**Original Article**

# A Study on the Mechanism of the Protective Effect of GuangeFang on Sepsis-Associated Acute Kidney Injury

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## Abstract

**Objective:** The objective of this study was to explore the mechanism of Guan Gefang (GGF) ; raw rhubarb 30 g, cassia arboreal 30 g, raw oyster 30 g, ground elm 60 g, and dandelion 30 g) kidney protection. **Materials and Methods:** Thirty-six Sprague Dawley rats were randomly divided into a control group (Group N), a sepsis control group (Group S), and a sepsis + GGF group (Group G). For Group N, 8 ml/kg 0.9% NaCl was used as an enema; for Group S, cecal ligation and puncture (CLP) method was used for modeling and 8 ml/kg 0.9% NaCl was used as an enema; and Group G, CLP was used for modeling and 8 ml/kg GGF was used as an enema. All of the enemas were applied once daily for 4 days. The indices of serum creatinine (SCr), blood urea nitrogen (BUN), uric acid (UA), mammalian target of rapamycin (mTOR), Janus kinase 2 (JAK2) were compared across each group. **Results:** Compared to Group S, Group G had lower levels of SCr, BUN, and UA ($P < 0.05$), while the activities of mTOR and JAK2 were significantly inhibited. **Conclusion:** GGF may have inhibited the JAK2 or mTOR signaling pathways to protect the rats' kidneys, which had sepsis-associated acute kidney injury.

**Keywords:** GuangeFang, Janus kinase 2, mammalian target of rapamycin, sepsis-associated acute kidney injury

## INTRODUCTION

Sepsis is a clinically common acute and severe disease. Due to its high incidence and mortality, it has become a focus for clinical and basic studies. Acute kidney injury (AKI) has many causes,[1] among which sepsis and infectious toxic shock are the primary.[2] Sepsis-associated AKI (S-AKI) is a common complication in critically ill patients. S-AKI is associated with 40%–50% of sepsis patients in intensive care units, and the disease is also associated with extremely high sepsis mortality.[3-5] Beijing Chinese medicine hospital professor Qing-Quan Liu found through many years of clinical practice that the pathogenesis of S-AKI is mainly about the poison resistance, loss of Yin, and deficiency of Yang. Subsequently, he created GuangeFang (GGF), which has generated interest with respect to its clinical effect. The results of the preliminary clinical study of this project showed that GGF enema can help reduce serum creatinine (SCr) and blood urea nitrogen (BUN) in patients with S-AKI, increase the volume of urine produced by the patients for 24 h, and play a protective role for patients who have S-AKI. In this study, animal experiments were conducted to explore the mechanism of GGF's protective effect on acute kidney injury in sepsis.

## MATERIALS AND METHODS

### Materials

#### Blood components of GuangeFang

GGF is composed of raw rhubarb (30 g), cassia arboreal (30 g), raw oyster (30 g), ground elm (60 g), and dandelion (30 g). To understand its mechanisms of action, the blood components of GGF were analyzed in the early stage of this experiment. The extract of GGF was prepared according to the management standard of clinical decocting room for Chinese traditional medicine. The decoction samples were analyzed by reversed-phase chromatography (RGC) using waters, and the time-of-flight tandem mass spectrometer equipped with electrospray ion source was used for mass spectrometry analysis. Through these analyses, it was

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**How to cite this article:** Li S, Liu QQ. A study on the mechanism of the protective effect of guangefang on sepsis-associated acute kidney injury. World J Tradit Chin Med 2021;7:414-8.
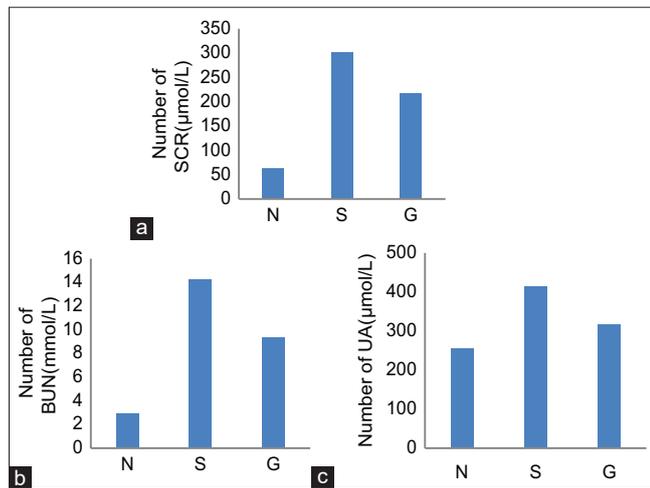
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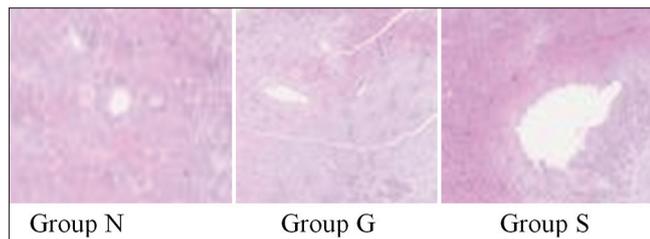
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**DOI:**
10.4103/2311-8571.328618

**Figure 1:** Blood sample result. (a) Compared to Group S, Group G has lower level in SCr ($P < 0.05$). (b) Compared to Group S, Group G has lower level in BUN ($P < 0.05$). (c) Compared to Group S, Group G has lower level in UA ($P < 0.05$). BUN: Blood urea nitrogen, UA: Uric acid, SCr: Serum creatinine



**Figure 2:** Pathological findings of renal medulla. Compared to Group S, Group G has smaller area in edema and vacuolar degeneration. Pallet method was used to score the severity of renal tubular injury: Group G score was lower than Group S score
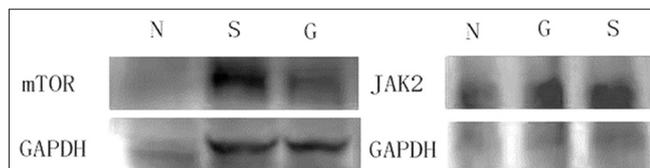


**Figure 3:** WB result. Compared to Group S, Group G has lower expression in mTOR and JAK2. Compared to Group S, the activity of mTOR and JAK2 in Group G was significantly inhibited. mTOR: Mammalian target of rapamycin, JAK2: Janus kinase 2, WB: Western blot

concluded that the blood components of GGF mainly include six kinds [Table 1].

### The network pharmacology analysis of GuangeFang

In the early stage of this study, the active ingredients and targets of GGF were predicted and screened based on databases. Cytoscape 3.7.1 National Institute of General Medical Sciences (NIGMS), US software was used to construct the key-active component-action target network, the String platform was used to construct the target protein interaction network, and the biological process of target gene ontology (GO) and metabolic pathways in the Kyoto

Encyclopedia of Genes and Genomes were analyzed through the biological information annotation database (DAVID). Six active ingredients in GGF were screened from the Sig formula, which acted on 22 targets. Because the mammalian target of rapamycin (mTOR) and Janus kinase 2 (JAK2) signaling pathways were at the front of the predictive pathway, these were chosen for further investigation in the following animal experiments.

### Animals

Healthy-specific pathogen-free Sprague Dawley (SD) 8-week-old male rats, weighing 180–220 g, animal production license no. SCXK (Shanghai) (2017-0005), were purchased from Shanghai Slake Experimental Animals Co., Ltd. The rats were kept in laminar flow racks and fed and drank freely. Twelve hours before modeling, the rats were alternately fed day and night for 7 days to maintain a constant temperature. All animal procedures were carried out in compliance with the guidelines for scientific animal procedures approved by the Ethics Committee of the Beijing Institute of Traditional Chinese Medicine (TCM), and the approval number is No. 2019060104.

### Drugs

GGF (consisting of raw rhubarb [30 g], cassia arboreal [30 g], raw oyster [30 g], ground elm [60 g], and dandelion [30 g]) were immersed in 4000 ml pure water for 2 h and then heated for 30 min. A second 4000 ml of pure water was then added to the fluid and fry cooked for 30 min to ensure dissolution, and the volume was reduced to 800 ml. The extracted drugs were stored in a refrigerator at −20°C in the dark.

### Reagents

SCr, BUN, and uric acid (UA) were sourced from the Nanjing Jiancheng Bioengineering Research Institute; MTOR antibodies and JAK2 antibodies were sourced from Abcam, USA.

## Methods

### Grouping and model preparation
#### Establish cecum ligation puncture model

Cecum ligation puncture (CLP) is the most commonly used animal model in S-AKI.[6,7] Consequently, we used the CLP model to study the mechanism of the protective effect of GGF on S-AKI. After CLP, SCr was tested to demonstrate the success of the model: If the index of SCr was two times of the index of SCr in Group N, the model was deemed a success.

### Model preparation of sham operation group

Laparotomy was performed with a 1–1.5 cm longitudinal incision in the middle of the lower abdomen with a scalpel. The muscular layer, fascia, and peritoneal layer are opened layer by layer. The cecum was located and then returned to the abdominal cavity, followed by closure of the abdomen.

### Grouping

Thirty-six SD rats were selected and randomly divided into the control group (Group N), the sepsis control group (Group S), and the sepsis + GGF group (Group G), with 12 rats in each group.

## Table 1: Analysis of blood components in GuangeFang

| | ESI +/− | Observed neutral mass (Da) | Observed m/z | Mass error (mDa) | Mass error (ppm) | Observed RT (min) | Detector counts | Response | Adducts |
|---|---|---|---|---|---|---|---|---|---|
| Rhubarb phenol | − | 254.0562 | 253.0489 | −1.7 | −6.8 | 10.18 | 706 | 617 | −H |
| | + | 254.0576 | 255.0649 | −0.3 | −1.3 | 10.18 | 87 | 87 | +H |
| β-incense tree | − | 426.3833 | 471.3815 | −2.9 | −6.2 | 18.58 | 244 | 244 | +HCOO |
| | + | 426.3834 | 427.3907 | −2.7 | −6.4 | 18.59 | 1145 | 843 | +H |
| Oppose coumaric acid | − | 164.0469 | 163.0397 | −0.4 | −2.5 | 1.59 | 787 | 671 | −H |
| | + | 164.0473 | 165.0546 | 0 | −0.2 | 1.6 | 5606 | 5174 | +H |
| Celery alcohol | − | 270.0526 | 269.0453 | −0.3 | −1 | 7.34 | 330 | 330 | −H |
| | + | 270.0526 | 271.0598 | −0.3 | −1 | 7.34 | 124 | 124 | +H |
| Coumaric acid | − | 148.0521 | 147.0448 | −0.3 | −2.3 | 3.58 | 1397 | 1281 | −H |
| | + | 148.0525 | 149.0598 | 0.1 | 0.4 | 3.59 | 702 | 651 | +H |
| Rhein | − | 284.031 | 283.0238 | −1.1 | −3.7 | 12.63 | 3356 | 1575 | −H |
| | + | 284.0323 | 285.0395 | 0.2 | | 12.64 | 79 | 79 | +H |

ESI: Emergency Stop Indicator; RT: radiographic testing
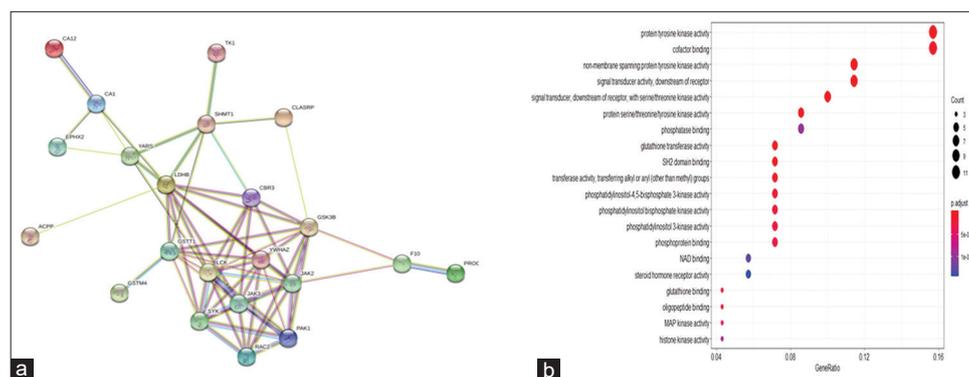


**Figure 4:** The network pharmacology analysis of GGF. (a) Compound-target network of GGF consisting of 22 notes and 63 interactions. (b) mTOR signaling pathway and JAK2 signaling pathway were at the front of the predictive pathway, GGF: GuangeFang, mTOR: Mammalian target of rapamycin

## Administration method
### The control group (Group N)
Group N was anesthetized by intraperitoneal injection of 0.35 ml/100 g chloral hydrate before enema, followed by inverted suspension. A 15 cm length, 2 mm diameter polypropylene tube was slowly inserted into the anus for 8 cm, and 3 ml/kg sterilization fluid was injected to clean. After wiping perianal, 8 ml/kg sodium chloride was injected as an enema, and the rats were put into the cage and fed normally after they were awake. The above enema was performed once daily for 4 days.

### In the sepsis group (Group S)
The CLP method was used for modeling 1 h before the first enema. One hour after modeling on the 1st day, rats were given 8 ml/kg of 0.9% normal saline directly. Subsequently, rats were given an enema once daily for 4 days, as described above.

### Sepsis + GuangeFang group (Group G)
The CLP method was used for modeling 1 h before the first enema. One hour after modeling on the 1st day, rats were given 8 ml/kg GGF enema directly. Subsequently, rats were given an enema once daily for 4 days.

## Detection indices and methods
### Specimen collection
Serum specimens: One hour after the last administration of the experiment, the rats in the three groups were anesthetized. Blood was collected from the abdominal aorta, and two yellow tubes (4 ml/tube) were placed at room temperature until the serum was separated. The blood samples were centrifuged at 3000 RPM for 10 min to prepare serum. Renal tissue specimen: The kidney was rinsed with cold saline, and the capsule, connective tissue, and blood vessels were removed. One kidney was longitudinally cut and preserved in formalin for subsequent sectioning for light microscopy. The other was put into liquid nitrogen and frozen in the tube for kidney tissue homogenization and Western blot.

### Determination of serum biochemical indices
Renal injury indices (Cr, BUN, and UA) were detected according to the kit instructions.

### Detection of renal pathological changes
Renal tissue was used to observe the pathological changes

under a light microscope. Microscopic indices, including tubular epithelial hyperplasia, brush edge disappearance, vacuolar degeneration, and epithelial detachment, were observed.

### Western blotting was used to detect the expression of mammalian target of rapamycin and Janus kinase 2 proteins in renal tissue

The whole protein of kidney tissue was extracted and the protein content in the supernatant was determined according to the kit instructions. After incubation with the upper sample buffer solution, equal amounts of protein were taken from each group for gel electrophoresis and then transferred to PVDF membrane semi-dry. After blocking, primary antibody was added and hybridized at 4°C. The next day, secondary antibody was added at room temperature for incubation. ECL developer was added for development imaging, and gray value semi-quantitative analysis was performed according to ImageJ software National Institutes of Health.US.

### Statistical methods

SPSS version 18.0 Statistical Product and Service Solutions, US statistical software was used for statistical analyses. Measurement data consistent with normal distribution were expressed as mean ± standard deviation (X ± s). One-way ANOVA was used for comparison between groups, and $t$-tests were performed for multiple comparisons. $P < 0.05$ was considered statistically significant.

## RESULTS

### Result of serum biochemical indices

As shown in Figure 1 that the levels of Cr, BUN, and UA in the experimental group (Group G) were lower than those in the control group (Group S) (all P < 0.05).

### Result of pathological findings of the renal medulla

As shown in Figure 2, compared to control group (Group S) the renal medulla pathological findings have less Edema, degeneration in experimental group (Group G).

### Result of Western blot for mammalian target of rapamycin and Janus kinase 2

As shown in Figure 3, compared to Group S, the activity of mTOR and JAK2 in Group G was significantly inhibited.

## DISCUSSION

### Research status of sepsis-associated acute kidney injury

The mortality rate of patients with S-AKI is higher than that of patients with nonsepsis acute kidney injury,[5] so early intervention of S-AKI is very important. The treatment of S-AKI using Western medicine has demonstrated that protectin DX[6] can alleviate S-AKI by inhibiting the activity of NF-kB. Furthermore, glycyrrhizic acid[7] and ulinastatin[8] can reduce kidney damage of sepsis mice to a certain extent. ABT-719[9] does not reduce the incidence of AKI in clinical trials, and the most promising drug to treat S-AKI to date, human

recombinant alkaline phosphatase, has been associated with a negative result in JAMA 2018.[10] Therefore, at present, there remains a lack of effective treatment for S-AKI.

In the TCM understanding of S-AKI, it is named after Guan Ge. This etymology was derived from Yu Jiayan[11] "Medical Law Guan Ge Theory," which said that Zhang Zhongjing's theory on Guan Ge "three great laws of reopening… Probably in fear of its vanity." Studies have shown that asthenia syndrome septic shock is more likely to be combined with acute kidney damage,[12] and TCM treatment includes promoting blood circulation to remove blood stasis.[13]

### Renal protective effect of GuangeFang on acute kidney injury in sepsis

This study used GGF, because in professor Qing-Quan Liu's experience, he believed that the sepsis and the pathogenesis of S-AKI is mainly stasis poison resistance, debility Yin, and harm Yang loss.[14] GGF uses raw rhubarb to relieve spilled hot, to the pathogens in a way, and uses cassia twig to temperature Yang, and raw oysters to supply Yin.

Preliminary clinical study results of this study demonstrated that GGF enema can assist in reducing creatinine and urea nitrogen in patients with S-AKI, and increase the 24-h urine volume of patients, which has a protective effect on the kidneys of patients.[15] In addition, GGF can reduce the inflammatory response in patients with S-AKI to a certain extent. However, the target and the mechanisms of action of GGF remain unclear. As shown in figure 4, mTOR signaling pathway and JAK2 signaling pathway were at the front of the network pharmacology analysis. We guess mTOR and JAK2 signaling pathway maybe the mechanism of kidney protection of GGF. So in this study, animal experiments were conducted to explore the mechanism of kidney protection of GGF.

Thirty-six SD rats were selected and randomly divided into a control group (Group N), a sepsis control group (Group S), and a sepsis + GGF group (Group G) with 12 rats in each group. The rats in Group N were treated with 8 ml/kg of 0.9% sodium chloride enema, and the rats in Group S were treated with 8 ml/kg of 0.9% sodium after 1 h of CLP modeling. The rats in Group G were treated with GGF enema after 1 h of modeling. These enemas were applied once daily for a total of 4 days. The indices of Cr, BUN, UA, mTOR, and JAK2 in each group were compared. The results showed that the levels of Cr, BUN, and UA in the experimental group (Group G) were lower than those in the control group (Group S) (all $P < 0.05$). Compared to Group S, the activity of mTOR and JAK2 in Group G was significantly inhibited.

The experimental results suggest that GGF can protect rats with S-AKI by inhibiting the JAK2/mTOR signaling pathways. This is similar to the results found by Wang Fei *et al*.[16] who demonstrated through animal experiments that inhibition of the JAK2 signaling pathway can alleviate acute kidney injury after hepatic cold ischemia-reperfusion in rats.

## Conclusion

GGF may improve S-AKI in rats by inhibiting JAK2 or mTOR signaling pathways.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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