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ORIGINAL ARTICLE

Dexmedetomidine Alleviates Ferroptosis Induced by Sepsis-Induced Renal Injury by Activating Keap1-Nrf2 Signaling Pathway

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SUMMARY

Background: **The purpose of this study was to investigate the protective effect of dexmedetomidine (DEX) on sepsis-induced acute kidney injury (AKI) and its possible mechanisms.**
Methods: **A total of 40 mice were randomly divided into the control group (C group), lipopolysaccharide treatment group (LPS group), LPS+DEX group, and ferrostatin-1 group (LPS+Fer-1 group). Mice in the LPS group were intraperitoneally injected with LPS (10 mg/kg), while mice in the LPS+DEX and LPS+Fer-1 groups were intraperitoneally injected with Dex (30 μg/kg) and Fer-1 (10 mg/kg), 1 hour before LPS injection, respectively. Mice in the control group were infused with the same volume of saline. Serum creatinine (SCr), blood urea nitrogen (BUN) and the levels of superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) in renal tissue were measured. HE staining was used to evaluate the degree of kidney tissue injury. Immunohistochemistry and western blot were used to detect the protein expressions of FTH, TFR, Keap1, and Nrf2 in kidney tissue.**
Results: **Compared with the control group, the serum levels of SCr and BUN were significantly increased, the levels of SOD and T-AOC in the kidney were decreased, the MDA level and renal injury score were increased, the expression of FTH and Nrf2 protein was reduced, and the expression of TFR and Keap1 protein was increased in the LPS group ($p < 0.05$). Compared with the LPS group, the serum levels of SCr and BUN were significantly decreased, the levels of SOD and T-AOC in the kidney were increased, the MDA level and renal injury score were decreased, the expression of FTH and Nrf2 protein was increased, and the expression of TFR and Keap1 protein was decreased in the LPS+DEX group ($p < 0.05$).**
Conclusions: **Dex can alleviate sepsis-associated acute kidney injury by activating the Keap1/Nrf2 pathway.**
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KEYWORDS

dexmedetomidine, sepsis-associated acute kidney injury, ferroptosis, Keap1-Nrf2 signaling pathway

INTRODUCTION

Sepsis is a systemic inflammatory response syndrome, usually caused by infection, which can lead to multiple organ dysfunction, and the kidney is one of the most common organs infected, which is known as sepsis-associated acute kidney injury (SA-AKI). SA-AKI is associated with high morbidity and mortality, and sepsis remains one of the major diseases with high mortality worldwide [1,2]. SA-AKI is closely related to microcirculation dysfunction, cell metabolic reprogramming, and inflammatory dysregulation, and its prognosis faces great challenges [3]. So, we are urgently looking for effective treatments to reduce and prevent kidney injury caused by sepsis. Ferroptosis is an oxidative cell necrosis catalyzed by iron, which is mainly manifested by the accumulation of iron and the massive production of lipid peroxidation products and ROS [4]. Studies have found that inhibiting ferroptosis can reverse kidney injury and protect the kidney in different AKI models, but the specific mechanism is still unclear [5].

Dex, a highly selective α_2 adrenoceptor agonist, can inhibit the activity of the sympathetic nervous system and reduce the release of inflammatory mediators, thereby reducing inflammatory damage to the kidney. Numerous studies have shown that Dex reduces ferroptosis-related inflammation and injury [6-11]. Therefore, we hypothesized that Dex could alleviate sepsis-associated renal injury by inhibiting the occurrence of ferroptosis. This study expands the theoretical basis of Dex protecting the kidney and provides a stronger basis for the application of Dex in patients with sepsis. We established an LPS-induced acute kidney injury (SA-AKI) mouse model to explore the renoprotective effect of Dex, aiming to elucidate the underlying mechanism of Dex attenuating ferroptosis in sepsis-associated renal injury.

MATERIALS AND METHODS

Medicines and reagents

DEX (Hengrui Pharmaceutical Co., Ltd., Jiangsu, China); LPS (Merck, USA); Fer-1 (Selleck, USA); SCr and BUN detection kits (Jiancheng, Nanjing, China); MDA, SOD and T-AOC detection kits (Beyotime, Jiangsu, China); Ferritin heavy chain (FTH), transferrin (TFR), Kelch-like Ech-associated protein 1 (Keap1), nuclear factor E2-related factor 2 (Nrf2) protein, and β-actin (Affinity, Jiangsu, China) were used.

Experimental animals

The animal study was approved by the Laboratory Animal Management and Ethics Committee of Bengbu Medical University (approval number: 2023370). Male C57 mice, 7 - 8 weeks of age, were obtained from Jiangsu Wukong Biotechnology Co., Ltd.

Grouping and administration

After 7 days of adaptive feeding, 40 mice, weighing 25 ± 1 g, were selected and divided into the control group, LPS Group, LPS+DEX group, and LPS+Fer-1 group. There were 10 mice in each group. Mice in the LPS group were intraperitoneally injected with LPS (10 mg/kg), while mice in the LPS+DEX and LPS+Fer-1 groups were intraperitoneally injected with Dex (30 μg/kg) and ferroptosis inhibitor (10 mg/kg), 1 hour before LPS injection, respectively. Mice in the control group were infused with the same volume of saline. The 3 R principle was followed for animal rearing and experimental procedures.

12 hours after administration, the mice were anesthetized with 4% paraformaldehyde, and eyeball venous blood was collected. Part of the mouse kidneys were fixed in 10% neutral formaldehyde for 24 hours and sent to the Department of Pathology at the First Affiliated Hospital of Bengbu Medical University. The remaining kidneys were collected in sterile tubes, snap-frozen in liquid nitrogen, and stored at -80℃. The mice were then euthanized.

Renal pathology

Renal tissues fixed with 4% paraformaldehyde were cut into 4 μm thick slices and embedded in paraffin. The eosin staining solution was used to stain the sections. Images were taken by using a light microscope at a magnification of 400 ×. The degree of renal injury was judged by three senior physicians of the Pathology Department of our hospital according to the Paller scoring standard; under a light microscope in a blind way, the renal tubular scores were calculated and the average value was taken.

Detection of blood indexes

The detection kits of SCr and BUN were used to detect the levels of SCr and BUN in mice, and all steps were carried out in strict accordance with the instructions of the kit.

Renal inflammation and oxidative stress

The levels of MDA, SOD, and T-AOC in renal tissues were measured. The working solution was prepared according to the instructions of the kit, and the corresponding working solution and the samples to be tested were added in sequence. The mixed samples were centrifuged, and the absorbance values were detected at 523 nm, 450 nm, and 520 nm, respectively.

Immunohistochemistry

Renal tissues fixed with 4% paraformaldehyde were sectioned into 4 μm thick segments and embedded in paraffin. Sections were deparaffinized with xylene and rehydrated. Sections were treated with 3% hydrogen

peroxide to block endogenous peroxidase activity and inactivated with 10% goat serum for nonspecific binding. The sections were incubated with the primary antibody overnight at 4℃, followed by incubation with the secondary antibody for 1 hour at 37℃. Color development was observed by the addition of a fresh DAB reaction mixture. Sections were dehydrated and counterstained with hematoxylin and sealed. Finally, each section was photographed using a light microscope. Blinded observers quantified the ratio of positive areas in each section.

Western blot

Protein expression levels in kidney tissues of mice were determined by western blot, and the tissues were lysed in RIPA buffer containing 1% PMSF to extract kidney tissue proteins. After gel electrophoresis, transmembrane, blocking, and protein samples were rinsed with TBST and incubated with different primary antibodies overnight at 4℃. The membranes were incubated with secondary antibodies (Affinity) for 2 hours at room temperature, after washing with TBST. Finally, the target bands were visualized by using a chemiluminescent imaging system (5200, Tanon, China) combined with a Beyo ECL Plus kit (Beyotime Biotechnology) and quantified by using Image J software (NIH, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were performed by using SPSS 27.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Quantitative data were presented as $\bar{x} \pm s$. The *t*-test and ANOVA were applied to compare quantitative data between the groups. $p < 0.05$ was considered significant. GraphPad Prism 10.1.2 software was used for data statistical analysis and statistical chart drawing.

RESULTS

Kidney function in mice

The levels of SCr and BUN in the LPS group were significantly higher than those in the control group ($p < 0.05$). Compared with the LPS group, the levels of SCr and BUN in the LPS+Dex group were significantly decreased ($p < 0.05$) (Table 1). After the use of ferroptosis inhibitor, the abovementioned indexes also showed significant improvement. The results showed that Dex could reduce sepsis-associated acute kidney injury and improve renal function.

Pathological changes of kidney in mice

In the LPS group, renal tubular epithelial cells were flat, interstitial hyperemia and edema, cell necrosis, and a large number of inflammatory cell infiltrations were observed, and the injury was very significant. Compared with the LPS group, the inflammation was significantly reduced after Dex pretreatment. The renal injury score in the LPS group was higher than that in the control group ($p < 0.05$). In addition, the pathological injury score in the LPS+Dex group was lower than that in the LPS group ($p < 0.05$) (Figure 1). The results showed that Dex could significantly reduce LPS-induced sepsis-associated renal injury.

Table 1. The effect of Dex on SCr and BUN in mice.

Group	SCr (μmol/L)	BUN (mmol/L)
Control	15.52 ± 1.40	12.01 ± 0.99
LPS	31.34 ± 2.72 **	29.64 ± 6.61 **
LPS + DEX	21.03 ± 2.60 *##	20.54 ± 1.46 **##
LPS + Fer-1	18.88 ± 2.22 *##	21.28 ± 2.14 **##

Compared with control group - * $p < 0.05$, ** $p < 0.01$, compared with LPS group - ## $p < 0.01$.

Changes in MDA, SOD, and T-AOC levels in mouse kidneys

The level of MDA in renal tissue in the LPS group was significantly higher than that in the control group and the levels of SOD and T-AOC were lower than those in the control group (all $p < 0.05$). Compared with the LPS group, the MDA level was decreased and the SOD and T-AOC levels were significantly increased in the LPS+Dex group (all $p < 0.05$) (Table 2). At the same time, we found that ferroptosis inhibitor Fer-1 could also inhibit oxidative stress and enhance the antioxidant capacity of the kidney, which confirmed that the protective effect of Dex on the kidney may be mediated by inhibiting ferroptosis.

Expression of FTH and TFR in mouse kidney

The protein expression levels of ferritin heavy chain (FTH) and transferrin receptor (TFR) in renal tissue of mice were detected by immunohistochemistry and western blot. Compared with the control group, the expression of FTH protein in renal tissues was significantly decreased and the expression of TFR protein was significantly increased in the LPS group ($p < 0.05$). The LPS+Dex group had a significantly higher protein expression of FTH and a significantly lower protein expression of TFR than the LPS group ($p < 0.05$) (Figure 2). The results indicate that ferroptosis plays a key role in renal injury in sepsis and that Dex can reverse renal injury caused by ferroptosis by inhibiting the occurrence of ferroptosis.

Keap1 and Nrf2 expression in mouse kidney

The protein expression levels of Keap1 and Nrf2 in the renal tissue of mice were detected by immunohistochemistry and western blot. Compared with the control group, the expression level of Keap1 protein in the renal tissue of mice in the LPS group was significantly increased and the expression level of Nrf2 protein was significantly decreased ($p < 0.05$). The LPS+Dex group

Table 2. The effect of Dex on MDA, SOD, and T-AOC in mice.

Group	MDA (nmol/mg)	SOD (U/mg)	T-AOC (U/mg)
Control	2.69 ± 0.15	81.08 ± 4.85	5.94 ± 0.37
LPS	4.64 ± 0.29 **	49.38 ± 13.11 **	3.60 ± 0.21 **
LPS + DEX	3.50 ± 0.21 #	61.65 ± 6.67 *	4.45 ± 0.20 **#
LPS + Fer-1	3.34 ± 0.23 #	60.67 ± 8.72 *	4.56 ± 0.17 **#

Compared with control group - * p < 0.05, ** p < 0.01, compared with LPS group - # p < 0.05.

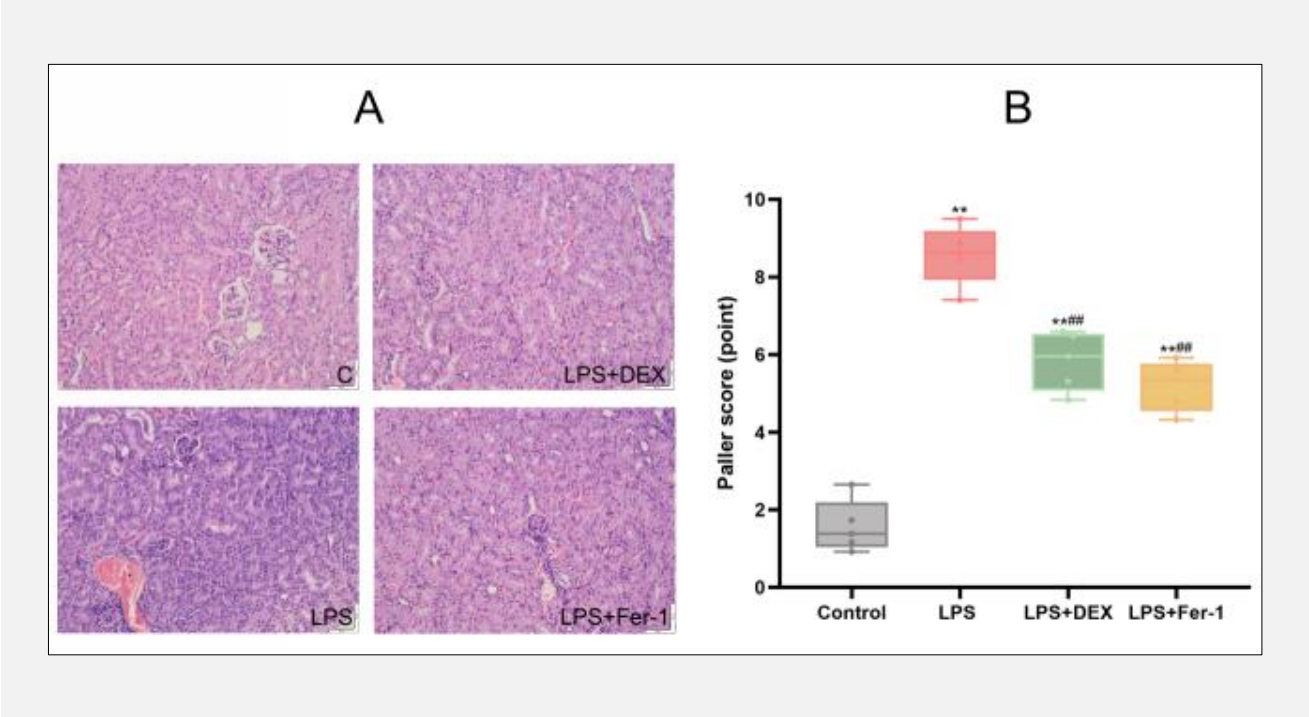

Figure 1. The pathological kidney changes in mice.

A - Renal pathological changes under the light microscope (HE × 400), B - pathological score of renal injury. Compared with control group - ** p < 0.01, compared with LPS group - ## p < 0.01.

had a significantly lower expression of Keap1 protein and a significantly higher expression of Nrf2 protein than the LPS group ($p < 0.05$) (Figure 3). The results indicate that ferroptosis plays a key role in renal injury in sepsis and that Dex can reverse renal injury caused by ferroptosis by inhibiting the occurrence of ferroptosis.

DISCUSSION

In this study, we measured the levels of renal function-related indicators and ferroptosis-related indicators in mice and confirmed that ferroptosis occurs in LPS-induced sepsis kidney injury and that Dex can effectively inhibit ferroptosis; the effect is similar to that of ferroptosis inhibitor, both can inhibit oxidative stress injury. This study accurately confirmed the key role of Dex in inhibiting sepsis and reducing kidney injury. At the same time, we identified a key pathway in this process, the Keap1-Nrf2 pathway, the activation of which has been shown to inhibit ferroptosis in vivo and in vitro [12-14]. Therefore, we propose the hypothesis that Dex can inhibit ferroptosis by activating the Keap1-Nrf2 pathway to alleviate renal injury induced by LPS. Dex, as our therapeutic agent of choice, decreased Keap1 protein expression, and the reduction of Keap1 expression further increased Nrf2 expression, thereby inhibiting ferroptosis and saving from septic kidney injury.

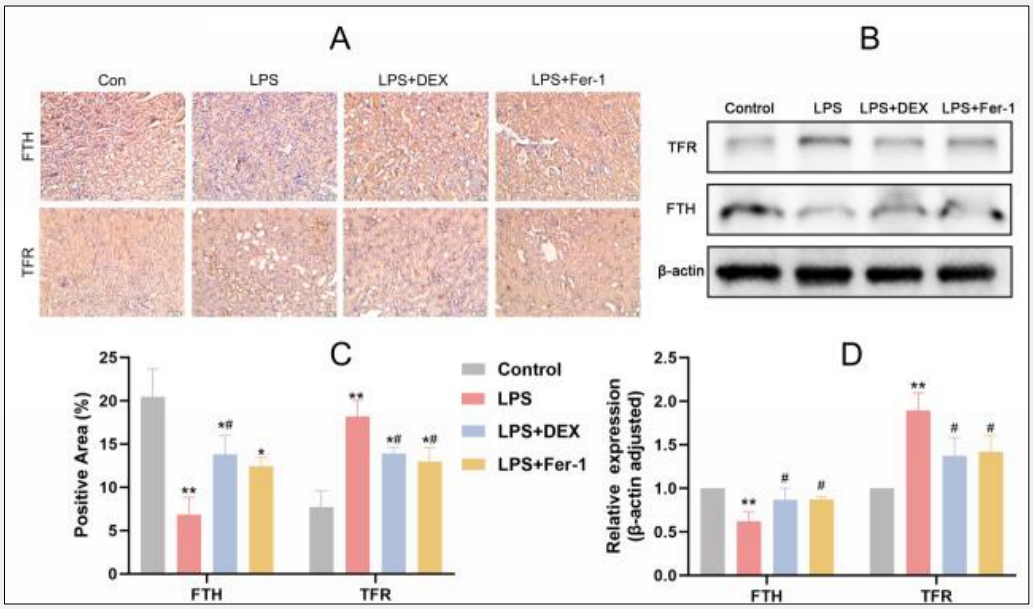

Figure 2. The effect of Dex on the expression of FTH and TFR in mice renal tissues.

A - Immunohistochemical results of renal tissues (IHC × 400), B - western blot band pattern, C - statistical map of the immunohistochemical positive area, D - chart of the statistical analysis of protein levels.
Compared with control group - $^{*}p < 0.05$, $^{**}p < 0.01$, compared with LPS group - $^{\#}p < 0.05$.

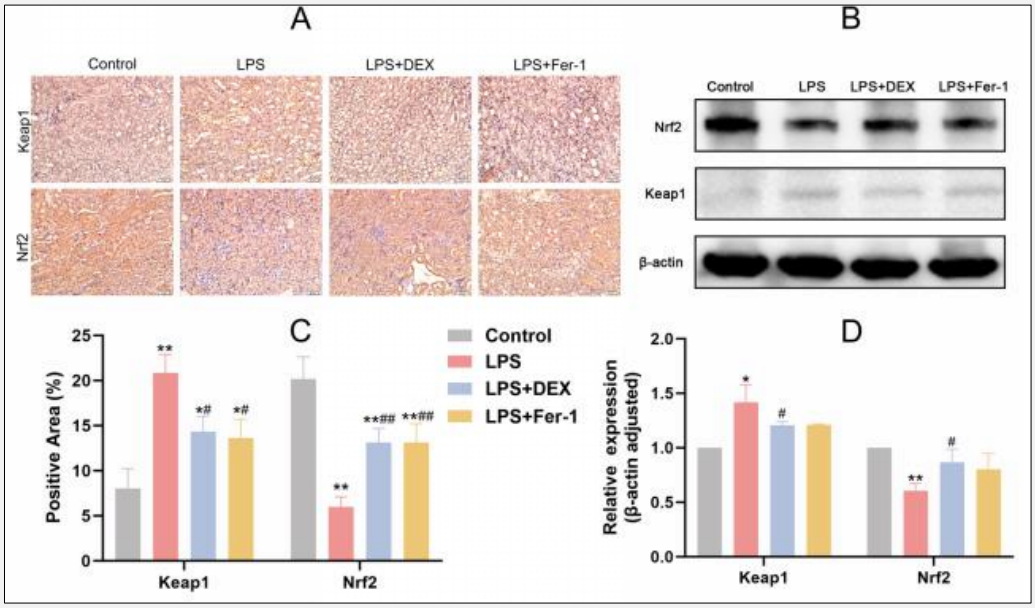

Figure 3. The effect of Dex on the expression of Keap1 and Nrf2 in mice renal tissues.

A - Immunohistochemical results of renal tissues (IHC × 400), B - western blot band pattern, C - statistical map of the immunohistochemical positive area, D - chart of the statistical analysis of protein levels.
Compared with control group - $^{*}p < 0.05$, $^{**}p < 0.01$, compared with LPS group - $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

These results indicate that Dex has great potential for the treatment of sepsis-associated kidney injury.

Sepsis is a life-threatening organ dysfunction and one of the most common causes of death in critically ill hospitalized patients. It can release bacterial toxins into the blood circulation, which can cause severe systemic infection. Severe sepsis can lead to multiple organ dysfunction, mainly heart, lung, and kidney dysfunction, and is the most common cause of acute kidney injury [15,16]. Studies have shown that the development of acute kidney injury is closely related to ferroptosis [17].

Ferroptosis is a novel form of cell death that has been shown to play an important role in a variety of diseases and is closely related to fatal infections [18]. The execution of ferroptosis is driven by iron-dependent phospholipid peroxidation, so we can modulate ferroptosis by controlling lipid peroxidation as well as various other cellular processes associated with lipid peroxidation [19]. Studies have shown that Dex can effectively improve a variety of disease damages caused by ferroptosis by reducing oxidative stress injury and can improve renal function and in-hospital survival in critically ill patients with SA-AKI [20-23].

FTH is a cytoplasmic iron storage protein that regulates several physiological processes, including ferroptosis, autophagy, oxidative stress, and inflammation [24]. In mice with acute kidney injury, the expression of FTH, one of the key ferroptosis-related proteins, is decreased. Previous studies have shown that Dex can inhibit ferroptosis by enhancing the expression level of FTH and can ultimately reduce myocardial I/R injury in mice [25]. In our study, we demonstrated that FTH protein degradation is a key mechanism for the occurrence of ferroptosis in a mouse model of acute sepsis.

TFR is a major regulator of iron uptake in cells and can be used as a major marker of ferroptosis [26]. It has been demonstrated that overexpression of TFR in rats promotes sepsis-induced ferroptosis in brain microvascular endothelial cells [27]. Recent studies have also found that LPS-induced inflammation in adipocytes disrupts iron homeostasis, leading to ferroptosis in adipocytes, which in turn leads to the significant upregulation of TFR expression [28]. In our study, we similarly demonstrated that elevated TFR protein expression is a key mechanism for the development of ferroptosis in a mouse model of acute sepsis.

Keap1 is a homodimeric protein, and Nrf2 is the main mediator of various cellular protective responses. The Keap1-Nrf2 pathway is a well-known protective mechanism and an important antioxidant pathway. It has protective effects against oxidative stress and electrophilic stress [29-31]. A large number of studies have found that regulating the Keap1-Nrf2 pathway can effectively reduce oxidative stress injury and ferroptosis injury, so studying this pathway is of great significance for clinical treatment of sepsis-induced acute kidney injury [32-35].

Our results suggest that Dex protects against sepsis-induced renal injury by inhibiting ferroptosis by regulating the expression of Keap1-Nrf2 pathway. Meanwhile, we found that ferroptosis inhibitor Fer-1 exerted similar renoprotective effects as Dex, which further confirmed that Dex may alleviate oxidative stress injury by inhibiting ferroptosis, thereby reducing acute kidney injury caused by LPS sepsis.

CONCLUSION

In conclusion, the present study provides evidence that Dex attenuates sepsis-associated renal injury by inhibiting ferroptosis. Our study demonstrated that ferroptosis mediated LPS-induced acute kidney injury. Dex can significantly upregulate the expression of FTH and downregulate the expression of TFR in sepsis-associated kidney injury by activating the Keap1-Nrf2 pathway. This study confirms that Dex can inhibit sepsis-associated renal injury through the Keap1-Nrf2 pathway and also provides new ideas and methods for clinical treatment of renal injury.

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Ethical Approval Statement:

The animal study was reviewed and approved by the Animal Ethics Committee of Bengbu Medical University (approval number: 2023370).

Declaration of Interest:

No potential conflicts of interest concerning this article were declared.

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