Frontiers in Bioscience Landmark

*Front. Biosci. (Landmark Ed)* **2023**; 28(11): 313
https://doi.org/10.31083/j.fbl2811313

*Original Research*

# Hugan Buzure Granule Alleviates Acute Kidney Injury in Mice by Inhibiting NLRP3/Caspase-1 Pathway and TLR4/NF-$\kappa$B Pathway

Chongwang Ran[1,2,†], Bing Yu[1,2,†], Hailong Yin[3], Yanfang Yang[1,2], Hezhen Wu[1,2,*], Qiang Yin[3,*]

[1]Faculty of Pharmacy, Hubei University of Chinese Medicine, 430065 Wuhan, Hubei, China
[2]Modern Engineering Research Center of Traditional Chinese Medicine and Ethnic Medicine of Hubei Province, 430065 Wuhan, Hubei, China
[3]Xinjiang Uygur Pharmaceutical Co., Ltd, 830026 Wulumuqi, Xinjiang, China
*Correspondence: hezh_wu@163.com (Hezhen Wu); yinqiang@renfu.com.cn (Qiang Yin)
[†]These authors contributed equally.
Academic Editor: Vesna Jacevic
Submitted: 29 November 2022 Revised: 28 April 2023 Accepted: 18 May 2023 Published: 28 November 2023

**Abstract**

**Background**: Hugan Buzure Granule (HBG) is a traditional prescription of Uygur nationality in China mainly used to treat liver cold, stomachache, spleen and rib pain, arthralgia, rheumatism and urinary system diseases. Its mechanism of action in treating acute kidney injury (AKI) continues to remain unconfirmed. This study's objective was to investigate the pharmacodynamics and mechanism of HBG in the management of AKI. **Methods**: The damage to the kidney tissue was examined by using H&E (Hematoxylin-eosin) staining. The BUN (Blood Urea Nitrogen) and Cr (Creatinine) in serum were examined by biochemical kit. The content of ROS (Reactive oxygen species) in kidney tissue was determined by ROS frozen section staining, while the amount of MDA (Malondialdehyde), GSH (Glutathione), and the enzymes of CAT (Catalase) and SOD (Superoxide dismutase) were assessed by using a biochemical kit. The tissue apoptosis was seen by using the TUNEL assay. ELISA kit was utilized to assess the content of IL-6, TNF-$\alpha$, and IL-1$\beta$ in serum. Immunohistochemistry and Western blot were utilized to identify the translation of proteins associated to the NLRP3/Caspase-1 pathway and the TLR4/NF-$\kappa$B pathway in various tissues. **Results**: HBG considerably improved the renal injury in mice and decreased their kidney coefficient in contrast with the Control group. Immunohistochemistry and Western blot demonstrated that the translation of NLRP3, Caspase-1, IL-18, IL-1$\beta$, TLR4, NF-$\kappa$B, IL-6, TNF-$\alpha$ were down-regulated in HBG groups. **Conclusions**: HBG may have a protective effect against AKI through anti-oxidative stress, inhibition of apoptosis and reduction of serum inflammatory factor levels. The mechanisms involved inhibiting NLRP3/Caspase-1 pathway and TLR4/NF-$\kappa$B pathway.

**Keywords:** hugan buzure granule; acute kidney injury; inflammation; apoptosis; oxidative stress

## 1. Introduction

Acute kidney injury (AKI) is a urinary system disease that can be brought on by a number of circumstances. It leads to a rapid deterioration in kidney function as well as a reduction in urine volume or nitrogen metabolism (urea, creatinine). There are approximately 13 million AKI patients worldwide each year. Hospital mortality is 8.8%, and patients with severe AKI have a greater risk of dying there [1]. Studies have shown that some patients with AKI who suffered from chronic inflammation for a long time, transformed to chronic kidney injury [2]. Clinically, the most important treatment of AKI is kidney replacement therapy, namely hemodialysis, which has infection risks in the process of treatment. Clinical use of statins, such as pravastatin alleviates the symptoms of kidney ischemia/reperfusion injury [3]. The body produces too much ROS, leading to oxidative stress, which causes inflammation and apoptosis, so antioxidants can be used to treat AKI. Inflammation is the main pathogenic factor of AKI, so anti-inflammatory drugs also are good at anti-AKI. In recent years, Chinese medicine has achieved remarkable results in the treatment of nephropathy. Cai *et al*. [4] found that Huangqi Danggui Mixture composed of Huangqi and Danggui has protective effect on AKI induced by ischemia/reperfusion. In the treatment of chronic glomerulonephritis, it has been illustrated that Tripterygium wilfordii has immunosuppressive and anti-inflammatory properties [5]. Kang *et al*. [6] discovered and summarized that many natural products can prevent and treat Chronic Kidney injury (CKI). Traditional Chinese medicine provides more clear advantages than western medicine in the treatment of nephropathy, including lower costs and fewer complications.

Studies have shown that after intraperitoneal injection of a certain amount of cisplatin, the renal function, nitrogen metabolism and urine volume of experimental animals continue to decline, renal tubules and glomeruli are damaged, which is consistent with the pathological manifestations of AKI, and is a good AKI model [7,8]. Oxidative stress, which is intimately associated with the occurrence and progression of AKI, is brought on by an excessive production of ROS or for other factors. Overexpression of inflammatory factors is an important manifestation of AKI. These

inflammatory factors mainly include IL-6, IL-1$\beta$, TNF-$\alpha$ and so on [9]. In the pathogenesis of AKI, the expression of NLRP3 is related to cell death, increases. Initiating the cell death process by utilizing pro-caspase-1 and caspase-1, NLRP3 further promotes the synthesis and release of IL-1$\beta$ and IL-18 [10]. TLR4 is the main receptor of the innate immune system recognition receptor. When AKI occurs, the expression of TLR4 increases, and its further activation of NF-$\kappa$B promotes the upregulation of IL-6 and TNF-$\alpha$, leading to inflammatory cascade reactions, resulting in apoptosis of kidney cells and tissue damage [11].

Hugan Buzure Granule (HBG) is derived from traditional Uyghur medicine classic prescription Hugan Buzure, it is clinically used to treat liver cold, stomachache, spleen and rib pain, arthralgia, rheumatism and urinary system diseases. The Uyghur Medicine Department of the Ministry of Health of the People's Republic of China includes its prescription formulation and indications in its drug standard (China Pharmacopoeia Commission, 1998). HBG consists of seven herbal medicines: (1) Fennel (Foeniculi Fructus, the fruit of *Foeniculum vuLgare* Mill.) 106 g, (2) Fennel root bark (Cortex Foeniculi, the root bark of *Foeniculum vuLgare* Mill.) 212 g, (3) Cuscuta chinensis (Cuscutae semen, the seed of *Cuscuta chinensis* Lam.) 53 g, (4) Chicory (the seed of *Cichorium intybus* L.) 212 g, (5) Chicory root (the root of *Cichorium intybus* L.) 106 g, (6) Celery seed (Fructus Apii, the seed of *Apium graveolens* L.) 106 g, (7) Celery root (the root of *Apium graveolens* L.) 212 g. These seven herbal remedies are listed in the Chinese Pharmacopoeia (China Pharmacopoeia Commission, 2015) and the Encyclopedia of Chinese Medicine Uygur Medicine. According to the literature review, the herbs in HBG have therapeutic effects on diseases of the urinary system. For example, Wu Song and other studies have shown that Cuscuta chinensis ethanol extract has protective effect on mice with AKI induced by lipopolysaccharide [12]. Fennel has some certain curative effects on nephritis and renal deficiency [13]. Chicory is mainly used for damp heat hepatitis, nephritis, urinary incontinence and systemic edema. However, there are few references on the therapeutic effects of HBG in AKI, which is our innovation. We want to put it on a solid scientific foundation so that it can be used in clinical practice. The objectives of this study are to demonstrate that HBG can ameliorate cisplatin-induced AKI in mice and to investigate its potential pharmacodynamics and mechanisms.

## 2. Materials and Methods

### *2.1 Materials*

Animals and drugs: 72 SPF balb/c male mice, aged at 4–6 weeks, weighed at 18–20 g (Experimental Animal Center of China Three Gorges University, Yichang, China). HBG (Cat# 200568, Xinjiang Uygur Pharmaceutical Co., Ltd., Wulumuqi, China). Cisplatin (Cat# N0624A, Dalian Meilun Biotechnology Co., Ltd., Dalian, China). SKP (Cat# JP17095, Tianjin Tongrentang Group Co., Ltd., Tianjin, China). The standards of quercetin (Cat# 19082203), kaempferol (Cat# 20082105), quercitrin (Cat# 18082102), apigenin (Cat# PS011063) and chlorogenic acid (Cat# 516E023) were acquired from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China).

Kits and instruments: Cr (Cat# C011-2-1) and BUN (Cat# C013-2-1) kit (Nanjing Jiancheng Pharmaceuticals, Nanjing, China). MDA (Cat# A003-1-2), GSH (Cat# A005-1-2), SOD (Cat# A001-3-2) and CAT (Cat# A007-1-1) kits (Nanjing Jiancheng Pharmaceuticals, Nanjing, China). Mouse TNF-$\alpha$, IL-6, IL-1$\beta$ ELISA kit (Cat# E-EL-M3063, Cat# E-EL-M2453c, Cat# E-EL-H0149c, Elabscience, China). ECL luminescent liquid (Cat# MA0186-1, Dalian Meilun Biotechnology Co., Ltd., Dalian, China). An Agilent 1260 Infinity system (Agilent Technologies, USA) was used for the High Performance Liquid Chromatography (HPLC) analysis. Enzyme-labelled instrument (SPARK 10M, TECAN, Switzerland). High speed centrifuge (Centrifuge 5810R, Eppendorf, Germany). Chemiluminescence gel imaging system (NIKON DS-U3, NIOKN, Japan).

### *2.2 Analysis of HBG by HPLC*

Accurately weighing 1.6 g of HBG was dissolved in 80% methanol, extracted by ultrasonic wave (power: 350 W, frequency: 53 kHz) for 40 min, and fixed the volume to 10 mL. The organic microporous membrane was used for filtration, and the filtrate was the test solution.

The HPLC of Agilent 1260 was used, Eclipse XDB-$C_{18}$ (4.6 mm $\times$ 250 mm, 5 µm), setting the elution solvent A as acetonitrile, B as 0.1% formic acid water, the gradient elution regimen was designed as follows: 0$\backsim$15 min, A for 5% $\rightarrow$ 10%, 15$\backsim$35 min, A for 10% $\rightarrow$ 18%, 35$\backsim$50 min, A for 18% $\rightarrow$ 25%, 50$\backsim$60 min, A for 25% $\rightarrow$ 35%, 60$\backsim$70 min, A for 35% $\rightarrow$ 70%. The UV was 327 nm.

### *2.3 Experimental Animals*

72 Balb/c mice were caged in a climate-controlled room under specified SPF conditions. Day and night were alternated for 12 h. This research project was approved by the Ethics Committee of Hubei University of Traditional Chinese Medicine (NO. HUCMS202006011). The mice were randomly divided into six groups before the experiment. The control group received oral saline for eleven consecutive days, and a single intraperitoneal (i.p.) injection of saline was given on the seventh day. The cisplatin group received oral saline for eleven consecutive days, and a single i.p. injection of cisplatin (13 mg/kg) was also given on the seventh day. HBG groups: For eleven days straight, HBG (1.6, 3.2, or 6.4 g/kg/day) was given orally once, and on the seventh day, cisplatin was applied intraperitoneally just once. SKP group: For eleven days straight, SKP (1.2 g/kg/day) was administered orally. On the seventh day, cisplatin was delivered intraperitoneally once. The final day, all mice were gavaged, sedated with 0.3% sodium pento-

barbital (80 mg/kg body weight), and then put to death. All subsequent test samples were 10. The remaining kidneys were stored in reserve at –80 °C while a portion of the kidneys were removed and fixed in 4% paraformaldehyde.

### *2.4 Behavior Observation and Organ Coefficient Determination of Mice*

The weight of the mice in each group was recorded daily and all behavioral traits were scrutinized. The organ coefficient was computed after the liver and kidney of mice were weighed.

### *2.5 Serum Biochemical Indices*

The blood sample was centrifuged. Upper serum was taken out and kept at –80 °C. A commercial kit was utilized to detect the contents of serum Cr and BUN in accordance with the manufacturer's instructions.

### *2.6 Histopathology Examination*

The kidneys were taken out of 4% PFA, encased in paraffin, dehydrated with ethanol, and removed with xylene. H&E staining was applied to paraffin sections (5 µm) before being inspected under a light microscope (NIKON ECLIPSE CI, Tokyo, Japan).

### *2.7 ROS Frozen Section Staining*

The frozen sections of kidney were incubated in dark with ROS staining solution. The nuclei were stained with DAPI. A quenching agent for anti-fluorescence was employed to seal the film. Through using fluorescent microscope, the images were inspected and recorded.

### *2.8 Analysis of Kidney Oxidative and Anti-Oxidative Parameters*

The kidney and ice-cold normal saline were homogenized and centrifuged (1:10, w/v). Using commercial kits in accordance with the directions, the resulting supernatant was utilized to assess the concentrations of MDA, reduced GSH, SOD, and CAT in conjunction to their contents and activities.

### *2.9 TUNEL Aassay*

The kidney tissue's apoptosis was observed by using the TUNEL assay. After already being treated with proteinase K, kidney slices were cultured in a solution of nucleotides that contained both fluorescein dUTP and TDT. DAPI was applied to stain the nuclei. A quenching agent for anti-fluorescence was added to seal the film. Through using fluorescent microscope, the images were inspected and recorded.

### *2.10 Detection of Inflammatory Factors by ELISA Kit*

TNF-$\alpha$, IL-1$\beta$, and IL-6 content in serum were calculated by utilizing ELISA kit.

### *2.11 Immunohistochemistry*

After rehydrating and eliminating the paraffin from the kidney slices, endogenous peroxidase was blocked before being coated with BSA. A specific amount of the primary antibody made with PBS was dripped on the sections, which were then incubated overnight at 4 °C. PBS was utilized to rinse the sections. The sections were then covered with a second antibody from the relevant species, which was HRP-labeled, and they were allowed to legally sit at room temperature for 50 minutes. PBS was employed to rinse the sections. To parts, DAB chromogenic solution was used to produce color. Harris hematoxylin was dyed for about 3 min and used tap water to cleanse for 10 min. After being dried and sealed with neutral gum, the pieces were sanitized with ethanol and xylene. Scrutiny under a microscope, image gathering, and evaluation.

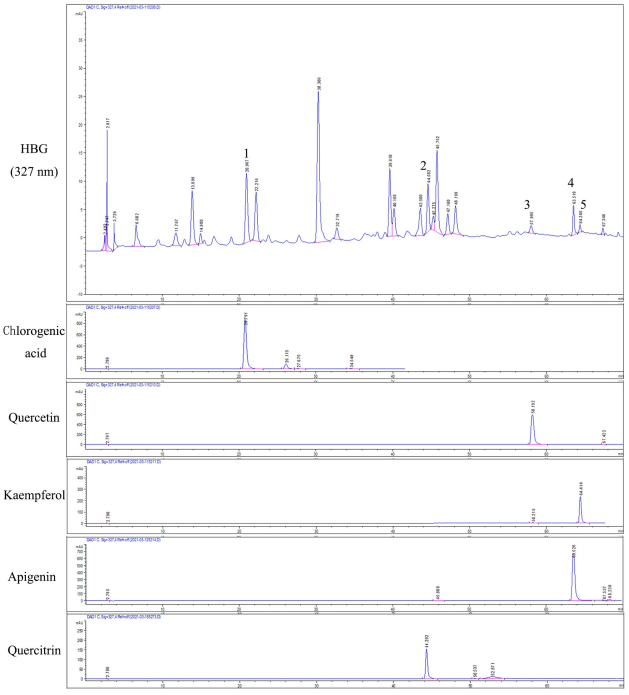


**Fig. 1. High Performance Liquid Chromatography (HPLC) of Hugan Buzure Granule (HBG) and reference standard (327 nm). 1 is Chlorogenic acid, 2 is Quercetin, 3 is Kaempferol, 4 is Apigenin, 5 is Quercitrin.**

### *2.12 Western Blot*

When creating a protein sample from kidney tissue, 5% 2-ME activation buffer and 5% loading buffer were added. The protein samples were placed onto an electrophoresis gel made of 10% SDS-polyacrylamide. The proteins were then transferred at 300 mA for 60 min to polyvinylidene difluoride membranes. The membranes were submerged in 5% skim milk (Sigma) for two hours. The membrane was in interaction with the diluted primary

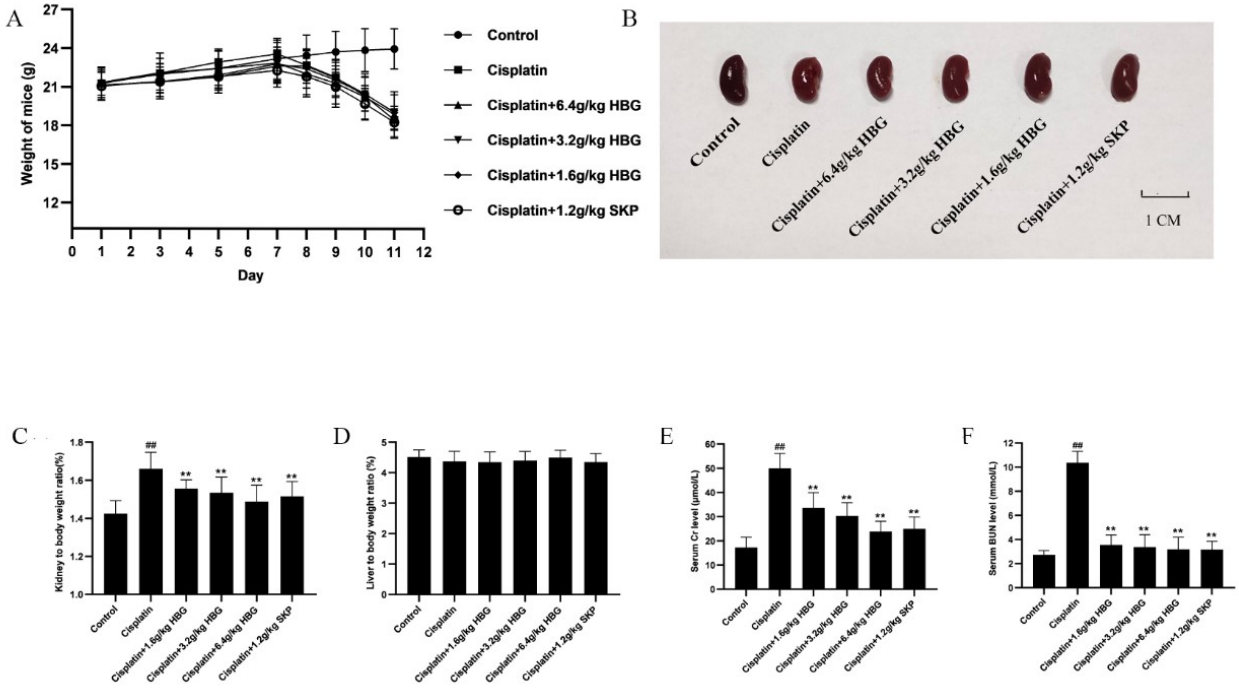


**Fig. 2. The effect of HBG on organ coefficient and serum Creatinine (Cr) and Blood Urea Nitrogen (BUN) in Chronic Kidney Injury (CKI) mice.** (A) The weight of mice changed with time. (B) Appearance of kidney in AKI mice. (C) Kidney coefficient. (D) Liver coefficient. (E) Serum Cr. (F) Serum BUN. Data were expressed as the mean ± SD. $^{\#\#}p < 0.01$ *vs.* the control group. $^{**}p < 0.01$ *vs.* the Cisplatin group.

IL-18, IL-1$\beta$, IL-6, and TNF-$\alpha$ antibodies for a number of hours. The membrane was detected for an hour with the suitable secondary antibody after being washed in TBST. The polyvinylidene difluoride membranes were colored by ECL luminescent liquid and was developed and photographed by chemiluminescence gel imaging system.

### *2.13 Statistic Evaluations*

One-way ANOVA and post hoc examination were utilized to contrast the results to the cisplatin group. Estimated to be a noteworthy value was $p < 0.05$. GraphPad Prism 8.0 (GraphPad Software, USA) was implemented to conduct the statistic evaluations.

## 3. Results

### *3.1 Component Analysis of HBG*

As shown in Fig. 1, HPLC of HBG and reference standard (327 nm), 1 is Chlorogenic acid, 2 is Quercetin, 3 is Kaempferol, 4 is Apigenin, 5 is Quercitrin.

### *3.2 The Influence of HBG on Behavior and Organ Coefficient in Mice*

The mice of Cisplatin group were dispirited, their hair color was dim and lusterless, their weight dropped sharply, their drinking water and eating decreased, and some mice had loose stools and their stomachs were swollen (Fig. 2A). The mental state of the mice in the HBG groups was noticeably better than that in the Cisplatin groups, their hair was smoother and their diet and drinking water were normal compared to the Cisplatin groups, and the phenomena of loose stools was diminished. The recovery of mice in Cisplatin + 6.4 g/kg HBG group was the fastest, and that in SKP group was the same as that in Cisplatin + 3.2 g/kg HBG group. The kidneys in the Control group were slender and long, and they were a dark red color. The kidneys in the Cisplatin group, in contrast hand, were plainly enlarged, and they were a lighter red color than the kidneys in the Control group (Fig. 2B). In HBG group, the enlargement of kidney was alleviated and the color gradually became dark. In contrast to the Control group (Fig. 2C), the Cisplatin group's kidney coefficient increased considerably ($p < 0.01$); The renal coefficient of HBG groups was markedly smaller than that of the Cisplatin group ($p < 0.01$). Relatively, the liver coefficient of Cisplatin group and HBG groups did not change significantly (Fig. 2D).

### *3.3 HBG Regulates Biochemical Indices of Kidney Function*

As shown in Fig. 2E,F, the serum Cr and BUN were considerably higher in Cisplatin group ($p < 0.01$). In comparison to the Cisplatin group, the serum Cr and BUN levels were considerably lower in the HBG and SKP groups ($p < 0.01$).

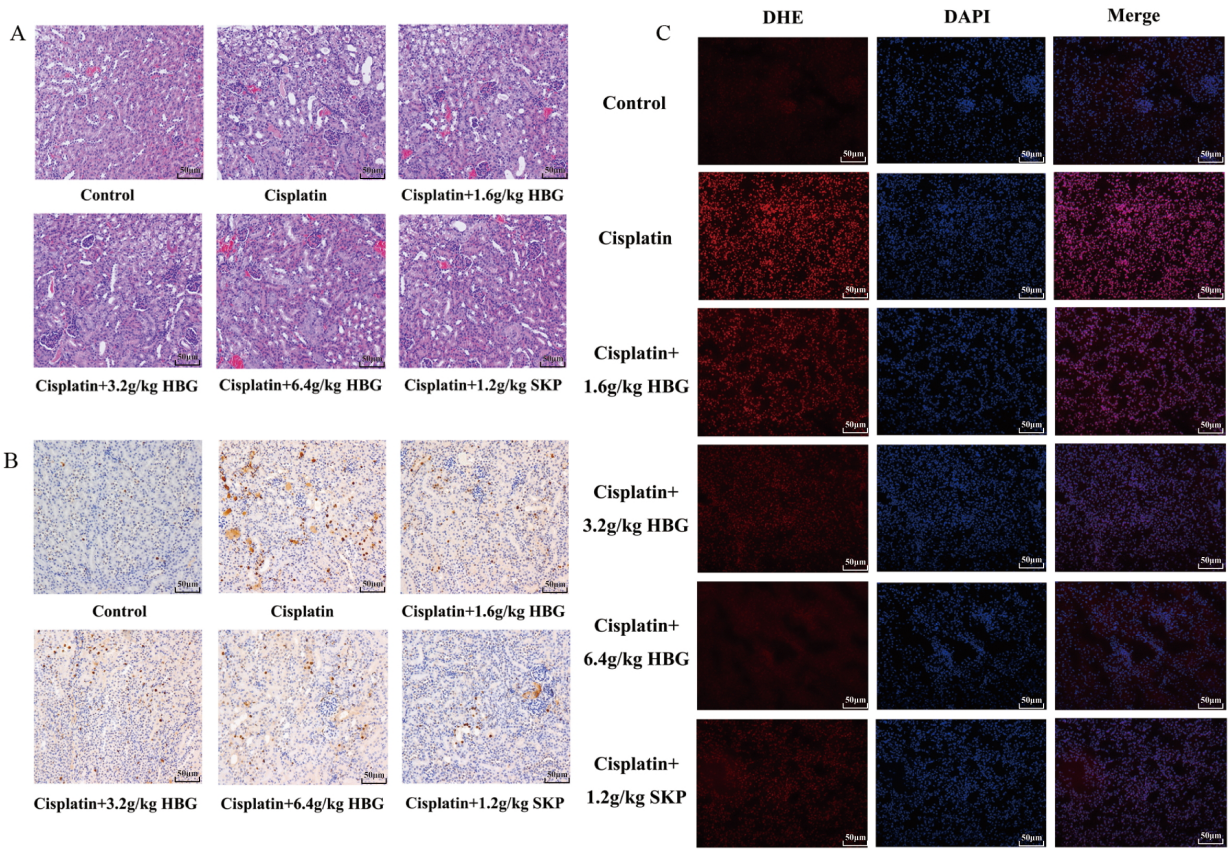


**Fig. 3. HBG influences the morphological change of kidney tissues in CKI mice.** (A) H&E staining section of kidney tissues (×200). (B) TUNEL assay of kidney tissues (×200). (C) ROS staining of kidney tissues (×200).

### *3.4 HBG Improves the Histology of Cisplatin Treated Mice*

The morphology of glomeruli and renal tubules was healthy, the structure of kidney tissue cells was ordinary, the arrangement of renal tubular cells was ordered and tight, and the boundary between cells was visible (Fig. 3A). The structure of kidney tissue in the Cisplatin group was obviously destroyed, many large vacuoles could be seen, the renal tubules were seriously dilated, and the arrangement of renal tubular cells was scattered. In comparison to Cisplatin group, the histological characteristics of the kidney injury in HBG groups were significantly improved, the number of vacuoles decreased, the expansion of renal tubules decreased, and the arrangement of renal tubular cells tended to be orderly. The Cisplatin + 6.4 g/kg HBG group showed more significant injury reduction. The recovery of SKP group was similar to that of Cisplatin + 3.2 g/kg HBG group.

### *3.5 HBG Attenuates Cisplatin-Induced Apoptosis in the Kidney*

As shown in Fig. 3B, brown was positive expression, indicating apoptosis. The positive expression of Cisplatin group was considerably increased. The positive expression of HBG groups was noticeably reduced, while the positive expression of SKP groups was even more noticeably reduced.

### *3.6 Calculation of Kidney Oxidative and Anti-Oxidative Parameters*

In contrast to the Control group, as seen in Fig. 3C, the fluorescence intensity of the Cisplatin group was much greater, indicating that the amount of ROS was dramatically higher. Compared with Cisplatin group, the fluorescence intensity was noticeably reduced in HBG groups, and the most obvious decrease was found in Cisplatin + 6.4 g/kg HBG group, the amount of ROS in SKP group was as similar to that in cisplatin + 3.2 g/kg HBG group.

As seen in Fig. 4A–D, the Cisplatin group's MDA content increased distinctly ($p < 0.01$), while the GSH content diminished markedly ($p < 0.05$). MDA levels in the HBG and SKP groups considerably dropped ($p < 0.01$), whereas GSH contents climbed. The activities of CAT and SOD in the Cisplatin group were considerably reduced ($p < 0.01$). The CAT and SOD activities in the HBG and SKP groups swelled notably ($p < 0.01$).

### *3.7 Detection of Inflammatory Factors by ELISA Kit*

As depicted in Fig. 4E–H, TNF-$\alpha$, IL-1$\beta$, and IL-6 contents were mounted up dramatically in the Cisplatin group ($p < 0.01$). TNF-$\alpha$, IL-1$\beta$, and IL-6 levels were diminished considerably in the HBG and SKP groups ($p < 0.01$ or $p < 0.05$).

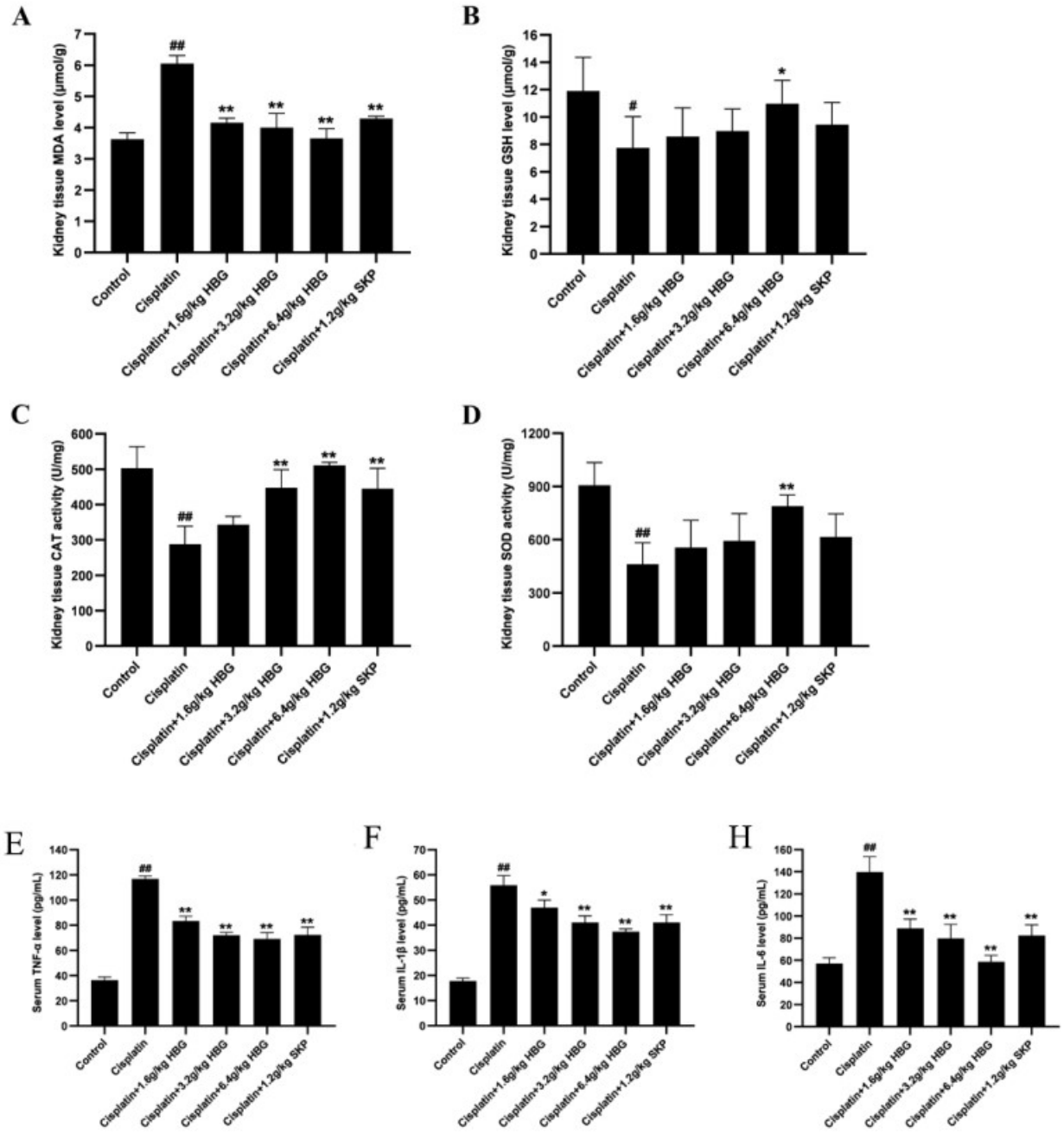


**Fig. 4. The level of oxidative stress associated to indicators in AKI mice' kidney tissue.** (A) is the MDA content, (B) is the GSH level, (C) is the activity of CAT, (D) is the content of SOD, (E) is the serum TNF-$\alpha$ level, (F) is the serum IL-1$\beta$ level and (H) is the serum IL-6 level. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* the Cisplatin group.

*3.8 Regulation of HBG on NLRP3/Caspase-1 Pathway in AKI Mice*

NLRP3 and Caspase-1 immunohistochemical staining of kidney tissues ($\times$200), brown is positive expression (Fig. 5A,B). The positive expression of Caspase-1 and NLRP3 in the Cisplatin group was increased substantially. The positive expression of NLRP3 and Caspase-1 in the HBG and SKP groups was reduced dramatically. These findings indicated that HBG diminished the expression of NLRP3 and Caspase-1 in AKI mice.

Results of IL-1$\beta$ and IL-18 Western blots in mouse kidney were displayed in Fig. 5C,D. The expression of IL-18 and IL-1$\beta$ in the Cisplatin group was increased markedly ($p < 0.01$). The expression of IL-18 and IL-1$\beta$ was sig-

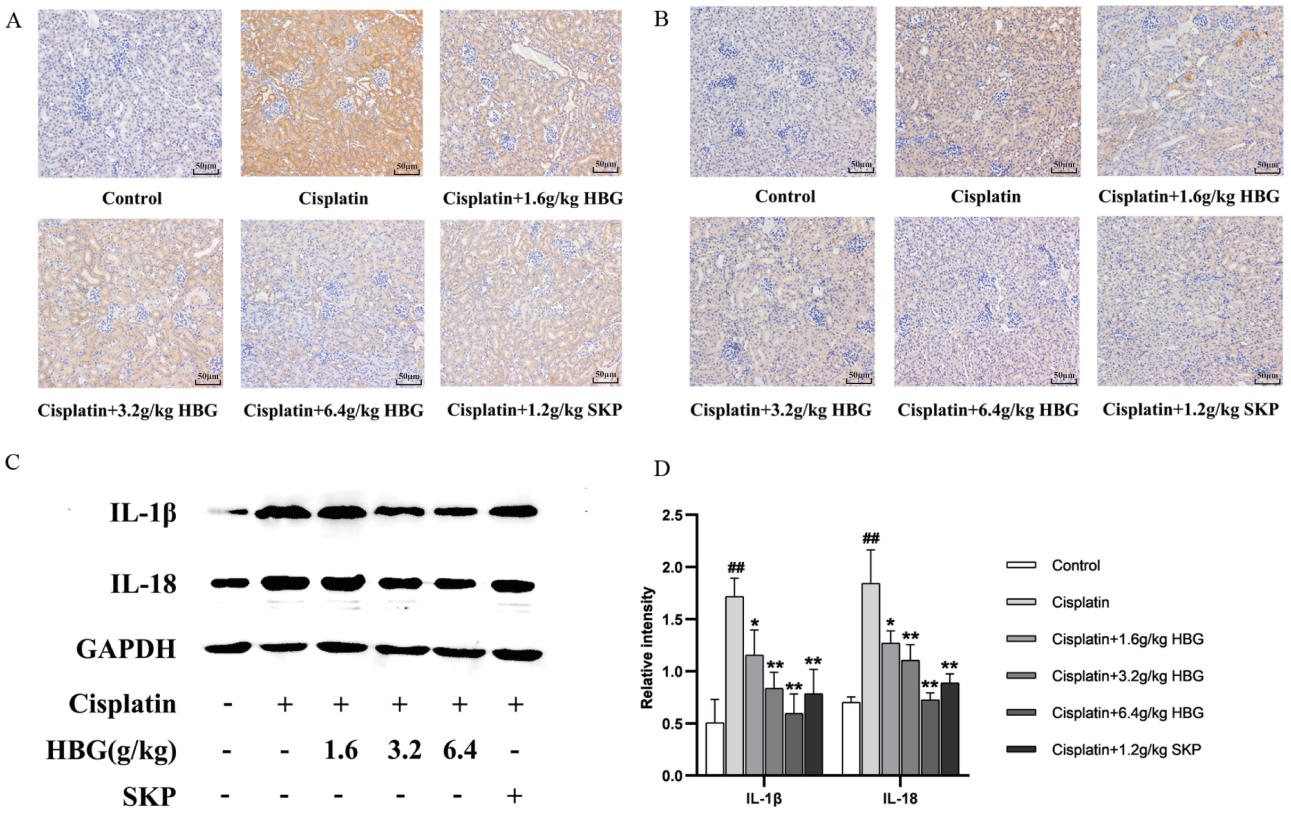


**Fig. 5. Expression of key proteins in NLRP3/Caspase-1 pathway.** (A) NLRP3 immunohistochemical staining of kidney tissues (×200). (B) Caspase-1 immunohistochemical staining of kidney tissues (×200). Western blot results of IL-1$\beta$ and IL-18. (C) is the protein translation of IL-1 $\beta$ and IL-18, (D) is western blot densitometric analysis. $^{\#\#}p < 0.01$ *vs.* the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* the Cisplatin group.

nificantly downregulated in the Cisplatin group. It demonstrated that in AKI mice, HBG decreased the translation of IL-18 and IL-1$\beta$.

### *3.9 Regulation of HBG on TLR4/NF-κB Pathway in AKI Mice*

The positive translation of TLR4 and NF-$\kappa$B in the Cisplatin group raised substantially (Fig. 6A,B). The positive translation of TLR4 and NF-$\kappa$B in the HBG and SKP groups was reduced dramatically. These detections suggested that in AKI mice, HBG decreased the translation of TLR4 and NF-$\kappa$B.

Results of Western blot for IL-6 and TNF-$\alpha$ in kidney were displayed in Fig. 6C,D. The translation of IL-6, TNF-$\alpha$ raised massively in the Cisplatin group ($p < 0.05$ or $p < 0.01$). The translation of IL-6, TNF-$\alpha$ in the HBG and SKP groups was dwindled considerably ($p < 0.01$ or $p < 0.05$). It demonstrated that in AKI mice, HBG brought down the expression of IL-6, TNF-$\alpha$.

## 4. Discussion

Through the analysis of HPLC, the results showed that HBG contained quercetin, kaempferol, chlorogenic acid and so on. The effectiveness of quercetin in treating AKI has been demonstrated [14,15], and inhibits TLR4/NF-$\kappa$B pathway [16]. Cheng *et al.* Kaempferol has been shown to prevent LPS-induced AKI in mice by Cheng *et al.* [17]. The ability of chlorogenic acid to reduce LPS-induced acute kidney injury has been confirmed by Ye *et al.* [18].

Cisplatin is a commonly used anti-tumor drug, however, 20–30% of patients have AKI after receiving cisplatin treatment [19]. In order to help study the complex mechanism of the development of kidney injury, the molecular mechanism of cisplatin metabolism and cisplatin nephrotoxicity have been created and extensively studied by using the animal model of cisplatin-induced AKI [20,21], likewise the potential medications for treating AKI. In this work, a mouse AKI model was created with once intraperitoneal dose of cisplatin (13 mg/kg). The cisplatin group were depressed, their hair color was dim and lusterless, their weight dropped sharply, and some mice had loose stools. HBG could improve the bad behavior of mice with AKI. Meanwhile, HBG group significantly reduced the kidney coefficient of mice, and improved the appearance of the kidney, indicating that HBG improved the swelling phenomenon of the kidney in mice with AKI. The H&E staining showed that HBG could improve the vacuoles, tubule dilation and scattered arrangement of renal tubular cells in

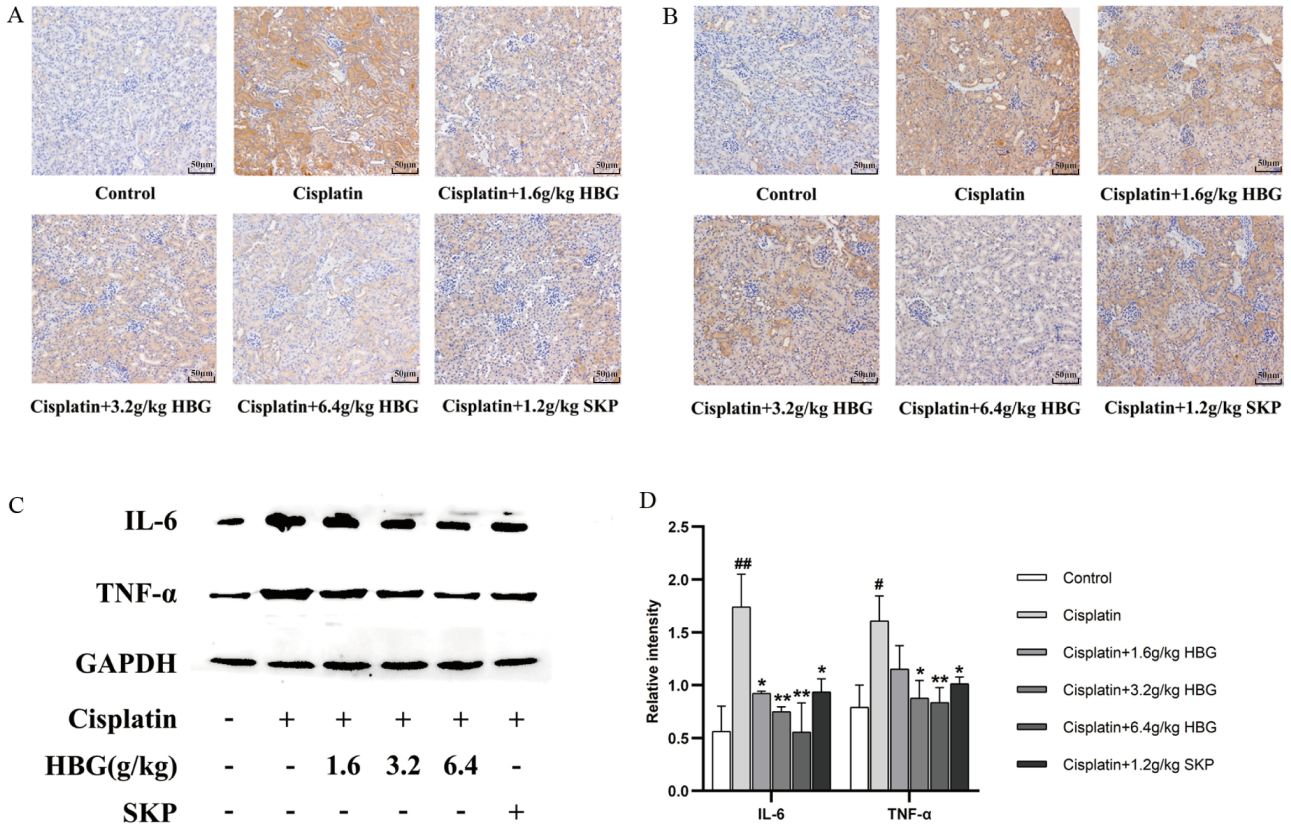


**Fig. 6. Expression of key proteins in TLR4/NF-$\kappa$B pathway.** (A) TLR4 immunohistochemical staining of kidney tissues ($\times$200). (B) NF-$\kappa$B immunohistochemical staining of kidney tissues ($\times$200). Western blot results of IL-6, TNF-$\alpha$ in AKI mice. (C) is the protein translation of IL-6, TNF-$\alpha$, (D) is western blot densitometric analysis. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* the Cisplatin group.

mice with AKI. The serum's Cr and BUN content was an important indicator of kidney function [22]. Mice in the HBG and SKP groups had vastly lower blood levels of Cr and BUN. It showed that HBG reduced the kidney function damage of mice with AKI.

Apoptosis of tissue cells, a rise in ROS (causing oxidative stress), and an amplification of inflammatory markers were frequently in relation to the course of AKI, aggravating kidney damage [23]. The apoptosis in the kidney of HBG groups was significantly decreased, indicating that HBG inhibited the apoptosis of kidney tissue cells in mice with AKI. Compared with Cisplatin group, the amount of ROS in HBG groups were lower, which indicated that HBG could reduce the content of ROS in kidney tissue. MDA is a biomarker of lipid peroxidation in living things, and it measured the level of lipid peroxidation in living creatures as well as the degree of cell damage in an indirect manner [24,25]. The biological defense system's primary enzyme, CAT, scavenges hydrogen peroxide in the body to shield cells from its toxicity [26]. All cells that metabolize oxygen had SOD, which shielded them against excessive superoxide. Two superoxide anions were converted into oxygen and hydrogen peroxide by the action of SOD [27]. The findings revealed that the HBG group's MDA content substantially dropped, while GSH content increased and CAT and SOD activity significantly enhanced. These results indicated that HBG reduced the oxidative stress in mice with AKI by reducing the amount of ROS and raising the antioxidant enzymes' rate of activity. The content of TNF-$\alpha$, IL-1$\beta$, and IL-6 was markedly diminished in the HBG group. These findings implied that HBG protected AKI animals by diminishing inflammatory factor levels in the serum.

In the pathogenesis of AKI, the increase of ROS induced oxidative stress and activated the NLRP3/Caspase-1 pathway which was related to cell death. When AKI occured, the expression of TLR4 increases, and its further activation of NF-$\kappa$B promoted upregulation of IL6 and TNF-$\alpha$, resulting in apoptosis of kidney cells and tissue damage. NLRP3, Caspase-1, IL-18, and IL-1$\beta$ related to the NLRP3/Caspase-1 pathway, along with IL-6, NF-$\kappa$B, TLR4, and TNF-$\alpha$ related to the TLR4/NF-$\kappa$B pathway, were all decreased by HBG. These results indicated that HBG reduced inflammation and apoptosis in AKI mice by inhibiting NLRP3/Caspase-1 pathway and TLR4/NF-$\kappa$B pathway, and ultimately achieved the purpose of reducing AKI.

## 5. Conclusions

This study established a model of cisplatin-induced AKI and treated it with HBG. It was confirmed that HBG had good anti-AKI pharmacodynamics. It was clarified that HBG could alleviate cisplatin induced AKI in mice by antioxidant stress, inhibiting the apoptosis of tissue cells, reducing the content of inflammatory factors in serum. The mechanism involved the inhibition of NLRP3/Caspase-1 pathway and TLR4/NF-$\kappa$B pathway. This study offered a solid scientific foundation for the use of HBG in clinical settings.

## Abbreviations

HBG, Hugan Buzure Granule; AKI, Acute kidney injury; HPLC, High Performance Liquid Chromatography; SKP, Shenyan Kangfu Pian; H&E, Hematoxylin-eosin; Cr, Creatinine; ROS, Reactive oxygen species; MDA, Malondialdehyde; GSH, Glutathione; CAT, Catalase; SOD, Superoxide dismutase; IL-6, Interleukin-6; TNF-$\alpha$, Tumor necrosis factor-$\alpha$; IL-1$\beta$, Interleukin-1$\beta$; IL-18, Interleukin-18; TLR4, Toll like receptor 4; NF-$\kappa$B, Nuclear factor kappa-B; PFA, Paraformaldehyde; DAPI, 4′,6-diamidino-2-phenylindole; SPF, Specific Pathogen Free; ELISA, Enzyme linked immunosorbent assay; BUN, Blood Urea Nitrogen.

## Availability of Data and Materials

For reasonable requirements, the data related to this study can be requested from the corresponding author.

## Author Contributions

HZW and QY designed the research study. CWR and BY performed the research. HLY and YFY provided help and advice on the ELISA experiments. CWR analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This research project was approved by the Ethics Committee , Hubei University of Traditional Chinese Medicine, Wuhan, China (NO. HUCMS202006011).


## Acknowledgment

Not applicable.

## Funding

This work was supported by National Key Research and Development Program of China plan key project of TCM Modernization Research (2017YFC1701000).


## Conflict of Interest

The authors declare no conflict of interest. This research was supported by Xinjiang Uygur Pharmaceutical Co., Ltd in which I had a financial interest. I have fully disclosed these interests to Qiang Yin and have developed an approved plan to manage any potential conflicts that arise from such an arrangement.

## References


[1] Xu X, Nie S, Liu Z, Chen C, Xu G, Zha Y, *et al*. Epidemiology and Clinical Correlates of AKI in Chinese Hospitalized Adults. Clinical Journal of the American Society of Nephrology. 2015; 10: 1510–1518.

[2] Black LM, Lever JM, Traylor AM, Chen B, Yang Z, Esman SK, *et al*. Divergent effects of AKI to CKD models on inflammation and fibrosis. American Journal of Physiology - Renal Physiology. 2018; 315: F1107–F1118.

[3] Haslinger-Löffler B. Multiple effects of HMG-CoA reductase inhibitors (statins) besides their lipid-lowering function. Kidney International. 2008; 74: 553–555.

[4] Cai Q, Li X, Wang H. Astragali and Angelica protect the kidney against ischemia and reperfusion injury and accelerate recovery. Chinese Medical Journal. 2001; 114: 119–123.

[5] Wan YG, Sun W, Zhen YJ, Che XY, Pu HP, Wang Y, *et al*. Multi-glycoside of Tripterygium wilfordii Hook. f. reduces proteinuria through improving podocyte slit diaphragm dysfunction in anti-Thy1.1 glomerulonephritis. Journal of Ethnopharmacology. 2011; 136: 322–333.

[6] Kang HG, Lee HK, Cho KB, Park SI. A Review of Natural Products for Prevention of Acute Kidney Injury. Medicina. 2021; 57: 1266.

[7] Behling EB, Sendão MC, Francescato HDC, Antunes LMG, Costa RS, Bianchi MDLP. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacological Reports. 2006; 58: 526–532.

[8] Perše M, Večerić-Haler Ž. Cisplatin-Induced Rodent Model of Kidney Injury: Characteristics and Challenges. BioMed Research International. 2018; 2018: 1462802.

[9] Jang HR, Rabb H. Immune cells in experimental acute kidney injury. Nature Reviews Nephrology. 2015; 11: 88–101.

[10] He WT, Wan H, Hu L, Chen P, Wang X, Huang Z, *et al*. Gasdermin D is an executor of pyroptosis and required for interleukin-1$\beta$ secretion. Cell Research. 2015; 25: 1285–1298.

[11] Tan Z, Shi Y, Yan Y, Liu W, Li G, Li R. Impact of endogenous hydrogen sulfide on toll-like receptor pathway in renal ischemia/reperfusion injury in rats. Renal Failure. 2015; 37: 727–733.

[12] Yang X, Yang J, He H, Hu J. Study on the effect of Uygur prescription Hugan buzure on acute liver injury of rats. Journal of Xinjiang Medical University. 2017; 40: 1479–1483+1491. (In Chinese)

[13] Zhang C, Zhang K, Tian X, Tian L, Mu D, Wang H, *et al*. Hepatoprotective effect of Hugan Buzure Keli on carbon tetrachloride induced-hepatic fibrosis in mice. Journal of Shihezi University. 2016; 34: 355–359. (In Chinese)

[14] Tan RZ, Wang C, Deng C, Zhong X, Yan Y, Luo Y, *et al*. Quercetin protects against cisplatin-induced acute kidney injury by inhibiting Mincle/Syk/NF-κB signaling maintained macrophage inflammation. Phytotherapy Research. 2020; 34: 139–152.

[15] Wang Y, Quan F, Cao Q, Lin Y, Yue C, Bi R, *et al*. Quercetin alleviates acute kidney injury by inhibiting ferroptosis. Journal of Advanced Research. 2021; 28: 231–243.

[16] Tan J, He J, Qin W, Zhao L. Quercetin alleviates lipopolysaccharide-induced acute kidney injury in mice by suppressing TLR4/NF-κB pathway. Journal of Southern Medical University. 2019; 39: 598–602. (In Chinese)
[17] Cheng X, Yang Y, Li W, Liu M, Wang Y, Du G. Effect and Possible Mechanism of Kaempferol on Acute Kidney Injury in LPS-Stimulated Mice. Chinese Pharmaceutical Journal. 2020; 55: 1439–1443. (In Chinese)
[18] Ye HY, Jin J, Jin LW, Chen Y, Zhou ZH, Li ZY. Chlorogenic Acid Attenuates Lipopolysaccharide-Induced Acute Kidney Injury by Inhibiting TLR4/NF-κB Signal Pathway. Inflammation. 2017; 40: 523–529.
[19] Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nature Reviews Immunology. 2008; 8: 726–736.
[20] Ozkok A, Edelstein CL. Pathophysiology of cisplatin-induced acute kidney injury. BioMed Research International. 2014; 2014: 967826.
[21] Zhang J, Goering PL, Espandiari P, Shaw M, Bonventre JV, Vaidya VS, *et al*. Differences in immunolocalization of Kim-1, RPA-1, and RPA-2 in kidneys of gentamicin-, cisplatin-, and valproic acid-treated rats: potential role of iNOS and nitrotyrosine. Toxicologic Pathology. 2009; 37: 629–643.
[22] Di Leo L, Nalesso F, Garzotto F, Xie Y, Yang B, Virzì GM, *et al*. Predicting Acute Kidney Injury in Intensive Care Unit Patients: The Role of Tissue Inhibitor of Metalloproteinases-2 and Insulin-Like Growth Factor-Binding Protein-7 Biomarkers. Blood Purification. 2018; 45: 270–277.
[23] Fan H, Le JW, Sun M, Zhu JH. Pretreatment with S-Nitrosoglutathione Attenuates Septic Acute Kidney Injury in Rats by Inhibiting Inflammation, Oxidation, and Apoptosis. BioMed Research International. 2021; 2021: 6678165.
[24] Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochemical and Biophysical Research Communications. 2017; 482: 419–425.
[25] Niki E. Biomarkers of lipid peroxidation in clinical material. Biochimica et Biophysica Acta. 2014; 1840: 809–817.
[26] Sepasi Tehrani H, Moosavi-Movahedi AA. Catalase and its mysteries. Progress in Biophysics and Molecular Biology. 2018; 140: 5–12.
[27] Perry JJP, Shin DS, Getzoff ED, Tainer JA. The structural biochemistry of the superoxide dismutases. Biochimica et Biophysica Acta. 2010; 1804: 245–262.