**ORIGINAL ARTICLE**
Iran J Allergy Asthma Immunol
October 2024; 23(5):550-562.
DOI: 10.18502/ijaai.v23i5.16750

# Emodin-based Regulation and Control of Serum Complement C5a, Oxidative Stress, and Inflammatory Responses in Rats with Urosepsis via AMPK/SIRT1

**Juan Cui[1], Shufang Wang[1], Sicheng Bi[2], Hong Zhou[1], and Lichao Sun[3]**

*[1] Department of Emergency Medicine, Emergency General Hospital, Beijing, China*
*[2] Department of Urology Surgery, Emergency General Hospital, Beijing, China*
*[3] Department of Emergency, China-Japan Friendship Hospital, Beijing, China*

***Received: 25 January 2024; Received in revised form: 26 February 2024; Accepted: 5 May 2024***

## ABSTRACT

Emodin, derived from *Rheum officinale* and aloe, is known for its diverse benefits such as anti-inflammatory, antioxidant, and antibacterial properties. Currently, the impact of emodin on urosepsis is unclear. This study aims to investigate the mechanism of action of emodin in urosepsis.

Peripheral blood mononuclear cells (PBMCs) were purchased from Cloud-Clone Animal Inc. and treated with emodin. Cell viability and the lactate dehydrogenase (LDH) level were then assessed. In a separate experiment, a urosepsis model was established in Sprague Dawley rats which were subsequently treated with emodin. The levels of oxidative stress-related factors, serum complements and inflammatory factors were measured using commercial kits. Blood urea nitrogen and serum creatinine levels were determined using a fully automatic biochemical analyzer. The levels of pro-inflammatory proteins and AMP-activated protein kinase (AMPK)/Sirtuin 1 (SIRT1) pathway-related proteins were evaluated via Western blot.

PBMCs were unaffected by emodin concentrations below 60 μg/mL, and minimal LDH levels were detected in the cells. Emodin attenuated the effects of *Escherichia coli* and diminished the production of serum complements, oxidative stress-related proteins, and inflammatory factors in PBMCs. Notably, the effects of emodin were lessened by an AMPK pathway inhibitor. Additionally, emodin alleviated oxidative stress, complement system activation, inflammation, and kidney injury in urosepsis rats through the AMPK/SIRT1 signaling pathway.

Emodin improved kidney damage in urosepsis rats by activating the AMPK/SIRT1 signaling pathway, which reduced oxidative stress, inflammation, and complement system activation.

**Keywords:** AMP-activated protein kinase/Sirtuin 1 pathway; Emodin; Inflammatory responses; Oxidative stress; Urosepsis

## INTRODUCTION

Sepsis has an extremely high mortality rate, ranking as the second leading cause of death in ICU patients after cardiovascular disease.[1,2] Urosepsis accounts for approximately 9% to 31% of sepsis cases.[3] Urosepsis is a condition in which the body first becomes infected by pathogens, followed by the release of toxins from the

**Corresponding Author:** Hong Zhou, PhD;
Department of Emergency Medicine, Emergency General Hospital, BeiJing, China. Tel: (+86 134) 8872 4610, Email: zhouhong718@126.com

urogenital tract into the blood circulation, triggering a systemic inflammatory response syndrome (SIRS).[4] If not treated, SIRS can evolve into severe sepsis, multiorgan dysfunction, and septic shock. Within the urinary system, Gram-negative bacteria especially *Escherichia coli*, which accounts for 50% of cases, serve as the primary causative agents of urosepsis. In contrast, Gram-positive bacteria make up only about 15%.[5] Therefore, a crucial step in mitigating urosepsis-driven renal injury involves discovering a mechanism for alleviating oxidative stress and neuronal inflammation in urosepsis.

Emodin is a natural compound derived from anthraquinone, found in the roots and rhizomes of plants such as *Rheum officinale* and aloe. It exhibits potential for chemical prophylaxis and therapy, demonstrating anti-inflammatory, anticancer, and antimicrobial properties.[6-10] Previous studies have reported that emodin can alleviate sepsis-induced damage through autophagy,[11,12] and it can suppress the phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/glycogen synthase kinase 3β (GSK3β) pathway in the neuroblastoma SH-SY5Y cell line, thereby reducing inflammation and oxidative stress.[13] However, no study to date has clearly elucidated the effect and mechanism of action of emodin in urosepsis.

AMP-activated protein kinase (AMPK) is a key kinase that regulates bioenergy metabolism and plays an important role in regulating oxidative stress and cell apoptosis.[14,15] It is known to stimulate sirtuin 1 (SIRT1),[16] which is part of the class III histone deacetylase family and plays a major role in such physiological processes as cellular apoptosis, aging, oxidative stress, and inflammatory responses.[17-22] As previously reported, pterostilbene attenuates allergic airway inflammation and oxidative stress by regulating the AMPK/SIRT1 pathway.[23] However, it is largely unknown whether emodin mitigates oxidative stress and inflammatory responses in urosepsis by utilizing this pathway.

Thus, we hypothesized that emodin might influence the complement C5a, oxidative stress, and inflammatory responses in urosepsis rats via the AMPK/SIRT1 pathway. To validate this hypothesis, we first examined the in vitro toxicity of emodin and scrutinized its impacts on oxidative stress, complement system activation, and inflammatory reactions. We then delved into the effects of emodin on oxidative stress, complement system initiation, inflammation, and renal impairment in urosepsis rats through the AMPK/SIRT1 signaling pathway. Our findings could serve as a reference for emodin-based treatment to alleviate urosepsis.

## MATERIALS AND METHODS

### Cell Culture

Peripheral blood mononuclear cells (PBMCs, CSI165Ra01) were obtained from Cloud-Clone Animal Inc. (Wuhan, China). These PBMCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (11965092, Gibco, Grand Island, NY, USA) enriched with 10% fetal bovine serum (A5669701, Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (15140148, Gibco, Grand Island, NY, USA), and maintained at 37°C in a 5% $CO_2$ atmosphere. Subsequently, the PBMCs were subjected to an *E coli*-rich environment and cultured for 24 hours to stimulate the creation of in vitro urosepsis models. Following this, the cells were preincubated with 10 μmol/L Compound C (CC) (HY-13418A, MedChemExpress, Monmouth Junction, NJ, USA) for 60 minutes to assess the efficacy of the AMPK signaling pathway.

### Cell Counting Kit-8 Test

PBMCs were seeded into 96-well plates at a density of $2 \times 10^3$ cells/well and cultured for 24 hours. The original medium was then discarded, and the cells were treated with medium containing varying concentrations of emodin (0, 7.5, 15, 30, 60, and 120 μg/mL) (HY-14393, MedChemExpress, Monmouth Junction, NJ, USA), and incubated for 48 hours, respectively. Following this, the medium was replaced with a fresh one; each well was treated with 10 μL of the Cell Counting Kit-8 (CCK-8) solution (APE×BIO, Houston, Texas, USA), and incubated for an additional 4 hours. Finally, the absorbance of the cells was measured at 450 nm using a microplate reader (Bio-Rad, Sunnyvale, CA).

### Lactate Dehydrogenase Test

Cells cultured with varying concentrations of emodin were collected and tested using Lactate Dehydrogenase (LDH) assay kits (AAT-A13809, QiYi Biological Technology Co., Ltd., Shanghai) to determine LDH levels within the cells.

### Reactive Oxygen Species (ROS) Test

Cells and rat renal tissue homogenates were collected and incubated with 15 μmol/L

dihydroethidium (DHE) (810253P, Sigma-Aldrich, Milwaukee, WI, USA) for 30 minutes. Afterward, the ROS levels in the aforementioned cells or tissues were observed using a fluorescence microscope (BX53, Olympus, Tokyo, Japan).

**Malondialdehyde, Superoxide Dismutase, and Glutathione Peroxidase Tests**

First, the cells and rat renal tissue homogenates were collected, and then centrifuged at 10000*g* and 4°C for 10 minutes. Subsequently, they were tested with malondialdehyde (MDA) assay kits (S0131M, Beyotime, Shanghai, China), superoxide dismutase (SOD) assay kits (S0101S, Beyotime, Shanghai, China), and glutathione peroxidase (GSH-Px) assay kits (E-BC-K096-M, Elabscience, Wuhan, China) to determine the MDA, SOD, and GSH-Px levels.

**Enzyme-linked Immunosorbent Assay (ELISA)**

Cells in the log phase were inoculated into a 6-well plate and cultivated for 48 hours. The supernatant was then retained and centrifuged for 10 minutes at 4°C and 1000 rpm, after which the supernatant was collected. Subsequently, rat renal tissues were lysed by adding RIPA lysis buffer (R0010, Solarbio, Beijing, China) and centrifuged at 14,000 rpm at 4°C for 25 minutes. The supernatant was again collected. Finally, 2 types of supernatants were tested using commercial assay kits to measure the levels of C5, C5a, interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6. The C5 assay kits (XY-A14708) were purchased from X-Y Biotechnology (Shanghai, China), while the C5a (E-EL-H0190), IL-1β (E-EL-H0149), TNF-α (E-EL-H2305), and IL-6 (E-OSEL-H0001) assay kits were obtained from Elabscience (Wuhan, China).

**Western Blot**

Cells or rat renal tissues were treated with RIPA lysis buffer (R0010, Solarbio, Beijing, China) to extract proteins. These proteins were then placed in a solution with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel to separate and identify the target protein bands. The target protein bands were transferred onto a polyvinylidene fluoride (PVDF) membrane, accompanied with and later blocked for 1 hour, followed by incubation overnight with primary antibodies diluted to 1:1000 at 4°C. The primary antibodies used were against cyclooxygenase-2 (COX-2, MA5-14568), antibody against inducible nitric oxide synthase (iNOS, PA1-036), antibody against p-AMPK (PA5-104982), antibody against AMPK (PA5-105297), antibody against SIRT1 (PA5-17074), and antibody against glyceraldehyde-3-phosphate dehydrogenase (GADPH, PA1-988). The membrane was washed with Tris-buffered saline with Tween-20 (TBST) on the next day, then treated with a goat anti-rabbit secondary antibody (A32731) diluted to 1:10000 for 1.5 hours at room temperature. Finally, a super excellent chemiluminescent substrate (ECL) detection kit (E-IR-R308, Elabscience, Wuhan, China) was used to visualize the protein bands. All the antibodies were sourced from Thermo Fisher Scientific (Waltham, MA, USA).

**Animal Experiment**

Twenty Specific Pathogen Free (SPF) Sprague-Dawley male rats weighing 200 g (SCXK [Beijing] 2021-0011) were procured from the Institute of Traditional Chinese Medicine Health Industry under China Academy of Chinese Medical Sciences (Beijing, China). All rats were housed in the laboratory animal center for at least a week, with access to plentiful food and water. The environment was maintained between 20°C to 24°C and at 45% to 65% humidity, with 12-hour day and night cycles. The rats were randomly divided into a control group, a sham group, a model group, and a model+emodin group. Before the surgery, each rat was anesthetized with sodium pentobarbital. Following disinfection, a silk thread was used to ligate the left ureter. Then, *E coli* (at a concentration of $2.0\times10^7$ CFU/mL) was injected into the renal pelvis of each rat to form a urosepsis model.[24] The sham group underwent similar surgical procedures but without ligation. Then, 2 mL of 60 μg/mL emodin (HY-14393, MedChemExpress, Monmouth Junction, NJ, USA) was intraperitoneally injected one hour before the surgery. Postoperation, the living conditions and survival rates of the rats were closely monitored. After 24 hours, the rats were euthanized using $CO_2$ delivered at a steady rate (50% of the container volume per minute) for 5 minutes. Finally, the renal tissues were harvested 24 hours postoperation.

**Hematoxylin and Eosin Staining**

The isolated left renal tissues from rats were fixed with 4% paraformaldehyde and embedded in paraffin. These tissues were then cut into 4 μm thick slices and dried for 6 hours in an oven set at 65°C. Subsequently, the slices were conventionally deparaffinated, hydrated, and stained with Hematoxylin and Eosin (H&E, C0105S,

Beyotime, Shanghai, China). Ultimately, the renal tissues were examined under a microscope (APX100, Olympus, Tokyo, Japan) to detect any localized pathological alterations.

**Biochemical Analysis**

Rats were first injected with emodin. After 24 hours, we collected blood samples from these rats and assessed the levels of blood urea nitrogen (BUN) and serum creatinine (Cr) using a fully automatic biochemical analyzer (CS1200, Jason, Shanghai, China).

**Statistical Analysis**

The test results are presented as the mean ± standard deviation. Analysis of variance and the Student's *t* test was performed using the SPSS 26.0 software, with $p<0.001$ indicating a significant difference. Graphs were generated using the GraphPad Prism 9.0 software. Each test was conducted 3 or more times.

## RESULTS

**Emodin's Toxic Effect on PBMCs**

To confirm the toxic effect of emodin on PBMCs, PBMCs were exposed to a solution with gradient concentrations of emodin for 24 hours, followed by testing with CCK-8 reagent and LDH assay kits to assess cell activity and LDH levels. Within the concentration range of 7.5 to 60 μg/mL, emodin did not significantly affect the activity or LDH levels of PBMCs ($p>0.001$). However, after being treated with 120 μg/mL emodin, the cell activity decreased to less than 50%, while the LDH level significantly increased ($p<0.001$) (Figures 1A and 1B). Consequently, 60 μg/mL emodin was used in subsequent tests.

**Emodin's Suppression of *E coli*-stimulated Oxidative Stress in PBMCs**

To elucidate the influences of emodin on the oxidative stress in PBMCs, emodin was employed to intervene in the PBMCs stimulated by *E coli*. ROS test findings indicated that *E coli* amplified the production of ROS in PBMCs, this effect was moderately attenuated by emodin ($p<0.001$) (Figure 2A). Concurrently, the increase in MDA production induced by *E coli* was significantly curtailed by emodin, and it also visibly mitigated *E coli*'s inhibitory effect on the activities of GSH-Px and SOD ($p<0.001$) (Figures 2B-D). Therefore, emodin lessened the oxidative stress in PBMCs stimulated by *E coli*.

**Emodin's Inhibition of *E coli*-stimulated Complement System Activation in PBMCs**

The levels of serum complement C5 and C5a were evaluated using ELISA. Consequently, emodin was discovered to obstruct *E coli*'s enhancement of C5 and C5a levels ($p<0.001$) (Figure 3A and B). Through Western blot testing, it was found that emodin could boost the expressions of COX-2 and iNOS ($p<0.001$) (Figures 3C–E). Moreover, ELISA results indicated that *E coli* stimulated the production of IL-1β, TNF-α, and IL-6, while emodin partially hindered this effect ($p<0.001$) (Figures 3F–H). These results revealed that emodin repressed the activation of the complement system in PBMCs stimulated by *E coli*, thereby alleviating inflammatory responses in these cells.

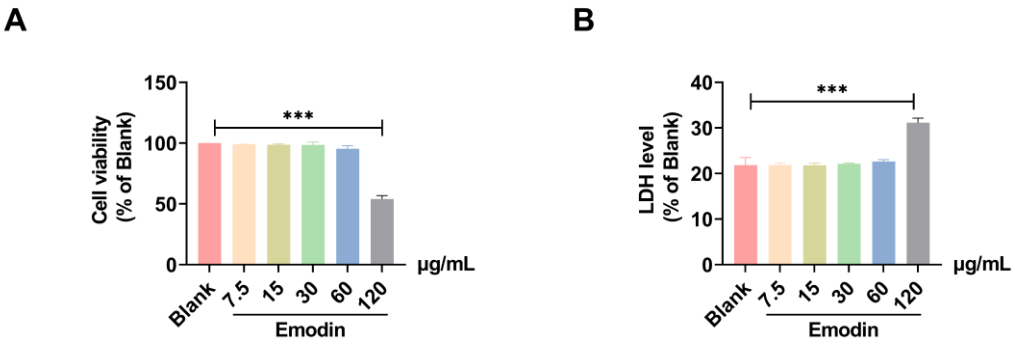



**Figure 1. Emodin's toxic effect on peripheral blood mononuclear cells (PBMCs). (A) The activity of PBMCs was assessed with the Cell Counting Kit-8 (CCK-8) method. (B) Lactate dehydrogenase (LDH) levels in PBMCs were tested using an applicable assay kit. The data are expressed as mean ± standard deviation; *** $p<0.001$.**

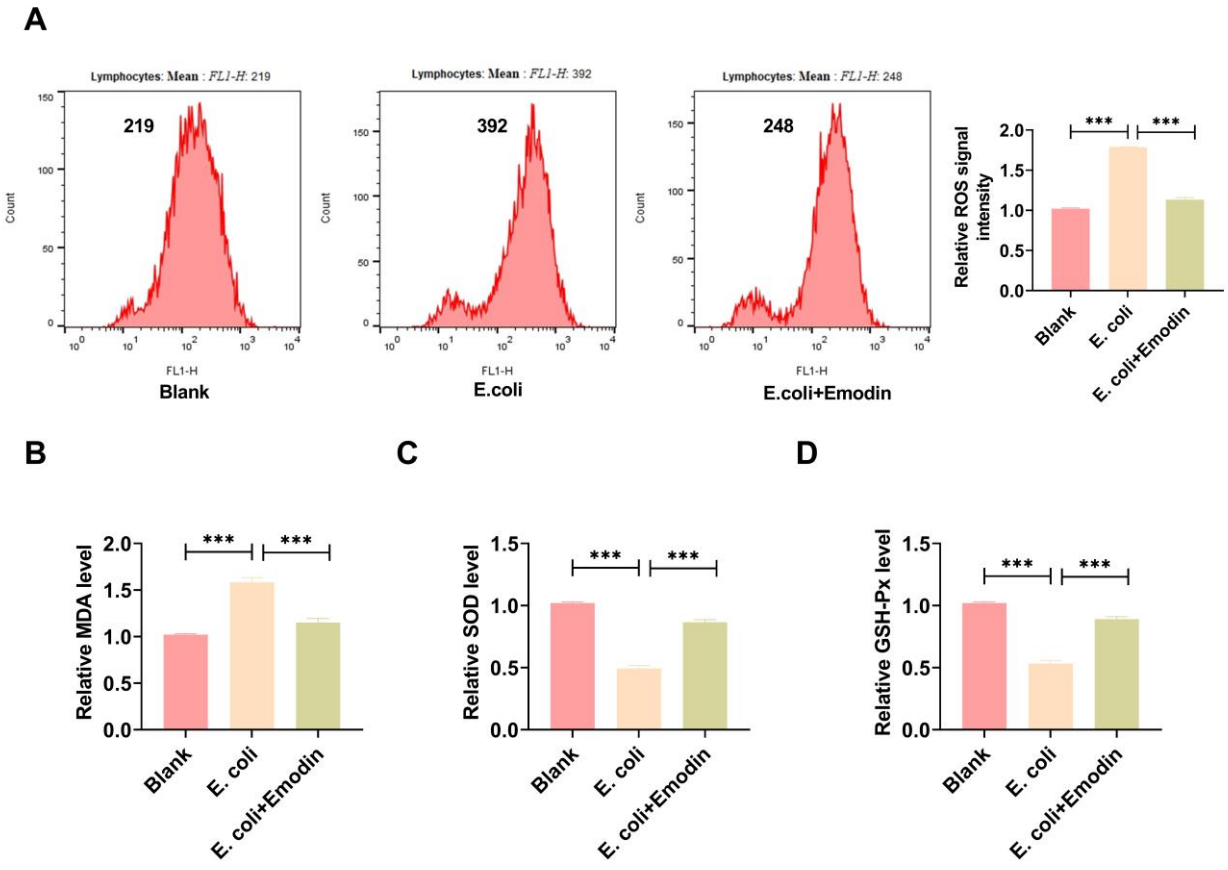



**Figure 2. Impact of emodin on the oxidative stress in peripheral blood mononuclear cells stimulated by *Escherichia coli*. (A) Reactive oxygen species levels were detected with the fluorescent probe labeling method. (B-D) Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels were measured using commercial assay kits. The data are expressed as mean ± standard deviation; ***$p < 0.001$.**

**Emodin's Regulation of the AMPK/SIRT1 Pathway**

To ascertain the regulation of the AMPK/SIRT1 pathway by emodin, we conducted a Western blot assay to study the expressions of AMPK, p-AMPK, and SIRT1 in PBMCs. We found that emodin counteracted *E coli*'s inhibition of p-AMPK and SIRT1 expressions in rat PBMCs, but with the addition of CC, this effect of emodin was reversed ($p$<0.001) (Figures 4A–C). Therefore, it can be stated that emodin activates the AMPK/SIRT1 pathway.

**Compound C (CC) can Weaken the Effect of Emodin**

After the intervention with CC and/or emodin, the *E coli*-stimulated PBMCs were tested using a fluorescence probe labeling method and commercial assay kits. Notably, CC drove the increase in the peak intensity of ROS signals and the MDA levels but mitigated the generation of GSH-Px and SOD ($p$<0.001) (Figures 5A-D). The ELISA revealed that intervention with CC notably heightened the production of C5 and C5a in PBMCs ($p$<0.001) (Figures 5E–F). Furthermore, as detected by Western blot testing, CC treatment significantly raised the levels of COX-2 and iNOS ($p$<0.001) (Figures 5G–I). In addition, the generation of IL-1β, TNF-α, and IL-6 were also enhanced by CC ($p$<0.001) (Figures 5J–L). These variations confirmed that emodin can alleviate the oxidative stress, complement system activation, and inflammatory responses triggered by *E coli* by activating the AMPK/SIRT1 pathway in PBMCs.

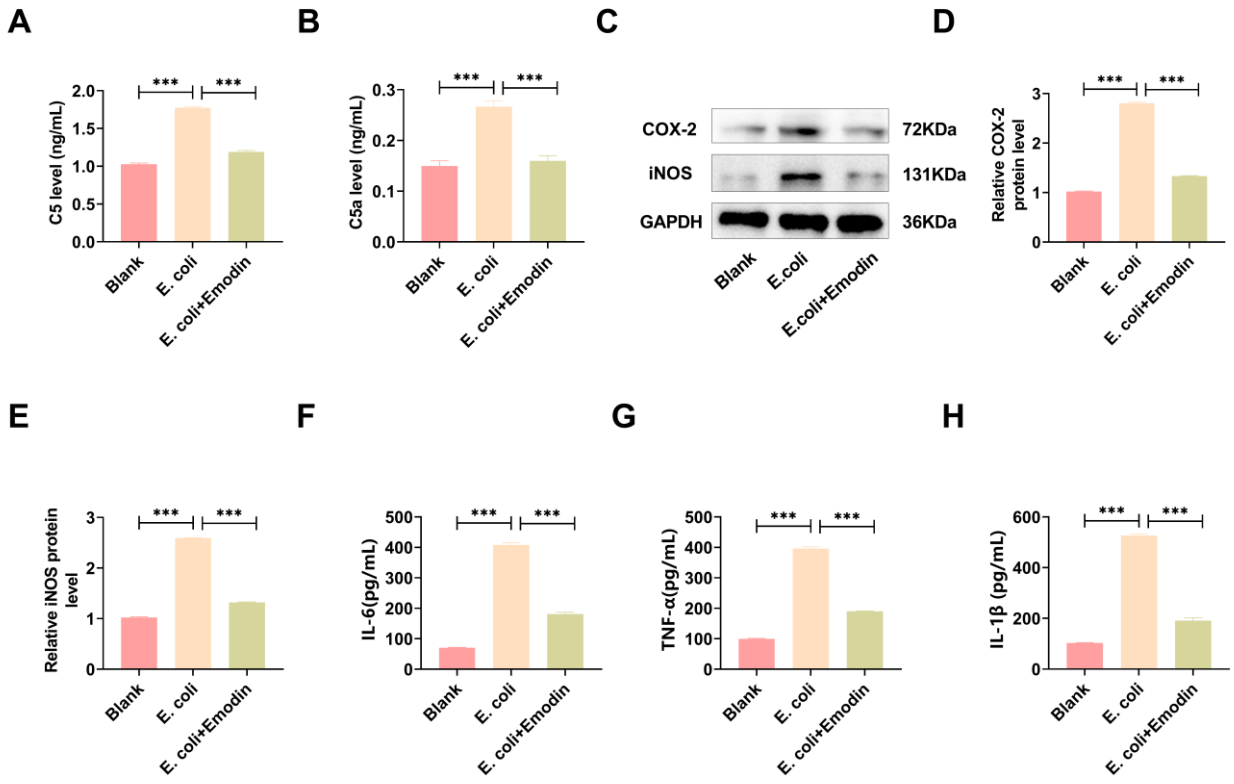



**Figure 3. Impacts of emodin on the complement system activation and inflammatory responses in PBMCs stimulated by *Escherichia coli*. (A-B) Levels of C5 and C5a were measured by enzyme-linked immunosorbent assay (ELISA). (C-E) Protein levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were assessed by Western blot analysis. (F–H) Levels of inflammatory cytokines were measured using ELISA kits. The data are expressed as mean±standard deviation; ***$p$<0.001.**

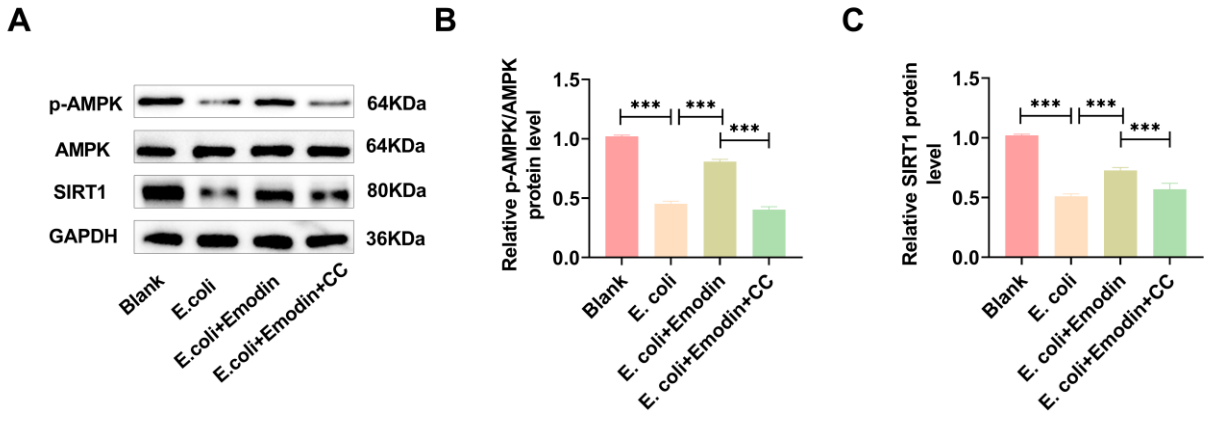
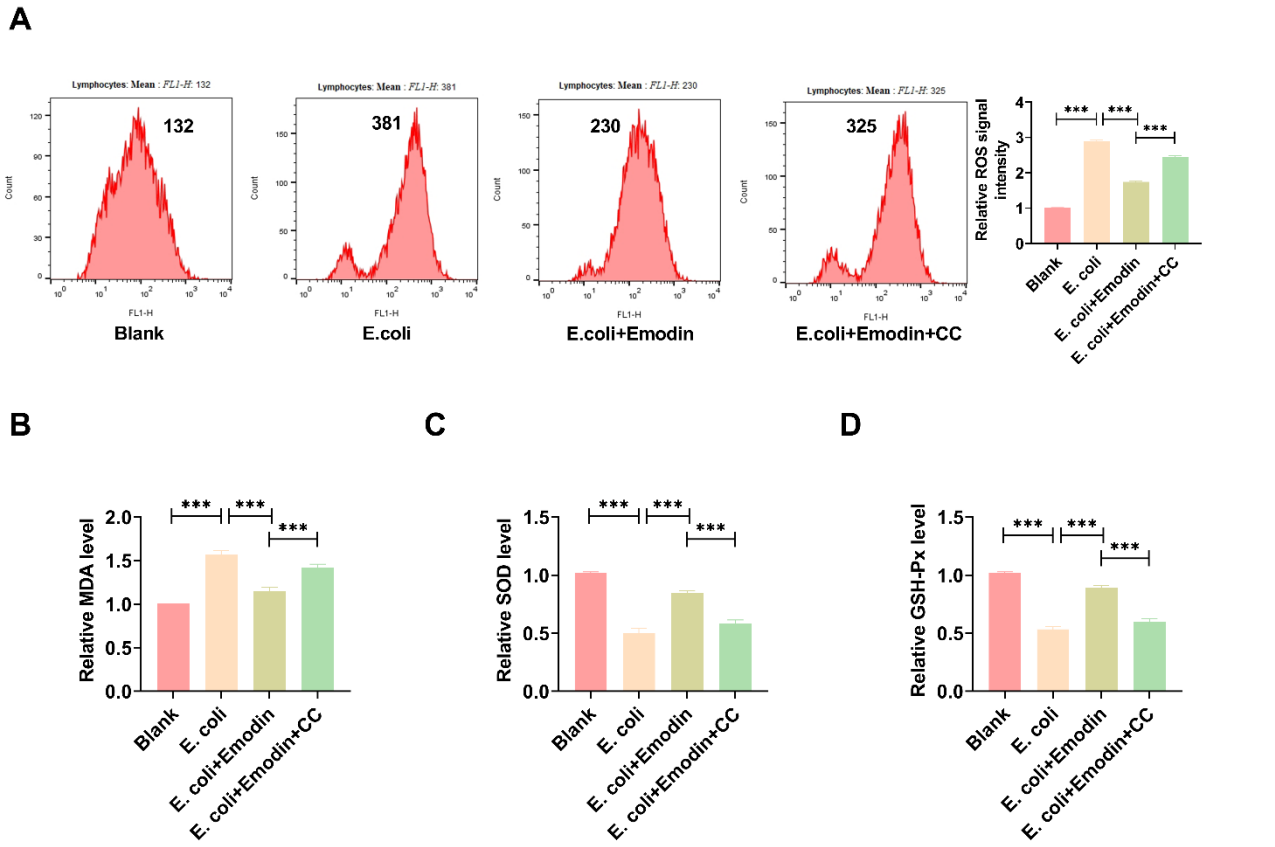



**Figure 4. Emodin's modulation of the AMP-activated protein kinase (AMPK)/Sirtuin 1 (SIRT1) pathway. (A-C) Expressions of p-AMPK, AMPK, and SIRT1 proteins were assessed by Western blot analysis. The data are expressed as mean±standard deviation; *** $p$<0.001.**

**Emodin Treats Urosepsis Rats by Activating AMPK/SIRT1 Signaling Pathway**

Rats in the urosepsis condition exhibited a notably low rectal temperature. However, after the administration of emodin, this temperature rose significantly ($p$<0.001) (Figure 6A). Emodin also reduced the levels of MDA and ROS in the renal tissues of rats with urosepsis while increasing the levels of SOD and GSH-Px ($p$<0.001) (Figures 6B–E). ELISA results showed that emodin significantly reduced the expressions of C5a, C5, IL-1β, TNF-α, and IL-6 in rats suffering from urosepsis ($p$<0.001) (Figures 6F-J). The increases in Cr and BUN levels observed in rats with urosepsis were prevented by emodin, as detected by the automatic biochemistry analyzer ($p$<0.001) (Figures 6K–L). According to H&E stained renal tissues, the control group and sham group of rats had well-organized renal structures, with clear glomeruli, renal capsule, and renal tubules, there was no inflammatory cell infiltration observed in the interstitium. On the other hand, rats with urosepsis showed disorganized renal tissue structures, abnormal glomerular structure, discernible enlarged renal capsules, dilated renal tubular lumens, and apparent necrotic tissue shedding, coupled with obstruction in the lumens. In addition, the renal interstitium was congested and ringed with numerous inflammatory cells. After administering emodin, these observed pathological changes in the renal tissues were mitigated (Figure 6M). Further, Western blot results indicated that emodin significantly increased the levels of SIRT1 and p-AMPK/AMPK in rats with urosepsis (Figures 6N–P). In summary, through the AMPK/SIRT1 pathway, emodin can reduce oxidative stress, complement system activation, and inflammatory responses, and could ultimately attenuate renal injury in rats with urosepsis.



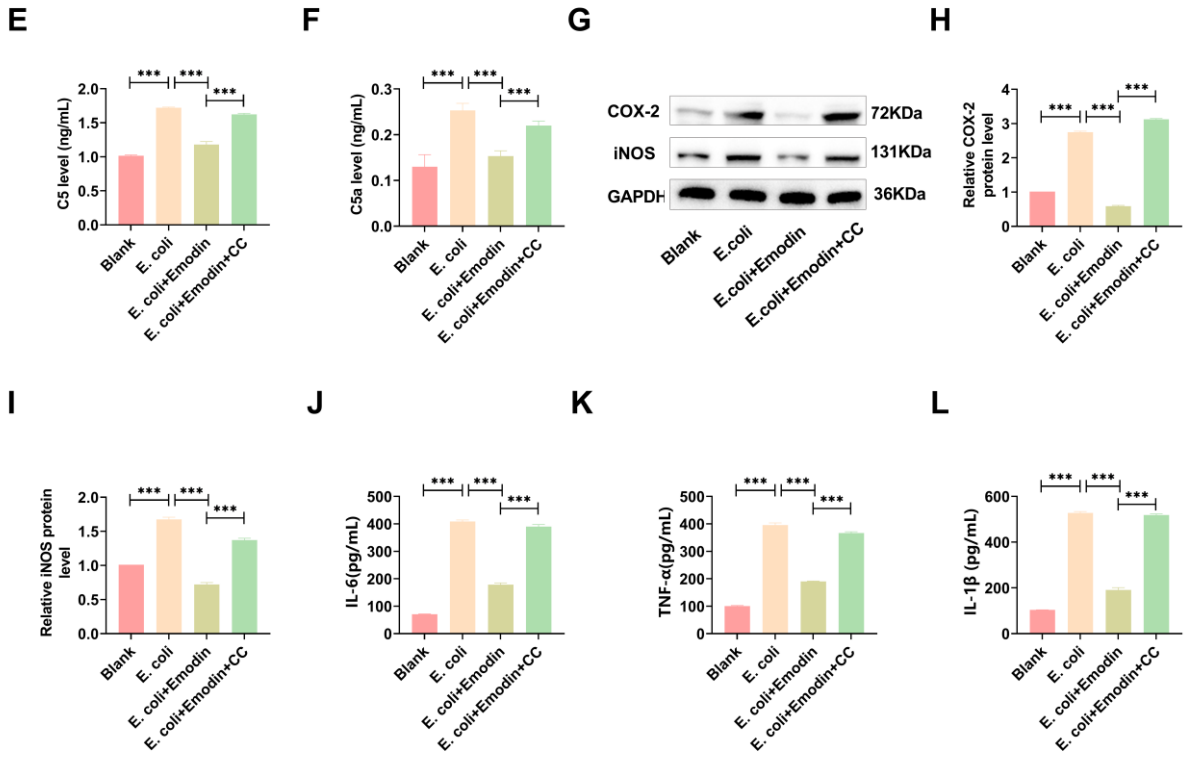



**Figure 5. Compound C (CC)-affected partial counteraction to emodin's attenuation of the *Escherichia coli*-stimulated oxidative stress, complement system activation, and inflammatory responses in peripheral blood mononuclear cells. (A) Reactive oxygen species (ROS) levels were detected by fluorescent probe labeling. (B–D) Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels were measured using commercial assay kits. (E–F) C5 and C5a levels were assessed by enzyme-linked immunosorbent assay (ELISA) (G–I) Protein levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were evaluated by western blot analysis. (J–L) Levels of inflammatory cytokines measured. The data are expressed as mean±standard deviation; *** $p$<0.001.**

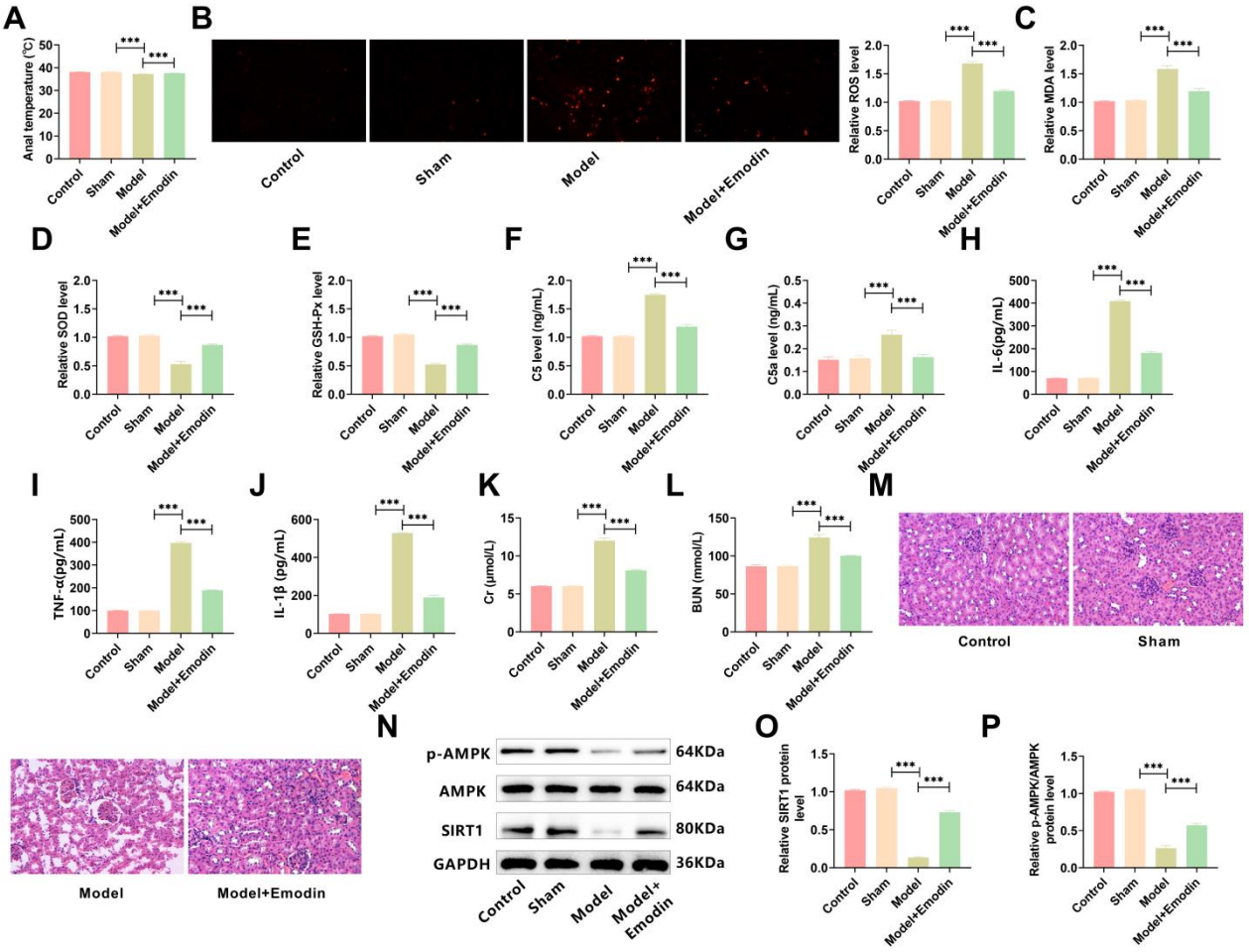



**Figure 6. Emodin's alleviation of oxidative stress, complement system activation, inflammatory responses, and renal injury in urosepsis rats via the AMP-activated protein kinase (AMPK)/Sirtuin 1 (SIRT1) pathway. (A) Rectal temperature of rats. (B) ROS levels detected by fluorescent probe labeling. (C–E) Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels were measured using commercial assay kits. (F–J) C5, C5a, IL-6, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) levels assessed by enzyme-linked immunosorbent assay (ELISA). (K–L) Creatinine (Cr) and blood urea nitrogen (BUN) levels were evaluated using an automatic biochemical analyzer. (M) Renal injury in rats assessed by hematoxylin and eosin staining. (N–P) Expressions of p-AMPK, AMPK, and SIRT1 were evaluated by western blot analysis. The data are expressed as mean±standard deviation; ***$p$<0.001.**

## DISCUSSION

Urosepsis is a severe, life-threatening infectious disease characterized by purulence and inflammation.[4] It commonly occurs in patients with functional or anatomical abnormalities in the urogenital tract who either receive improper treatment or develop urinary tract infections. Such patients may experience the spread of pathogens into their bloodstream, triggering unregulated bodily responses.[25] Urosepsis often results from a combination of urinary tract infection and obstruction of the upper urinary tract obstruction,[26-28] typically presenting as acute pyelonephritis.[29]

Sepsis is characterized by concurrent inflammation and oxidative stress, involving imbalances in iNOS, nitric oxide (NO), and glutathione.[30-32] Cells under oxidative stress often produce more ROS, which can damage their molecular structures such as DNA, membrane lipids, and proteins.[33] ROS primarily targets lipids and polyunsaturated fatty acids. MDA is the end

product of lipid peroxidation and reflects the severity of free radical attack.[34,35] GSH-Px and SOD can protect cells from oxidative damage by removing ROS.[27,36] The results of our study showed that when emodin concentrations were below 60 μg/mL, PBMC cell viability was higher and the LDH level was lower. Also, emodin reduced the *E.coli*-promoted increase in ROS and MDA production in PBMCs and dampened the *E.coli*-induced reduction in SOD and GSH-Px production in PBMCs. Therefore, emodin can lessen the oxidative stress on PBMCs.

During sepsis, the complement system is initially activated, leading to an abundant production of complement fragments such as C3a, C4a, and C5a. These fragments trigger inflammation, resulting in dysfunction in the liver, kidneys, heart, lungs, brain, and other organs.[37-39] Major pro-inflammatory proteins such as COX-2 and iNOS play a crucial role in the synthesis of prostaglandin E2 (PGE2) and NO.[40-43] In cases of inflammation, the levels of pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6 are significantly upregulated.[44,45] This study examined the effects of emodin on the generation of serum antibodies and inflammatory factors in PBMCs stimulated by *E coli*. Emodin noticeably suppressed the *E coli*-stimulated generation of serum antibodies (C5 and C5a), pro-inflammatory proteins (iNOS and COX-2), and inflammatory cytokines (IL-1β, TNF-α, and IL-6) in PBMCs. Thus, suggesting that emodin can attenuate inflammatory responses and complement system activation in the context of urosepsis.

AMPK can augment the increase in the NAD/NADH ratio, thus activating the SIRT1 deacetylase.[46] Bei et al, demonstrated that emodin, by modulating the PPARα/γ-AMPK pathway, can participate in fatty acid metabolism.[47] Song et al, reported that amelogenin could be used to regulate the AMPK/SIRT1/NF-κB pathway, thereby preventing sepsis-induced brain injury.[48] To understand the emodin-based control of the AMPK/SIRT1 pathway, Western blot tests were carried out to clarify the expressed levels of AMPK, p-AMPK, and SIRT1 in PBMCs. Consequently, the production of p-AMPK and SIRT1 was noticeably hindered with the addition of CC. CC stimulated the restoration of ROS and MDA levels but obstructed the production of SOD and GSH-Px; after administering CC, we observed elevated levels of C5, C5a, COX-2, iNOS, IL-1β, TNF-α, and IL-6. Therefore, emodin played a part in relieving *E coli*-induced oxidative stress, inflammatory responses, and complement system activation in PBMCs by controlling the AMPK/SIRT1 pathway in these cells.

In subsequent experiments using rat urosepsis models, we noticed anomalous glomerular structures, dramatically enlarged renal capsules, visibly necrotic tissue shedding, obstructions in the renal tubules, and infiltration of inflammatory cells in the interstitium. After administering emodin, the renal tissue lesions within urosepsis rats significantly improved. Notably, emodin impeded the production of ROS and MDA while enhancing the production of SOD and GSH-Px in urosepsis rats. At the same time, emodin diminished the expressions of C5a, C5, IL-1β, TNF-α, IL-6, Cr, and BUN, but conversely promoted the expressions of SIRT1 and p-AMPK. Overall, emodin can reduce oxidative stress, complement system activation, inflammatory responses, and renal injury in rats with urosepsis via the AMPK/SIRT1 pathway.

This study simply illustrates the significant role of emodin in the treatment of urosepsis. The use of emodin in clinical settings requires further exploration. A summary of the survey findings indicates that emodin effectively mitigated oxidative stress, complement system activation, and inflammatory responses in PBMCs in vitro, and pathological damage in renal tissues in vivo. Importantly, emodin inhibited oxidative stress in PBMCs and played an integral role in urosepsis rats through the AMPK/SIRT1 pathway. Despite these findings, additional investigation is necessary to elucidate how emodin triggers this pathway. Emodin is non-toxic for PBMCs. Emodin can lessen oxidative stress, complement system activation, and inflammatory responses in *E coli*-stimulated rat PBMCs by modulating the AMPK/SIRT1 pathway, thus improving the condition of renal injury in urosepsis rats.

## STATEMENT OF ETHICS

All procedures performed in this study involving human participants followed the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All subjects were approved by the Ethics Committee of Emergency General Hospital, Beijing (2023-KY-073).

## FUNDING

No institutional fundings were received for this study.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

Not applicable.

## REFERENCES

1. Reinhart K, Brunkhorst FM, Bone HG, Bardutzky J, Dempfle CE, Forst H, et al. Prevention, diagnosis, therapy and follow-up care of sepsis: 1st revision of S-2k guidelines of the German Sepsis Society (Deutsche Sepsis-Gesellschaft e.V. (DSG)) and the German Interdisciplinary Association of Intensive Care and Emergency Medicine (Deutsche Interdisziplinäre Vereinigung für Intensiv- und Notfallmedizin (DIVI)). Ger Med Sci. 2010.
2. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.
3. Levy MM, Artigas A, Phillips GS, Rhodes A, Beale R, Osborn T, et al. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. Lancet Infect Dis. 2012;12(12):919-24.
4. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama. 2016;31(5):801-10.
5. Wagenlehner FM, Weidner W, Naber KG. Pharmacokinetic characteristics of antimicrobials and optimal treatment of urosepsis. Clin Pharmacokinet. 2007;464(54):291-305.
6. Srinivas G, Babykutty S, Sathiadevan PP, Srinivas P. Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent. Med Res Rev. 2007. 27(5):591-608.
7. Tu YJ, Tan B, Jiang L, Wu ZH, Yu HJ, Li XQ, et al. Emodin Inhibits Lipopolysaccharide-Induced Inflammation by Activating Autophagy in RAW 264.7 Cells. Chin J Integr Med. 2021;27(5):345-52.
8. Wen Y, Han C, Liu T, Wang R, Cai W, Yang J, et al. Chaiqin chengqi decoction alleviates severity of acute pancreatitis via inhibition of TLR4 and NLRP3 inflammasome: Identification of bioactive ingredients via pharmacological sub-network analysis and experimental validation. Phytomedicine. 2020;7(9):153328.
9. Shuangsuo D, Zhengguo Z, Yunru C, Xin Z, Baofeng W, Lichao Y, et al. Inhibition of the replication of hepatitis B virus in vitro by emodin. Med Sci Monit. 2006;12(9):Br302-6.
10. Chang CH, Lin CC, Yang JJ, Namba T, Hattori M. Anti-inflammatory effects of emodin from ventilago leiocarpa. Am J Chin Med. 2004;242;139-42.
11. Guo R, Li Y, Han M, Liu J, Sun Y. Emodin attenuates acute lung injury in Cecal-ligation and puncture rats. Int Immunopharmacol. 2023;85:506626
12. Li Y, Guo R, Zhang M, Chen P, Li J, Sun Y. Protective effect of emodin on intestinal epithelial tight junction barrier integrity in rats with sepsis induced by cecal ligation and puncture. Exp Ther Med. 2020;196(18):3521-30.
13. Li R, Liu W, Ou L, Gao F, Li M, Wang L, Wei P, Miao F. Emodin Alleviates Hydrogen Peroxide-Induced Inflammation and Oxidative Stress via Mitochondrial Dysfunction by Inhibiting the PI3K/mTOR/GSK3β Pathway in Neuroblastoma SH-SY5Y Cells. Biomed Res Int. 2020;1562915.
14. Trefts E, Shaw RJ. AMPK: restoring metabolic homeostasis over space and time. Mol Cell. 2021;81(18):3677-90.
15. Wang J, Li Z, Gao L, Qi Y, Zhu H, Qin X. The regulation effect of AMPK in immune related diseases. Sci China Life Sci. 2018;61(5):523-33.
16. Shi HJ, Xu C, Liu MY, Wang BK, Liu WB, Chen DH, et al. Resveratrol Improves the Energy Sensing and Glycolipid Metabolism of Blunt Snout Bream Megalobrama amblycephala Fed High-Carbohydrate Diets by Activating the AMPK-SIRT1-PGC-1α Network. Front Physiol. 2018;1258.
17. Zhao Q, Tian Z, Zhou G, Niu Q, Chen J, Li P, et al. SIRT1-dependent mitochondrial biogenesis supports therapeutic effects of resveratrol against neurodevelopment damage by fluoride. Theranostics. 2020;10(11):4822-38.
18. Dou YQ, Kong P, Li CL, Sun HX, Li WW, Yu Y, et al. Smooth muscle SIRT1 reprograms endothelial cells to suppress angiogenesis after ischemia. Theranostics. 2020;10(3):1197-212.
19. He F, Li Q, Sheng B, Yang H, Jiang W. SIRT1 Inhibits Apoptosis by Promoting Autophagic Flux in Human Nucleus Pulposus Cells in the Key Stage of Degeneration via ERK Signal Pathway. Biomed Res Int. 2021:8818713.

20. Xu C, Wang L, Fozouni P, Evjen G, Chandra V, Jiang J, et al. SIRT1 is downregulated by autophagy in senescence and ageing. Nat Cell Biol. 2020;22(10):1170-9.
21. Rada P, Pardo V, Mobasher MA, García-Martínez I, Ruiz L, González-Rodríguez Á, et al SIRT1 Controls Acetaminophen Hepatotoxicity by Modulating Inflammation and Oxidative Stress. Antioxid Redox Signal. 2018;28(13):1187-208.
22. Liang D, Zhuo Y, Guo Z, He L, Wang X, He Y, et al. SIRT1/PGC-1 pathway activation triggers autophagy/mitophagy and attenuates oxidative damage in intestinal epithelial cells. Biochimie. 2020;170(4):10-20.
23. Xu C, Song Y, Wang Z, Jiang J, Piao Y, Li L, et al. Pterostilbene suppresses oxidative stress and allergic airway inflammation through AMPK/Sirt1 and Nrf2/HO-1 pathways. Immun Inflamm Dis. 94, 1406-1417 (2021).
24. Cao Y, Bai C, Si P, Yan X, Zhang P, Yisha Z, et al. A novel model of urosepsis in rats developed by injection of Escherichia coli into the renal pelvis. Front Immunol. 2022;13(4):1074488.
25. Wagenlehner FM, Lichtenstern C, Rolfes C, Mayer K, Uhle F, Weidner W, et al. Diagnosis and management for urosepsis. Int J Urol. 2013;20(10):963-70.
26. Bozkurt M, Yumru A E, Salman S, Assessment of perioperative, early, and late postoperative complications of the inside-out transobturator tape procedure in the treatment of stress urinary incontinence. Clin Exp Obstet Gynecol. 2015;42(1):82-9.
27. Chen D, Jiang C, Liang X, Zhong F, Huang J, Lin Y, et al. Early and rapid prediction of postoperative infections following percutaneous nephrolithotomy in patients with complex kidney stones. BJU Int. 2019;123(6):1041-7.
28. Xu G, He Y, Zhao H, Jiang X, Feng G, Yang W, et al. Mini-nephroscope combined with pressure suction: an effective tool in MPCNL for intrarenal stones in patients with urinary tract infections. Urolithiasis. 2016;44(5):445-50.
29. Dreger NM, Degener S, Ahmad-Nejad P, Wöbker G, Roth S. Urosepsis--Etiology, Diagnosis, and Treatment. Dtsch Arztebl Int. 2015;112(49):837-47.
30. Webber RJ, Sweet RM, Webber DS. Inducible Nitric Oxide Synthase in Circulating Microvesicles: Discovery, Evolution, and Evidence as a Novel Biomarker and the Probable Causative Agent for Sepsis. J Appl Lab Med. 2019;3(4):698-711.
31. Joseph LC, Kokkinaki D, Valenti MC, Kim GJ, Barca E, Tomar D, et al. Inhibition of NADPH oxidase 2 (NOX2) prevents sepsis-induced cardiomyopathy by improving calcium handling and mitochondrial function. JCI Insight. 2017;2(17):9428.
32. Lyons J, Rauh-Pfeiffer A, Ming-Yu Y, et al. Cysteine metabolism and whole blood glutathione synthesis in septic pediatric patients. Crit Care Med. 2001;294, 870-7.
33. Forman H J, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20(9):689-709.
34. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014:360438.
35. Toto A, Wild P, Graille M, Turcu V, Crézé C, Hemmendinger M, et al. Urinary Malondialdehyde (MDA) Concentrations in the General Population-A Systematic Literature Review and Meta-Analysis. Toxics. 2022;10(4):160.
36. Handy DE, Loscalzo J. The role of glutathione peroxidase-1 in health and disease. Free Radic Biol Med. 2022;188:146-61.
37. Fattahi F, Ward PA. Understanding Immunosuppression after Sepsis. Immunity. 2017;47(1)3-5.
38. Koivikko P, Arola O, Inkinen O, Tallgren M. One-Year Survival after Inhospital Cardiac Arrest-Does Prearrest Sepsis Matter? Shock. 50(1):38-43.
39. Libert C, Ayala A, Bauer M, Cavaillon JM, Deutschman C, Frostell C, et al. Part II: Minimum Quality Threshold in Preclinical Sepsis Studies (MQTiPSS) for Types of Infections and Organ Dysfunction Endpoints. Shock. 51(1):23-32.
40. Wang L, Gu J, Zong M, Zhang Q, Li H, Li D, et al. Anti-inflammatory action of physalin A by blocking the activation of NF-κB signaling pathway. J Ethnopharmacol. 2021;26(7):1134-90.
41. Han JM, Lee EK, Gong SY, Sohng JK, Kang YJ, Jung HJ. Sparassis crispa exerts anti-inflammatory activity via suppression of TLR-mediated NF-κB and MAPK signaling pathways in LPS-induced RAW264.7 macrophage cells. J Ethnopharmacol. 2019;231: 10-18.
42. Liao W, He X, Yi Z, Xiang W, Ding Y. Chelidonine suppresses LPS-Induced production of inflammatory mediators through the inhibitory of the TLR4/NF-κB signaling pathway in RAW264.7 macrophages. Biomed Pharmacother. 2018;107:1151-9.
43. Linghu KG, Ma QS, Zhao GD, Xiong W, Lin L, Zhang QW, et al. Leocarpinolide B attenuates LPS-induced inflammation on RAW264.7 macrophages by mediating NF-κB and Nrf2 pathways. Eur J Pharmacol. 2020;868:172854.

44. Liu S, Yang T, Ming TW, Gaun TKW, Zhou T, Wang S, et al. Isosteroid alkaloids with different chemical structures from Fritillariae cirrhosae bulbus alleviate LPS-induced inflammatory response in RAW 264.7 cells by MAPK signaling pathway. Int Immunopharmacol. 2020;84:106047.
45. Lee HH, Jang E, Kang SY, Shin JS, Han HS, Kim TW, et al. Anti-inflammatory potential of Patrineolignan B isolated from Patrinia scabra in LPS-stimulated macrophages via inhibition of NF-κB, AP-1, and JAK/STAT pathways. Int Immunopharmacol. 2020;86:106726.
46. Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature. 2009;458(7241):1056-60.
47. Bei Y, Tia B, Li Y, Guo Y, Deng S, Huang R, et al. Anti-influenza A Virus Effects and Mechanisms of Emodin and Its Analogs via Regulating PPARα/γ-AMPK-SIRT1 Pathway and Fatty Acid Metabolism. Biomed Res Int. 2021;9066938.
48. Cheng S, Feng C, Wingen LU, Cheng H, Riche AB, Jiang M, et al. Amarogentin has protective effects against sepsis-induced brain injury via modulating the AMPK/SIRT1/NF-κB pathway. Brain Res Bull. 2022;189:44-56.