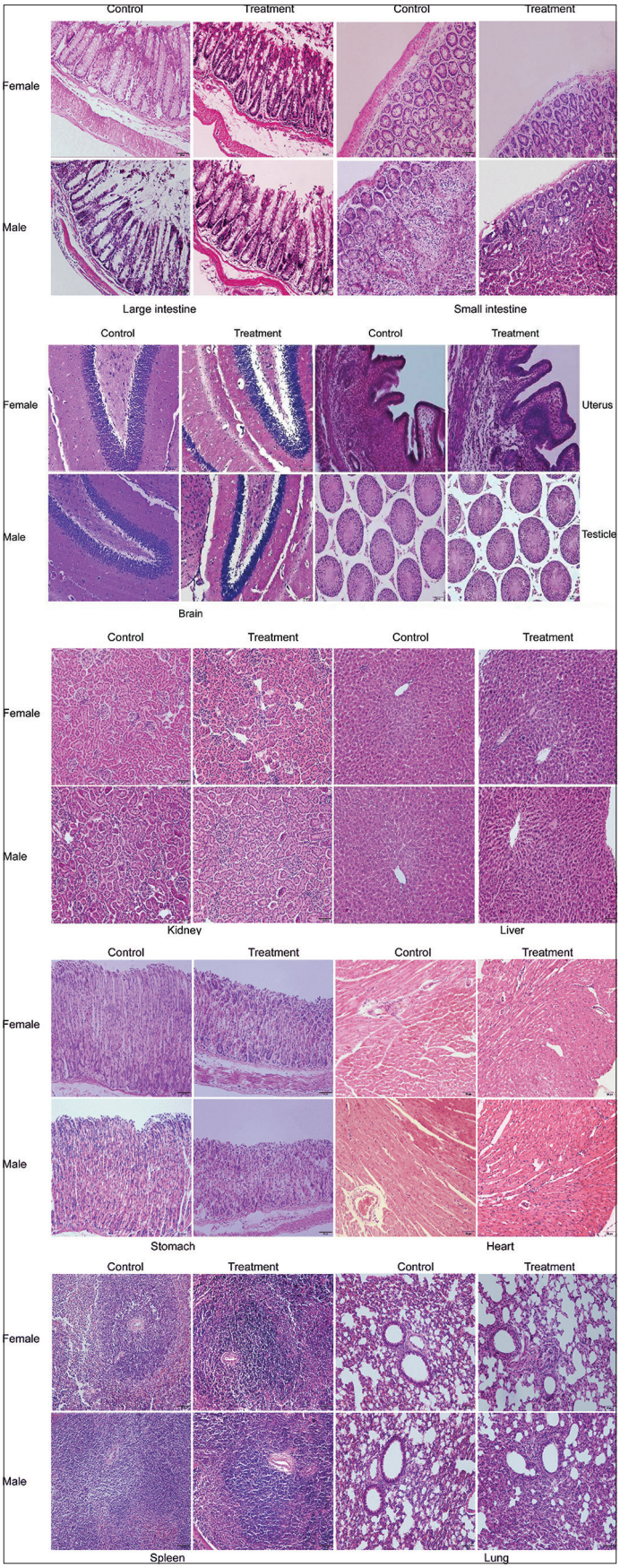

Figure 3: Light microscopic photographs from different tissue sections stained with H&E (×200)

Histopathological results

Hematoxylin-eosin staining

Figure 8 showed that the control group kidneys showed normal glomeruli (gl) covered with an epithelial layer and showed no inflammatory cell infiltration, blockage, bleeding, or interfacial injury. The Bowman's capsules (bs) exhibited regular structures, and the proximal tubular (pt) and distal tubules (dt) were typical. In the model groups were observed pt and dt were distorted and incomplete (green arrow), nuclear lysis (blue arrow), gl degeneration, atrophy (black arrow), cell proliferation in gl capillaries (black oval), detachment of the brush like border structure of rt (red arrow), inflammatory cell infiltration and damage were observed. In addition, bs exhibited irregular structures. The BYJ-L and BYJ-M groups showed varying degrees of improvement in the pathological phenomenon of the model group, and the BYJ-H group recovered to a level similar to the control group.

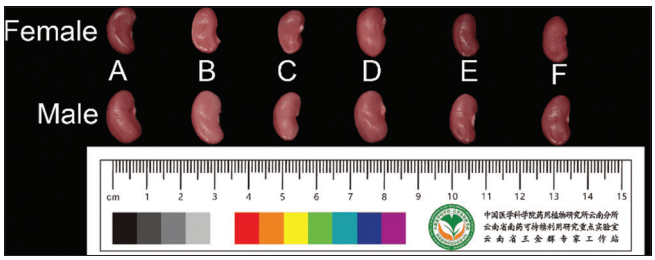

Figure 4: Morphology of kidneys. (A-F): Control, model, BYJ-low-dose group, BYJ-medium-dose group, BYJ-high-dose group, control + BaiYangJie group

Masson staining

Figure 9 showed that there were no histopathological

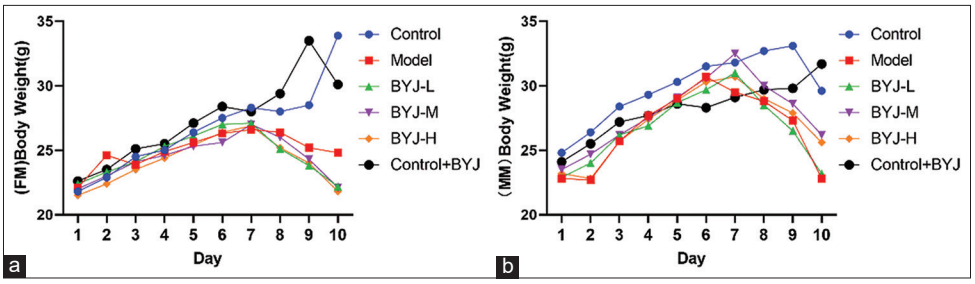

Figure 5: (a) Weight changes female mice, (b) Weight changes male mice. BYJ: BaiYangJie, BYJ-L: BYJ-low-dose group, BYJ-M: BYJ-medium-dose group, BYJ-H: BYJ-high-dose group

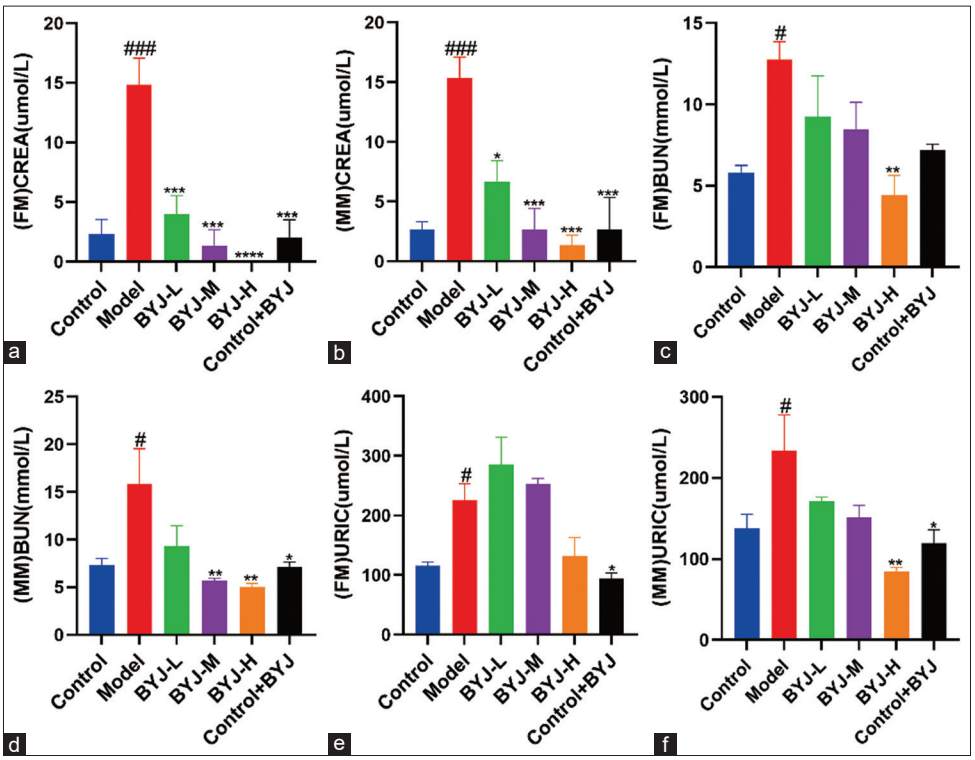

Figure 6: Changes in inflammatory factor values. Mean ± SEM values ($n = 5$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs. Model, and #$p < 0.05$, ###$p < 0.001$ vs. Control. (a and b) CREA value change, (c and d) BUN value change, (e and f) BUN value change. FM is female mice, MM is male mice. BYJ: BaiYangJie, BYJ-L: BYJ-low-dose group, BYJ-M: BYJ-medium-dose group, BYJ-H: BYJ-high-dose group

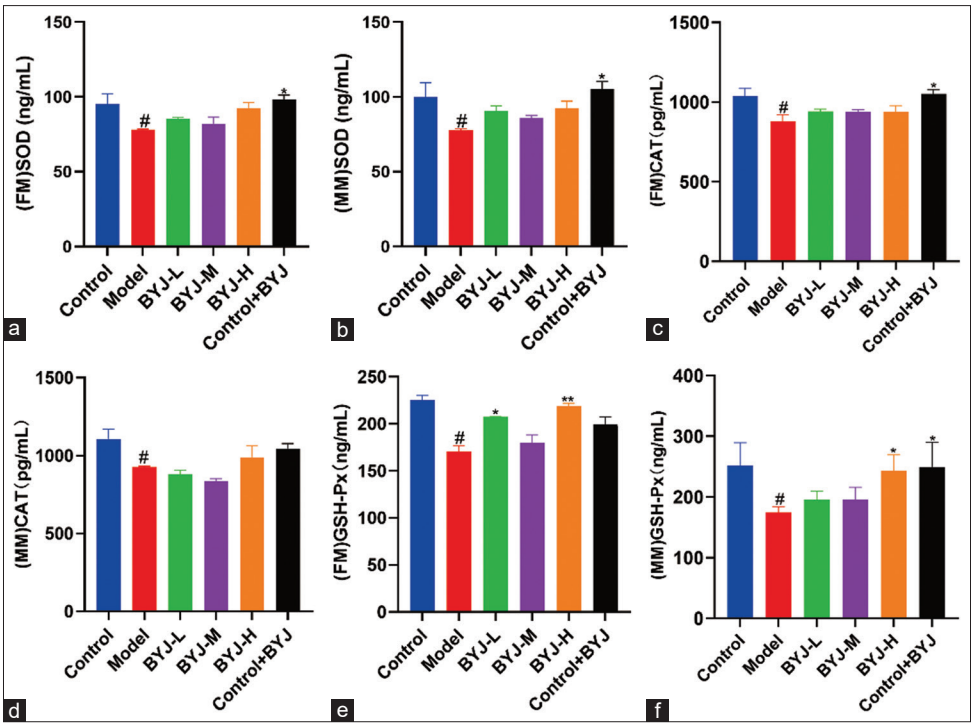

Figure 7: Changes in kidney SOD, CAT and GSH-Px values. Mean ± SEM values ($n = 5$), *$p < 0.05$, **$p < 0.01$ vs. Model, and #$p < 0.05$, vs. Control. (a and b) SOD value change, (c and d) CAT value change, (e and f) GSH-Px value change. FM is female mice, MM is male mice. BYJ: BaiYangJie, BYJ-L: BYJ-low-dose group, BYJ-M: BYJ-medium-dose group, BYJ-H: BYJ-high-dose group

changes in the control group, and the model group showed kidney interstitial fibrosis and reduced collagen deposition. All treatment groups significantly improved this phenomenon. The pathological phenomenon in the BYJ-H group was similar to that of the control group, and the effect was significant.

DISCUSSION

In the present study, the principle of dosage selection was based on the dosage given to Dai medicine in the third volume of the 2005 edition of the *Yunnan Province Standard for Traditional Chinese Medicine*, which was half and two times the dosage was converted into mouse dosage. The mice injected with CP showed typical clinical symptoms and pathological changes, such as weight loss and organ lesions. The treatment groups had a significant callback effect on kidney injury the BYJ-H group had the most significant pharmacology effect, followed by the BYJ-M and then the BYJ-L. This reflects the dose-dependent protective effect of BYJ on AKI.

There was no significant difference in the results of the Control, Control + BYJ group, and female and male in this experiment. This proves that BYJ does not cause harm to the body and its protective effect on the kidneys is not related to gender. ME-BYJ did not show significant weight recovery, which may be due to insufficient administration time after modeling. Corresponding adjustments will be made in subsequent experiments. Phytochemistry examination of the ME-BYJ showed that BYJ contains kaempferol, trans-pterostilbene, andrograpanin, dehydroevodiamine, kaempferol-3-O-glucoside, and other substances. It has been shown that kaempferol has a certain therapeutic effect on kidney injury.[8,9] Kaempferol, trans-pterostilbene, andrograpanin, dehydroevodiamine, kaempferol-3-O-glucoside, and other substances have also been confirmed to have anti-inflammatory and antioxidant activities.[10-13]

CONCLUSION

This article validated the pharmacodynamics of BYJ from the perspectives of anti-inflammatory, antioxidant, and histopathological studies using both female and male mice, and proved its safety through toxicological experiments. However, the specific mechanism is not yet clear, which may be related to the pharmacological effects of its own components. This will be the next research goal of our research group.

Acknowledgment

The authors thank financial support for Yunnan Province Wang Jinhui Expert Workstation (202105AF150057) and the "Rainforest Talent Support Program" for young talents in Xishuangbanna Prefecture (2023006).

Financial support and sponsorship

Wang Jinhui Expert Workstation; Yunnan Key Laboratory

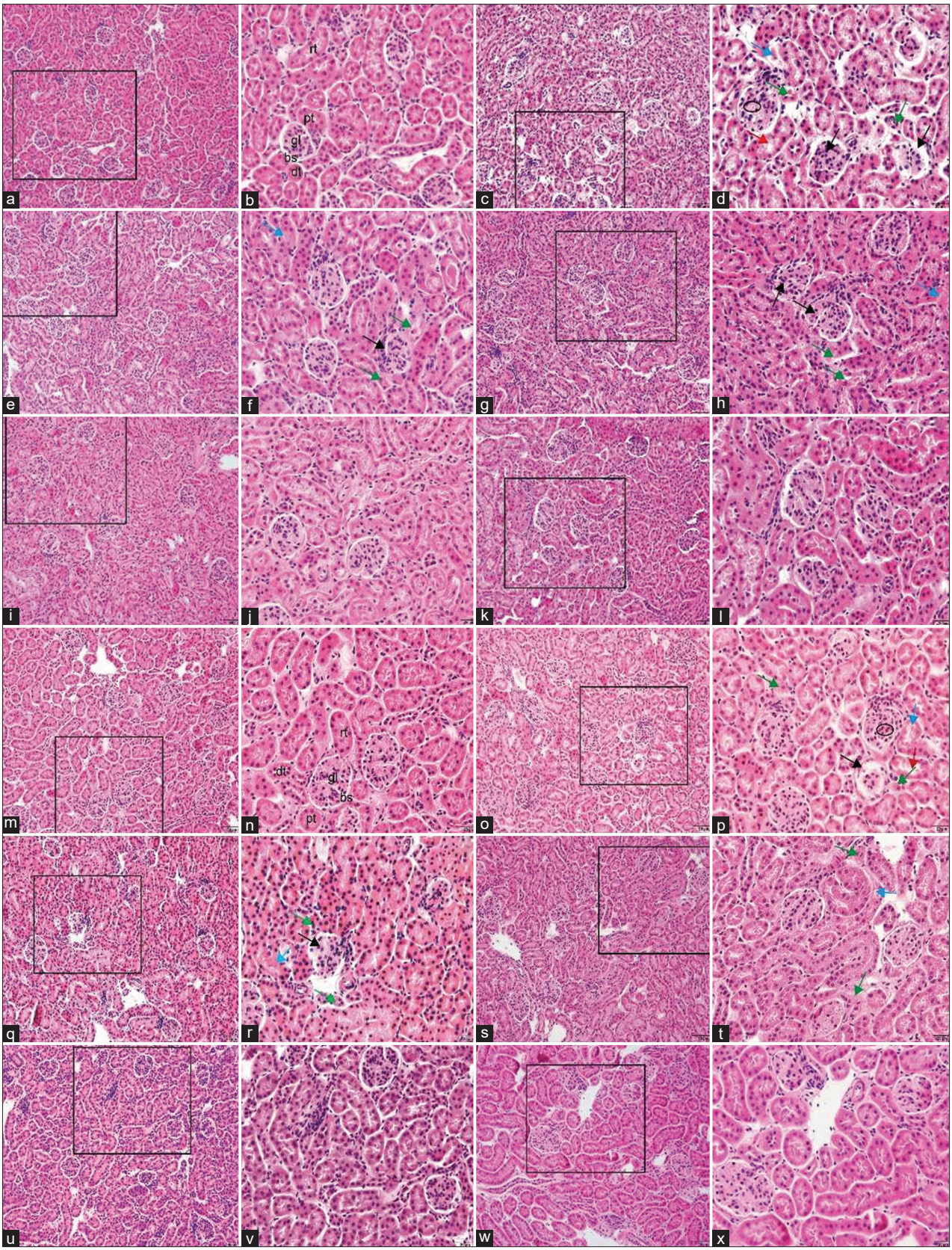

Figure 8: Light microscopic photographs from kidney tissue sections stained with H&E. (×200/×400), a-b/m-n: Control Group, c-d/o-p: Mode Group, e-f/q-r: BYJ-L Group, g-h/s-t: BYJ-M Group, i-j/u-v: BYJ-H Group, k-l/w-x: Control+BYJ Group, a~l shows female mice, m~x shows male mice, glomerules (gl), Bowman's capsules (bs), renal tubule (rt), the proximal (pt) and distal tubules (dt)

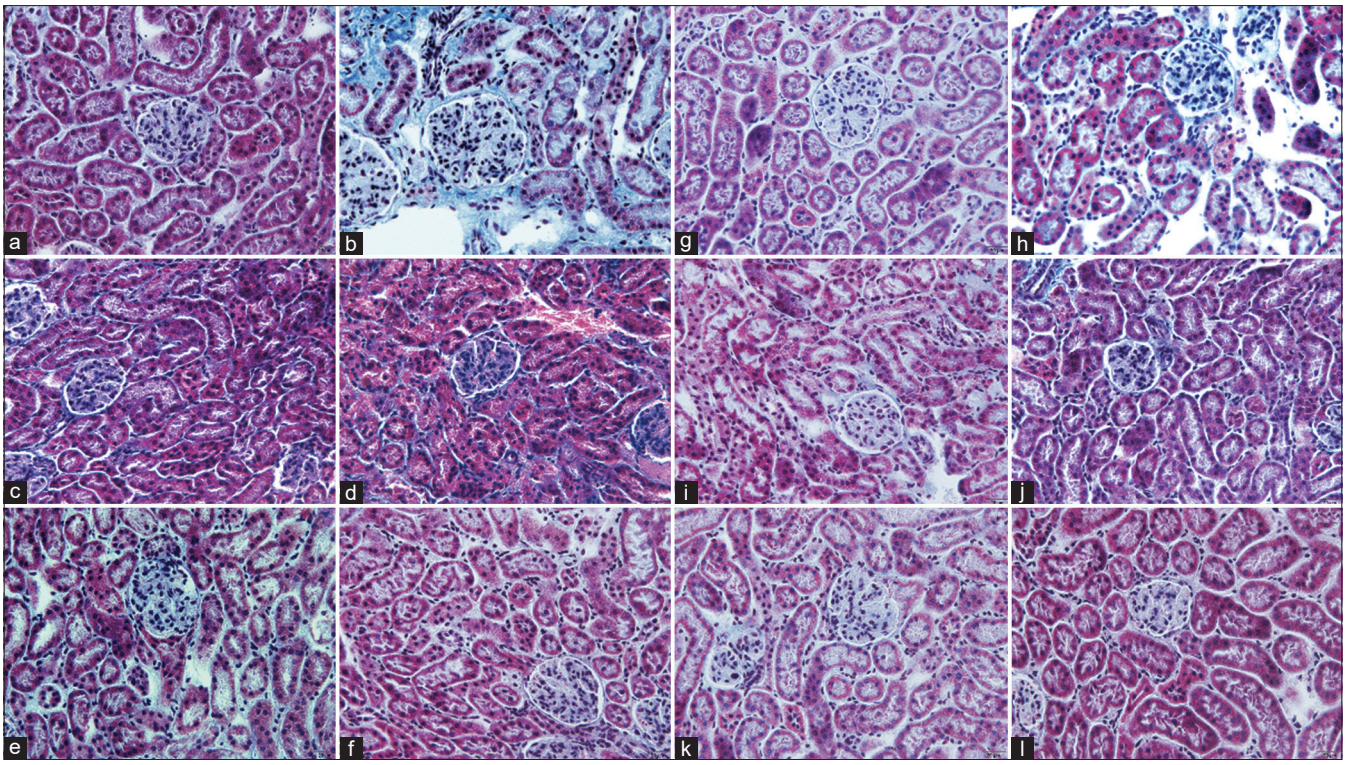

Figure 9: Light microscopic photographs from kidney tissue sections stained with Masson. (×400) a/g: Control Group, b/h: Mode Group, c/i: BYJ-L Group, d/j: BYJ-M Group, e/k: BYJ-H Group, f/l: Control +BYJ Group, a~f shows female mice, g~l shows male mice

of Southern Medicine Utilization; Young Talents under the "Rainforest Talent Support Program" in Xishuangbanna Prefecture.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Deng JS, Jiang WP, Chen CC, Lee LY, Li PY, Huang WC, *et al.* Cordyceps cicadae mycelia ameliorate cisplatin-induced acute kidney injury by suppressing the TLR4/NF-κB/MAPK and activating the HO-1/Nrf2 and sirt-1/AMPK pathways in mice. Oxid Med Cell Longev 2020;2020.
2. Li X, Zou Y, Xing J, Fu YY, Wang KY, Wan PZ, *et al.* Pretreatment with roxadustat (FG-4592) attenuates folic acid-induced kidney injury through antiferroptosis via Akt/GSK-3β/Nrf2 pathway. Oxid Med Cell Longev 2020;2020.
3. Soni SS, Pophale R, Ronco C. New biomarkers for acute renal injury. Clin Chem Lab Med 2011;49:1257-63.
4. Iqbal MO, Ahmed MM, Arshad S, Javaid U, Khan IA, Manzoor M, *et al.* Nephroprotective Effects of *Alhagi* camelorum against cisplatin-induced nephrotoxicity in albino wistar Rats. Molecules 2022;27:941.
5. Yan X, Tang B, Liu M. Phenanthrenes from *Arundina graminifolia* and *in vitro* evaluation of their antibacterial and anti-haemolytic properties. Nat Prod Res 2018;32:707-10.
6. Hu Q, Zhou B, Ye Y, Jiang Z, Huang X, Li-Y, *et al.* Cytotoxic Deoxybenzoins and Diphenylethylenes from Arundina graminifolia[J]. Journal of Natural Products 2013,76(10):1854-1859.
7. Zhu Y, Jin C, Tai H, Dao H, Zhang X. Research progress on Dai medicine *Arundina graminifolia*. Chin J Ethnomed Ethnopharm 2021;30:66-72.
8. Yuan P, Sun X, Liu X, Hutterer G, Pummer K, Hager B, *et al.* Kaempferol alleviates calcium oxalate crystal-induced renal injury and crystal deposition via regulation of the AR/NOX2 signaling pathway. Phytomedicine 2021;86:153555.
9. Chen D, Ma S, Ye W, Wang W, Sun D, Shang F, *et al.* Kaempferol reverses acute kidney injury in septic model by inhibiting NF-κB/AKT signaling pathway. J Food Biochem 2023;2023:1-11.
10. Raghavan R, Cheriyamundath S, Madassery J. Exploring the mechanisms of cytotoxic and anti-inflammatory property of andrographolide and its derivatives. Pharmacogn Rev 2018;12:56.
11. Herrera-Rocha KM, Rocha-Guzmán NE, Gallegos-Infante JA, González-Laredo RF, Larrosa-Pérez M, Moreno-Jiménez MR. Phenolic acids and flavonoids in acetonic extract from quince (*Cydonia oblonga* mill.): Nutraceuticals with antioxidant and anti-inflammatory potential. Molecules 2022;27:2462.
12. He X, Li C, Wei Z, Wang J, Kou J, Liu W, *et al.* Protective role of apigenin in cisplatin-induced renal injury[J]. Eur J Pharmacol 2016;789:215-221.
13. Wan J, Bao Q. The protective effect and mechanism of dehydrogenated *Tetradium ruticarpum* on gastric mucosa of experimental gastric ulcer rats. Chin Tradit Herb Drugs 2020;51:4698-703.