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ORIGINAL RESEARCH

Hispidulin protective impact on sepsis induced acute kidney injury is mediated by regulation of AKT and NF-κB pathway

Haihong Xu[1], Xiaomei Chen[1], Daoqin Liu[1], Yanlang Yang[1,*]

[1]Department of Nephrology, Wannan Medical College Affiliated Yijishan Hospital, 241000 Wuhu, Anhui, China

***Correspondence**
yyl0_dr@163.com
(Yanlang Yang)

Abstract

Sepsis-induced acute kidney injury is associated with inflammatory dysregulations within the kidney. This study aimed to explore the renal protective effect of hispidulin on suppressing the apoptosis rate, and inhibiting reactive oxygen species production and inflammatory response after cecal puncture (CLP) operation. In order to gain a deeper understanding of the relationship between sepsis and acute kidney injury, the CLP induced kidney injury animal model was established. The automated biochemical analyzer was used to measure the kidney function related biomarkers including serum cystatin C (ScysC), blood urea nitrogen (BUN), and serum creatinine (Scr). The pathological changes of damaged kidney tissues were detected by hematoxylin and eosin (H&E) staining. The expression of inflammatory cytokines including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) were detected by their corresponding test kits and Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). The level of reactive oxygen species production-related protein including myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) in kidney tissue from each group were quantized using Enzyme-linked immunosorbent assay (ELISA). The protein expression was measured using western blot and the apoptotic rate of kidney tissue was measured by terminal deoxynucleotidyl transferase Deoxyuridine Triphosphate (dUTP) nick end labeling (TUNEL) assay. Our results revealed that hispidulin has the protective ability in sepsis-induced acute kidney injury. The potential mechanism of hispidulin on sepsis-induced cell apoptosis, oxidative stress and inflammatory response was also investigated. Finally, our results highlighted that hispidulin exerted a protective effects on CLP-induced acute kidney injury by suppressing the protein kinase B (AKT) and Nuclear factor kappa B (NF-κB) signaling pathways. In summary, the current study provided a piece of novel evidence, that hispidulin can be explored as a potential drug in CLP-induced acute kidney injury by examining its effects on suppressed the oxidative stress, inflammatory responses, and apoptosis in kidney tissue.

Keywords

Sepsis; Acute kidney injury; Hispidulin; AKT; NF-κB

1. Introduction

Sepsis is the main reason of acute kidney injury initiated *via* the dysregulated host response to infection. It is prevalent in ~50% of acute kidney injury incidents in intensive care units (ICUs) [1]. Sepsis linked acute kidney injury predicts a 6- to 8-fold enhanced death probability in sepsis patients. Moreover, there is an enhanced occurrence in progression of chronic kidney disease found in the prognosis of sepsis [2]. Multiple complex mechanisms have role in development and progression of acute kidney injury due to sepsis, but the physiological mechanisms of acute kidney injury are not yet fully elucidated. Therefore, effective therapies cannot be identified. To improve the prognosis of patients with acute kidney injury caused by sepsis, it is imperative to explore new approaches and their potential mechanisms [3].

The downstream glycogen synthase kinase-3β can phosphorylate by the activation of Phosphoinositide 3-kinases (PI3K)/Akt and has role in modulating inflammatory responses or cellular oxidative stress in a variety of diseases [4–6]. Several studies have reported that the inflammation progression is regulated *via* NF-κB signaling pathway by modulating expression of pro-survival and pro-inflammatory genes for controlling the cell survival and immune response [7]. Hispidulin, a flavonoid found in many Chinese herbal

medicines, has multiple properties including anti-oxidant, anti-fungal, anti-tumor, anti-osteoporotic, anti-inflammatory and anti-mutagenic [8]. In an animal model of cerebral ischemia-reperfusion, pretreatment with hispidulin improves brain function and reduces infarct size and cerebral edema [9]. In a high-glucose-induced podocyte injury model, apoptosis progression inhibition and autophagy activation through hispidulin treatment could protect the damaging of cells induced by high-glucose. Nevertheless, protective impact of hispidulin on acute kidney injury induced by sepsis is not reported in mice.

In this work, the roles of hispidulin are demonstrated in acute kidney injury induced *via* CLP by examining whether kidney cell apoptosis, inflammatory response impairment and oxidative stress induced by CLP surgery are linked with AKT and NF-κB pathway activation.

2. Methods

2.1 Animal model

Twelve hours each of light and dark cycle was maintained at constant temperature to keep 50 adult BALB/c mice weighing 30–40 g of 6–8 weeks age. The operation of cecal ligation and puncture (CLP) used in this study was executed to activate kidney injury induced by sepsis as reported earlier [10]. All animals were distributed randomly into 5 groups named as Sham operated; CLP operated; CLP + hispidulin (20 mg/kg); CLP + hispidulin (40 mg/kg); and CLP + hispidulin (60 mg/kg). Hispidulin treatment was performed one hour after CLP surgery, with intraperitoneal injections of different concentrations of Hispidulin once a day for 3 days. Sigma-Aldrich provided the Hispidulin.

2.2 Staining by hematoxylin and eosin (H&E)

The H&E stained section was used to determine the pathology diagnosis of kidney dysfunction after CLP surgery. The kidney tissue was obtained after sacrificing the mice and fixed with 10% paraformaldehyde after experiments. Then, the mice's kidney tissue was sliced to five μm-thickness after embedding in paraffin. Hematoxylin and eosin (H&E) were employed to stain the sections. The kidney histological alters were observed with light microscope (Eclipse TE2000-U, Nikon, Tokyo, Japan), and imaged by the camera attached to microscope.

2.3 Kidney function determination

The collection of blood samples was made from each group, and the automated biochemical analyzer (AU 5400, Olympus, Tokyo, Japan) was employed to measure the expression levels of blood urea nitrogen (BUN), serum creatinine (Scr), levels were measured by commercial ELISA kits (DKM100, R&D System, US) in accordance with respective manufacturer's protocols.

2.4 Western blot

The kidney tissues from each group were collected, homogenized with Radioimmunoprecipitation Assay (RIPA) buffer, and centrifuged at 15,000 g for 10 min at 4 °C. 25 μg protein from each sample were separated on 8% Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, moved to nitrocellulose membranes, and incubated overnight at 4 °C with primary antibodies against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:10,000, sc-47724, Santa Cruz Biotechnology), P65 (1:3000, sc-109, Santa Cruz Biotechnology), p-P65 (1:2000, ab86299, Abcam), IκBα (1:5000, 9242, Cell Signaling Technology), p-IκBα (1:2000, 2859, Cell Signaling Technology), AKT (1:8000, ab8805, Abcam), p-AKT (1:3000, ab38449, Abcam), PI3K (1:8000, ab191606, Abcam), p-PI3K (1:2000, ab86714, Abcam), Poly (ADP-ribose) polymerases (PARP) (1:5000, 9542, Cell Signaling Technology), cleaved-PARP (1:2000, 5625, Cell Signaling Technology), Bax (1:8000, MA5-14003, Invitrogen), and B-cell lymphoma 2 (Bcl-2) (1:5000, MA5-11757, Invitrogen). The membrane was incubated for 1 hour at 37 °C with secondary antibody labelled with horseradish peroxidase. Enhanced chemiluminescence was utilized to visualize the proteins on membrane. GAPDH was taken as an internal control.

2.5 TUNEL assay

Mice kidney tissue from all groups was cut into 3 μm slices. Staining was made with TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling) kit (ab66110, Abcam, Cambridge, UK) as per manufacturer's guidelines. Tissue sections were stained by TUNEL mixture at 37 °C for 1 hour and put for 30 min in converter-peroxidase. Images were taken after drying for calculating the apoptosis-positive cells.

2.6 ELISA

The level of reactive oxygen species production-related protein including myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) in kidney tissue of every group were quantized at room temperature utilizing commercial ELISA kit as per manufacturer's procedure (Abcam). The inflammatory cytokines expression levels including interleukin 6 (IL-6), interleukin 1β (IL-1β), and tumor necrosis factor α (TNF-α) in kidney tissue of every group were quantified at room temperature utilizing ELISA as per manufacturer's procedure (R&D Systems).

2.7 qRT-PCR

Kidney tissue of every group was collected and treated for homogenization. Trizol (Invitrogen) digestion as per manufacturer's protocol was employed to isolate total RNA. The extracted RNA was measured for its concentration and purity through Nanodrop2000 (Thermo Fisher Scientific, Pennsylvania, USA). The cDNA was attained by reversing RNA *via* SuperScript III Reverse Transcriptase and SYBR Green Master Mix kits (4309155, Thermo Fisher Scientific, Pennsylvania, USA). GAPDH acted as the internal control. Comparative $2^{-\Delta\Delta Ct}$ method was used to calculate the RNA expression data. Primers of current work had the sequence as under (Table 1):

TABLE 1. Primers sequence.

Primer name	Sequence
TNF-α	Forward (5′-3′): GGGAGTAGACAAGGTACAAC Reverse (5′-3′): TCTCATCAGTTCTATGGCCC
IL-1β	Forward (5′-3′): ATGGCAACTGTCCCTGAACT Reverse (5′-3′): GTCGTTGCTTGTCTCTCCTT
IL-6	Forward (5′-3′): TGCTGGTGACAACCACGGC Reverse (5′-3′): GTACTCCAGAAGACCAGAGG
GAPDH	Forward (5′-3′): AACCCATCACCATCTTCCAGGAGC Reverse (5′-3′): ATGGACTGTGGTCATGAGCCCTTC

TNF: tumor necrosis factor; IL: interleukin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

2.8 Statistics

Every experiment was performed in triplicate. GraphPad Prism Software 7 (La Jolla, CA, USA) was utilized to examine the data. Quantitative data were reported as mean $\pm$ standard error of mean (SEM). The *t*-tests and one-way Analysis of variance (ANOVA) were employed for the comparisons. $p < 0.05$ was considered as the statistically significant difference.

3. Results

3.1 Hispidulin attenuates acute kidney injury induced by CLP

The animal model of acute kidney injury induced by sepsis was developed by widely used CLP-operated mice to evaluate hispidulin impact. Automated biochemical analyzer was used to measure acute kidney injury biomarkers Scr and BUN. The Scr and BUN expressions were obviously increased after CLP operation, compared to sham group. In contrast, a dose-dependent decline in Scr and BUN expressions was noted after the treatment by hispidulin (Fig. 1A,B). The influence of different dosages of hispidulin (20, 40 and 60 mg/kg) treatment on the recovery of kidney function following CLP operation was further investigated. Staining by H&E revealed that after CLP operation, renal tissue was remarkably edematous, with number of inflammatory cells infiltration in tissue, vague structure of glomerular, and narrowed renal cystic lumen. Furthermore, the renal tubules were deformed, and its brush border disappeared and the tubule epithelial cells shed. However, these histological changes were obviously attenuated in dose-dependent way after three consecutive days of hispidulin treatment (Fig. 1C). Taken together, the findings demonstrated that hispidulin mitigated acute kidney injury induced by CLP in dose-dependent manner.

3.2 Hispidulin reduced cellular apoptosis in CLP induced acute kidney injury

To explore protective effect of hispidulin on cellular apoptosis induced by CLP, mice underwent hispidulin treatment with different concentrations and then western blot and TUNEL assay were performed in kidney tissue from those mice. The results in Fig. 2A showed that CLP operation remarkably increased the apoptosis-linked Cleaved-PARP and protein Bax expression levels, while suppressing the level of Bcl-2 and PARP. As expected, hispidulin ameliorated the CLP operation induced apoptosis in dose-dependent way. Similar result was also found in the TUNEL assay. The quantity of apoptosis positive cells in CLP group was markedly enhanced compared to sham group. Cotreatment with hispidulin obviously minimized the quantity of apoptosis-positive cells in dose-dependent way contrary to CLP group (Fig. 2B). The outcomes proposed that hispidulin could obviously suppress cell apoptosis of the kidney during CLP-induced sepsis.

3.3 Hispidulin reduces CLP induced oxidative stress

To evaluate conservatory impact of hispidulin on oxidative stress induced by CLP in kidney tissue, levels of reactive oxygen species produced from kidney tissue was detected. The indirect markers expressions of oxidative stress including MDA and MPO in mice operated by CLP were substantially greater compared to those in sham group (Fig. 3). Conversely, anti-oxidant biomarkers including SOD and GSH in mice operated by CLP were considerably decreased. Consecutive treatment of hispidulin reduced MDA and MPO expressions, and increased the anti-oxidant biomarkers expression levels compared to CLP group (Fig. 3). The findings illuminate that hispidulin may have role in the regulation activities of oxidative stress induced by CLP.

3.4 Hispidulin suppresses CLP induced kidney inflammation

To further explore how hispidulin regulates kidney inflammatory response induced by CLP, change in the proinflammatory cytokines was evaluated through ELISA and real-time PCR. The findings in Fig. 4A showed that interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) expressions in mice operated by CLP were substantially increased in CLP group. This stimulatory effect of CLP surgery was completely attenuated in dose-dependent way by hispidulin (Fig. 4). Consistent with outcomes from real-time PCR, the result of ELISA showed that hispidulin treatment minimized proinflammatory cytokines levels including IL-6, IL-1β and TNF-α, induced by CLP operation (Fig. 4). These results demonstrated that hispidulin may be able to alleviate the injury related to sepsis-induced kidney inflammation.

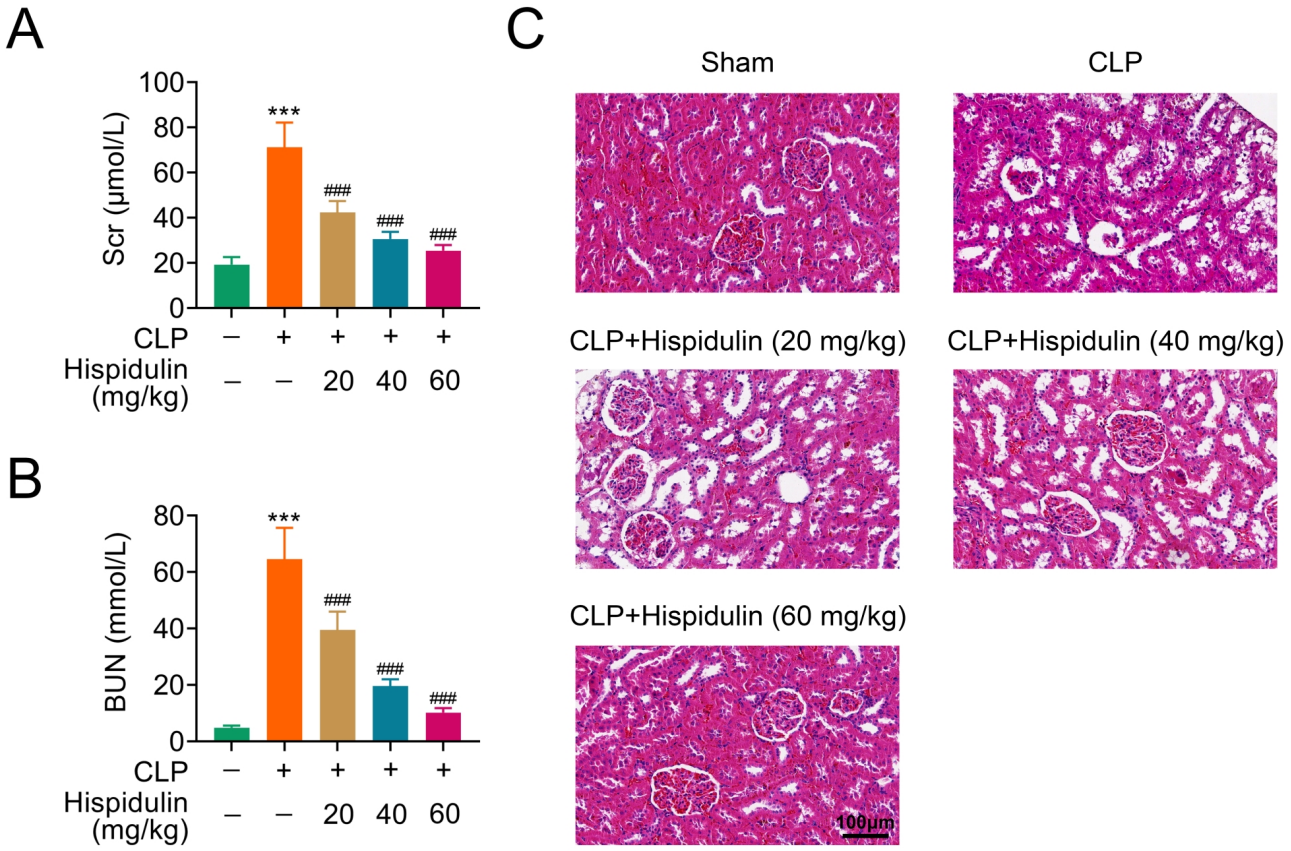

FIGURE 1. Hispidulin impacts on acute kidney injury induced by CLP in mice. (A,B) Automatic biochemical analyzer measured the expression levels of Scr and BUN. (C) Histopathological changes measured after CLP operation and hispidulin treatment by staining with H&E. ###$p < 0.005$ *vs.* CLP. ***$p < 0.005$ *vs.* sham. Data were presented as mean ± SEM. CLP: cecal ligation and puncture; Scr: serum creatinine; BUN: blood urea nitrogen.

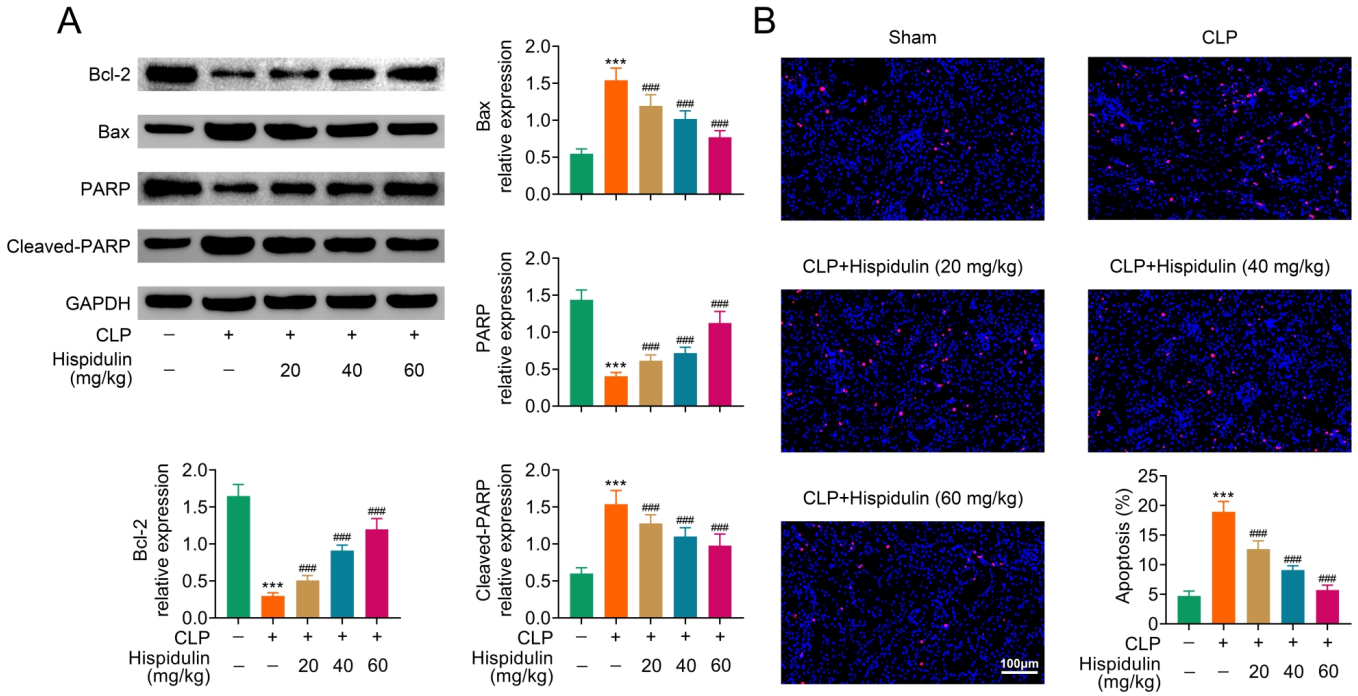

FIGURE 2. Impact of hispidulin on cellular apoptosis induced by CLP in kidney tissue. (A) Western blot analysis examined the protein expression of Bax, Bcl-2, PARP and Cleaved-PARP. (B) TUNEL assay evaluated apoptosis cell rate of kidney tissue. ###$p < 0.005$ *vs.* CLP. ***$p < 0.005$ *vs.* sham. Data were presented as mean ± SEM. CLP: cecal ligation and puncture; Bcl: B-cell lymphoma; PARP: Poly (ADP-ribose) polymerases; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

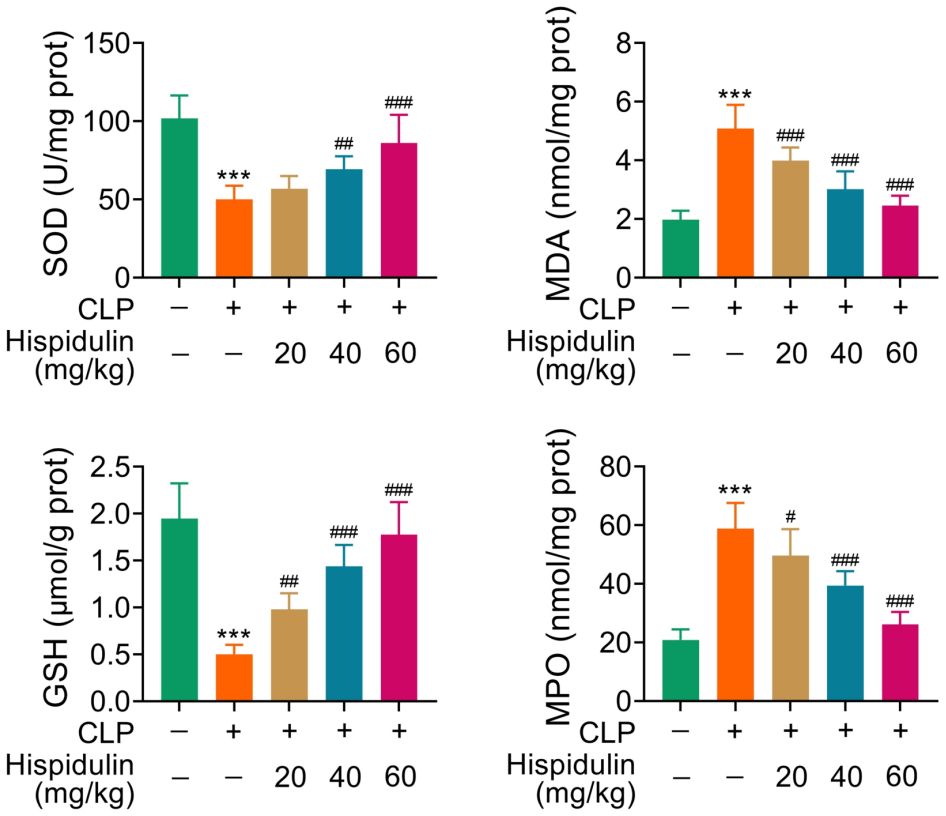

FIGURE 3. Impact of hispidulin on oxidative stress induced by CLP. Quantitative result of the expression level of protein related to reactive oxygen species. ***$p < 0.005$ *vs.* sham. #$p < 0.05$ *vs.* CLP. ##$p < 0.001$ *vs.* CLP. ###$p < 0.005$ *vs.* CLP. Data presented as mean ± SEM. CLP: cecal ligation and puncture; SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione; MPO: myeloperoxidase.

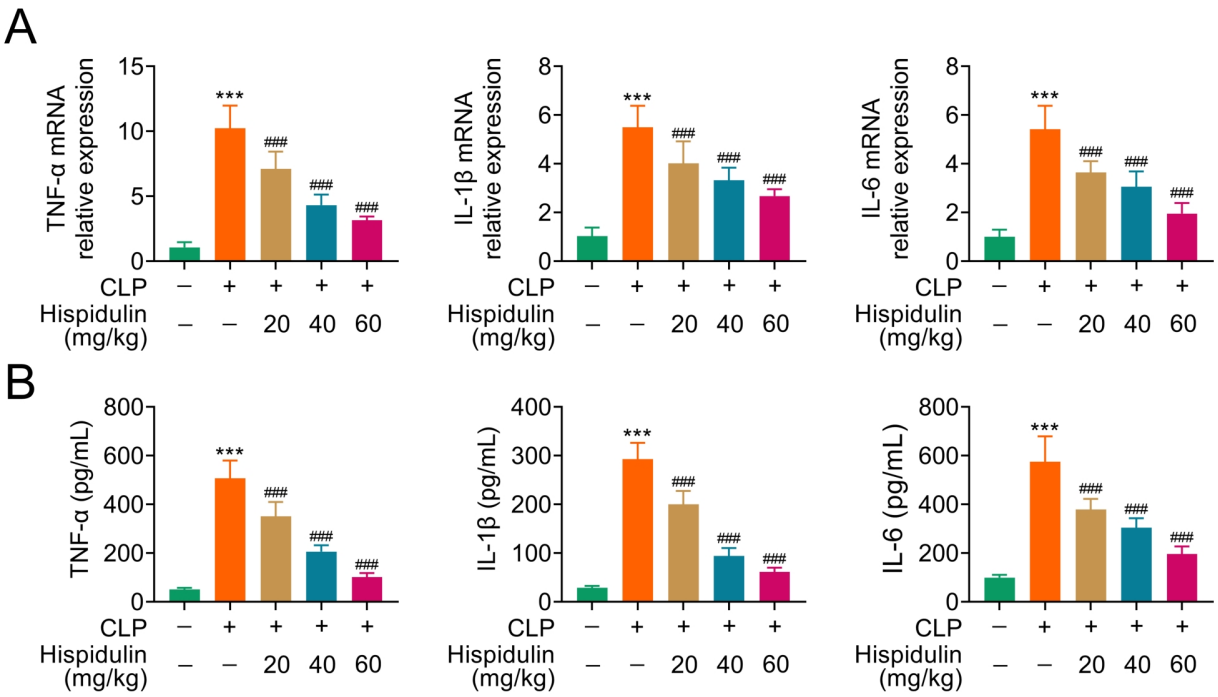

FIGURE 4. Impact of hispidulin on pro-inflammatory cytokines production induced by CLP in kidney tissue. (A) The mRNA expression levels of TNF-α, IL-6 and IL-1β induced by CLP were detected by RT-qPCR in kidney tissue. (B) The protein expression of IL-6, IL-1β and TNF-α was examined through ELISA. ###$p < 0.005$ *vs.* CLP. ***$p < 0.005$ *vs.* sham. Data were presented as mean ± SEM. CLP: cecal ligation and puncture; TNF: tumor necrosis factor; IL: interleukin.

3.5 Hispidulin attenuates AKT and NF-κB signaling pathways activation stimulated by CLP operation

We next explored whether hispidulin protective impacts were rescued by AKT and NF-κB signaling pathways inhibition through CLP operation and hispidulin treatment. The protein level of phosphorylation of PI3K, AKT, IκBα and P65 were remarkably increased in CLP than in sham group, which reversed in dose-dependent way by hispidulin treatment (Fig. 5). The findings reflected that hispidulin against acute kidney injury induced by CLP through modulated AKT and NF-κB pathways.

4. Discussion

Acute kidney injury induced by sepsis is associated with inflammatory dysregulation within the kidney which is induced by multiple risk factors such as bacterial infection [11]. In the kidney, unrestricted activation of inflammatory response could exacerbate the infection and lead to multiple organ failure and organ fibrosis [12]. The pathophysiology of sepsis-related acute kidney injury involves multiple factors, including the abnormal production of ROS (reactive oxygen species), overexpression of inflammatory cytokine release, intrarenal hemodynamic changes, and endothelial dysfunction. Previous study has shown that in septic patients, the pro-inflammatory cytokines including IL-6, TNF-α and IL-1β promote progression of kidney dysfunction; conversely, suppressing pro-inflammatory cytokines release contributes to ameliorating survival outcomes [13]. Hispidulin shows remarkable anti-inflammatory, anti-osteoporotic activities, analgesic, anti-oxidative and antipyretic effects [9]. In addition, hispidulin also exerts anti-proliferative effects in solid tumors like pancreatic cancer, renal cell carcinoma and ovarian cancer [14].

In this study, BALB/c mice were treated with hispidulin after CLP operation induced acute kidney injury to assess protective impacts of this compound in this particular disease. Hispidulin treatment showed effective protection against CLP-induced acute kidney injury. When CLP surgery mice was exposed to hispidulin, significant attenuation of the histological changes was observed in the kidney tissue, indicating that exposure to hispidulin could reduce the CLP induced acute kidney injury in dose-dependent way. Elevated reactive oxygen species caused more extensive and irreversible cell damage through pro-inflammatory cytokine release, DNA damage, and cellular necrosis or apoptosis [15]. Pro-inflammatory molecules produced by reactive oxygen species under stressful conditions cause an inflammatory response. Acute kidney injury is induced by an exaggerated expression level of inflammatory factors like IL-6 and TNF-α, including tissue damage or aggravated apoptosis [16]. Sepsis induces apoptosis through reactive oxygen species release, and reduce anti-inflammatory cytokine levels in kidney tissue [17]. During sepsis, inflammation is a necessary step against the pathogen, and immune cell overactivation is avoided through this sternly controlled immune response.

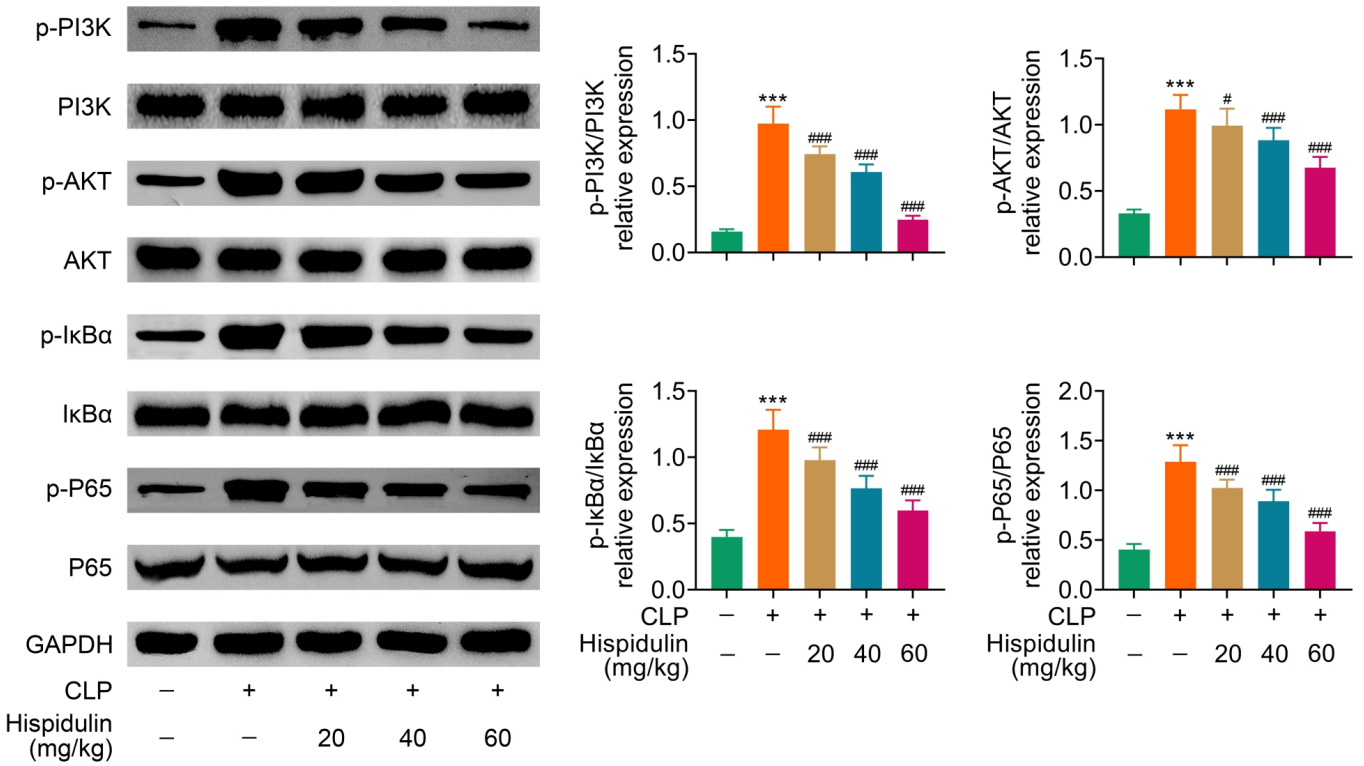

FIGURE 5. Impact of hispidulin on NF-κB and AKT signaling pathways activation induced by CLP. Protein expressions of p-P65, P65, p-IκBα, IκBα, p-AKT, AKT, p-PI3K and PI3K were evaluated by Western blot analysis. ***$p < 0.005$ *vs.* sham. #$p < 0.05$ *vs.* CLP. ###$p < 0.005$ *vs.* CLP. Data were presented as mean ± SEM. PI3K: Phosphoinositide 3-kinases; AKT: protein kinase B; CLP: cecal ligation and puncture; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

The results from this study were in line with these observations. CLP operation led to elevated pro-inflammatory cytokines expression levels compared with those of the sham groups. Contrarily, hispidulin treatment significantly reduced the oxidative stress, apoptosis ratio and inflammatory factors expression in mice induced by the CLP surgery. Based on these findings, hispidulin may suppress apoptosis by inhibiting reactive oxygen species production and inflammatory response. The NF-κB pathway is particularly controversial because it contradicts the well-known inflammatory function. NF-κB regulated inflammatory cytokines production, and extensive NF-κB inhibition may delay tissue repair and prolong the inflammatory response [18]. Previous studies indicated that the extracellular signal-regulated kinase mitogen activated PI3K/Akt pathways play an essential role in cisplatin-induced kidney injury [19]. The PI3K/Akt is crucial proliferation signaling which coordinates numerous cellular functions, such as transcriptional regulation and cell survival [20]. These effects prompted us to investigate whether hispidulin attenuates renal system damage caused by CLP surgery *via* the modulation of NF-kB and AKT pathway. The results first showed that protein levels of phosphorylation of P65, IκBα, AKT and PI3K were remarkably increased in CLP group, which got reversed by treating with hispidulin in dose-dependent manner. This finding suggested hispidulin repressed the inflammatory mediators expression, oxidative stress and CLP-induced apoptosis by suppressing NF-κB and AKT signaling activation.

There are still some limitations in this study, such as lacking define the extent of AKI, which may affect the outcome. Additionally, we have not conducted an extensive exploration of the association between inflammatory factors and the AKT signaling pathway. Future clinical studies are essential for fully validation of findings in the animal model of this work.

5. Conclusions

In summary, current research supplied new evidence for hispidulin serving as a potential drug in acute kidney injury induced by CLP through exploring its effects on suppressed oxidative stress, inflammatory responses, and apoptosis in kidney tissue.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

HHX—designed the research study. XMC—performed the research. DQL—analyzed the data. YLY—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of Wannan Medical College Affiliated Yijishan Hospital (Approval No. LLSC-2022-142).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Watchorn J, Huang D, Bramham K, Hutchings S. Decreased renal cortical perfusion, independent of changes in renal blood flow and sublingual microcirculatory impairment, is associated with the severity of acute kidney injury in patients with septic shock. Critical Care. 2022; 26: 261.

[2] Yue S, Li S, Huang X, Liu J, Hou X, Zhao Y, *et al*. Machine learning for the prediction of acute kidney injury in patients with sepsis. Journal of Translational Medicine. 2022; 20: 215.

[3] Ren Q, Guo F, Tao S, Huang R, Ma L, Fu P. Flavonoid fisetin alleviates kidney inflammation and apoptosis *via* inhibiting Src-mediated NF-κB p65 and MAPK signaling pathways in septic AKI mice. Biomedicine & Pharmacotherapy. 2020; 122: 109772.

[4] Lin WH, Jiang WP, Chen CC, Lee LY, Tsai YS, Chien LH, *et al*. Renoprotective effect of *pediococcus acidilactici* GKA4 on cisplatin-induced acute kidney injury by mitigating inflammation and oxidative stress and regulating the MAPK, AMPK/SIRT1/NF-κB, and PI3K/AKT pathways. Nutrients. 2022; 14: 2877.

[5] Liu L, Shi Q, Wang K, Qian Y, Zhou L, Bellusci S, *et al*. Fibroblast growth factor 10 protects against particulate matter-induced lung injury by inhibiting oxidative stress-mediated pyroptosis *via* the PI3K/Akt/Nrf2 signaling pathway. International Immunopharmacology. 2022; 113: 109398.

[6] Wang Y, Wu Y, Peng S. Resveratrol inhibits the inflammatory response and oxidative stress induced by uterine ischemia reperfusion injury by activating PI3K-AKT pathway. PLOS ONE. 2022; 17: e0266961.

[7] Lee HW, Choi IW, Ha SK. Immunostimulatory activities of theobromine on macrophages *via* the activation of MAPK and NF-κB signaling pathways. Current Issues in Molecular Biology. 2022; 44: 4216–4228.

[8] Liu K, Zhao F, Yan J, Xia Z, Jiang D, Ma P. Hispidulin: a promising flavonoid with diverse anti-cancer properties. Life Sciences. 2020; 259: 118395.

[9] An P, Wu T, Yu H, Fang K, Ren Z, Tang M. Hispidulin protects against focal cerebral ischemia reperfusion injury in rats. Journal of Molecular Neuroscience. 2018; 65: 203–212.

[10] Ruiz S, Vardon-Bounes F, Merlet-Dupuy V, Conil J, Buléon M, Fourcade O, *et al*. Sepsis modeling in mice: ligation length is a major severity factor in cecal ligation and puncture. Intensive Care Medicine Experimental. 2016; 4: 22.

[11] Song C, Adili A, Kari A, Abuduhaer A. FSTL1 aggravates sepsis-induced acute kidney injury through regulating TLR4/MyD88/NF-κB pathway in newborn rats. Signa Vitae. 2021; 17:167–173.

[12] Djudjaj S, Boor P. Cellular and molecular mechanisms of kidney fibrosis. Molecular Aspects of Medicine. 2019; 65: 16–36.
[13] Abdelnaser M, Alaaeldin R, Attya ME, Fathy M. Modulating Nrf-2/HO-1, apoptosis and oxidative stress signaling pathways by gabapentin ameliorates sepsis-induced acute kidney injury. To be published in Naunyn-Schmiedeberg's Archives of Pharmacology. 2023. [Preprint].
[14] Liu K, Zhao F, Yan J, Xia Z, Jiang D, Ma P. Hispidulin: a promising flavonoid with diverse anti-cancer properties. Life Sciences. 2020; 259: 118395.
[15] Wang X, Fan D, Cao X, Ye Q, Wang Q, Zhang M, *et al*. The role of reactive oxygen species in the rheumatoid arthritis-associated synovial microenvironment. Antioxidants. 2022; 11: 1153.
[16] Chen Y, Zhou X, Wu Y. The miR-26a-5p/IL-6 axis alleviates sepsis-induced acute kidney injury by inhibiting renal inflammation. Renal Failure. 2022; 44: 551–561.
[17] Zhang B, Zeng M, Li B, Kan Y, Wang S, Cao B, *et al*. Arbutin attenuates LPS-induced acute kidney injury by inhibiting inflammation and apoptosis *via* the PI3K/Akt/Nrf2 pathway. Phytomedicine. 2021; 82: 153466.
[18] Liu D, Zhong Z, Karin M. NF-κB: a double-edged sword controlling inflammation. Biomedicines. 2022; 10: 1250.
[19] Potočnjak I, Marinić J, Batičić L, Šimić L, Broznić D, Domitrović R. Aucubin administered by either oral or parenteral route protects against cisplatin-induced acute kidney injury in mice. Food and Chemical Toxicology. 2020; 142: 111472.
[20] Karimian A, Mir SM, Parsian H, Refieyan S, Mirza-Aghazadeh-Attari M, Yousefi B, *et al*. Crosstalk between phosphoinositide 3-kinase/Akt signaling pathway with DNA damage response and oxidative stress in cancer. Journal of Cellular Biochemistry. 2019; 120: 10248–10272.

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