Journal of Inflammation Research

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Large Chengqi Decoction Improves Sepsis-Related Intestinal Damage by Inhibiting Inflammatory Response Through the HMGB1-TLR4 Signaling Pathway

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Purpose: This study aimed to evaluate the therapeutic efficacy of Large Chengqi Decoction(LCD) in attenuating sepsis-related intestinal injury by targeting the HMGB1-TLR4 signaling pathway.

Methods: Seventy-five Sprague-Dawley (SD) rats were utilized to establish a septic intestinal injury model, randomized into sham operation, model control, and three treatment groups (LCD0.1, LCD1, LCD10). HMGB1, TLR4, IL-6, and MCP-1 levels in intestinal tissues were assessed via ELISA and Western blotting. Histopathological changes were examined using HE staining of ileum sections.

Results: Compared to the sham group, the model group showed significant elevation of inflammatory markers, confirming successful model establishment. In the LCD1 group, HMGB1 levels were notably higher at 3 and 5 days, accompanied by consistent TLR4 downregulation. IL-6 levels were significantly reduced at 3 days, and MCP-1 levels were lower compared to the sham group. LCD10 group exhibited decreased HMGB1 levels at 5 days and reduced IL-6 levels at 3 days. Immunohistochemical analysis at 3 days post-modeling indicated that LCD1 group expressions of HMGB1, TLR4, NF-κB, and MCP-1 resembled the sham group and significantly differed from the model group. Both LCD1 and LCD10 groups showed improved ileal damage and reduced edema compared to the model group.

Conclusion: LCD effectively mitigates inflammatory responses in septic rats by modulating the HMGB1-TLR4/NF-κB pathway, thereby promoting intestinal repair. Concentrations of 1g/mL and 10g/mL present promising therapeutic strategies for sepsis-related intestinal injury, highlighting the potential of traditional Chinese medicine in sepsis treatment research.

Keywords: large Chengqi decoction, high mobility group protein 1, toll-like receptor 4, sepsis-related intestinal injury, traditional Chinese medicine

Introduction

Sepsis is a life-threatening organ dysfunction with high mortality. A worldwide survey found cause-of-death data from 109 million individual death records to calculate sepsis-related mortality for up to 282 potential causes of death.[1,2] Sepsis causes damage to the intestinal mucosa, leading to bacterial translocation and aggravating local and remote organ damage.[3,4] Research shows that when sepsis occurs, excessive inflammation damages intestinal epithelial cells, leading to increased apoptosis of intestinal epithelial cells, and then damage to the intestinal mucosal barrier, causing intestinal microorganisms and their products to enter the blood from the intestine, promoting bacterial migration and the spread of toxins will further stimulate the host's inflammatory response, leading to multiple organ failure and life-threatening clinical symptom.[5,6]

Received: 5 September 2024
Accepted: 29 March 2025
Published: 23 April 2025

However, our current understanding of the mechanisms of intestinal injury in sepsis is still lacking. Some scholars have found that the levels of inhibitory factors for intestinal epithelial regeneration and defense are increased in the intestinal mucosa of mice after septic stress, as well as in the intestinal mucosa of patients with inflammatory bowel disease and sepsis.[7] At the same time, inflammatory responses are often associated with various severe traumas, which recruit immune cells into target organs, causing systemic inflammatory responses and leading to sepsis.[8] A key function of epithelial cells is to maintain a barrier that protects the body from pathogens, irritating luminal contents, and microbiota. Disruption of this barrier can lead to inflammation of the intestinal mucosa and, in severe cases, sepsis.[9]

In recent years, research on the HMGB1-TLR4 signaling pathway has made great progress in reducing inflammatory responses and improving sepsis-related intestinal injury.[10] HMGB1 is an endogenous inflammatory mediator that triggers inflammatory responses by connecting with TLR4, leading to damage to important organs.[11] Studies have found that inhibiting the HMGB1-TLR4 signaling pathway can alleviate the symptoms of sepsis-related intestinal injury, improve intestinal barrier function, and reduce intestinal toxin translocation rate,[12] thereby improving the prognosis of sepsis.[13] It can be seen that inhibiting the HMGB1-TLR4 signaling pathway is an important means to treat sepsis-related intestinal injury. Some scholars have found that the protective effect of ulinastatin on the intestinal mucosal barrier against sepsis is mediated through the TLR4/MyD88/NF-κB pathway.[14] Some scholars have achieved an improvement in intestinal edema in mice with endotoxin-induced systemic inflammatory response by inhibiting the TLR4 and NLRP3 signaling pathways.[15] A study on sepsis-related liver injury found that the inflammatory response was alleviated by inhibiting the HMGB1/TLR4/NF-κB signaling pathway.[16] This study noted that sepsis-related liver injury and intestinal injury are related to the pathogenesis of endotoxins, increased inflammatory factors, etc.

Large Chengqi Decoction (LCD) is a classic Chinese medicine prescription. The formula contains rhubarb, Magnolia officinalis, citrus aurantium, and sodium salt ("Health Treasure Book"). It has the functions of laxative, heat-clearing and detoxification.[17] Sepsis-related intestinal injury is a modern medical disease. Based on its clinical manifestations such as abdominal distension, nausea, constipation, and vomiting, it can be classified into categories such as "abdominal pain", "bloating", "fullness", "stomach pain" and "constipation", The current consensus among TCM experts points out that the main causes of sepsis are exogenous six evils, endogenous heat poison, blood stasis, conflict between good and evil, damage to healthy qi, and mixture of deficiency and excess, ultimately leading to disease.[18] Therefore, when patients with sepsis are complicated by intestinal damage, they should follow the treatment methods of clearing away heat and clearing the bowels, detoxifying and removing blood stasis, so that evil qi can be removed, stomach qi can be generated, and the balance of yin and yang in the body can be restored.[19] LCD comes from Zhang Zhongjing's. Treatise on Febrile Diseases and its medicinal composition is: rhubarb, sodium salt, citrus aurantium, and Magnolia officinalis. In the prescription, rhubarb is used as a monarch drug for purging heat and laxative, cleansing the stomach and intestines; Glauber's salt is a ministerial drug for helping to purge heat and laxative, and can soften hardness and moisturize dryness; Fructus Aurantii and Magnolia officinalis are used as assistants, having the effect of promoting qi and dispersing stagnation.[20] This prescription has the effect of clearing away heat and unblocking the viscera, and is mainly used to treat Yangming viscera syndrome. Its main pulse syndromes are: abdominal fullness and pain, fever without aversion to cold, difficulty in defecation, umbilical pain, slow or large pulse, etc.[21] Among them, it is rich in the emodin can regulate the HMGB1/TLR4/NF-κB signaling pathway and improve the levels of inflammatory factors in cerebral hemorrhage model rats.[22] When used in rat models of severe pancreatic cancer, honokiol can inhibit the HMGB1-TLR4/NF-κB signaling pathway, thereby achieving the goal of inhibiting the levels of inflammatory factors.[23] Therefore, this study hypothesizes that LCD and its rich active ingredients can inhibit the activation of the HMGB1/TLR4 signaling pathway and effectively reduce the body's inflammatory response. This study aimed to observe through animal experiments whether LCD can reduce the inflammatory response of sepsis-related intestinal injury and its effect on the HMGB1/TLR4 signaling pathway.

Materials and Methods

Animal Source

75 SPF-grade healthy male Sprague-Dawley (SD) rats, 4 to 6 weeks old, weighing 190 to 220 g. Provided by the Experimental Animal Center of our hospital. Use standard rodent feed and drinking water, and use independent feeding and drinking methods. The ambient temperature and humidity in the laboratory are set at 20~25°C and 55%~65%

respectively. The ambient light time was set to 12 h/d, and the feeding process followed the feeding operation rules for clean-level experimental animals, and pre-feeding was conducted for 7 days before modeling.

Preparation of LCD Freeze-Dried Powder Preparation

Citrus aurantium (batch number: 221205037; producing area: Hunan, China; Kangmei Pharmaceutical Co., Ltd).

Rhubarb (batch number: 230100149; producing area: Sichuan, China; Kangmei Pharmaceutical Co., Ltd).

Magnolia officinalis (batch number: 230160851; producing area: Sichuan, China; Kangmei Pharmaceutical Co., Ltd).

Citrus aurantium, Rhubarb and Magnolia officinalis were purchased from The First Affiliated Hospital of Wenzhou Medical University, and authenticated by Professor Qiandong Zhu (Departments of Hepatobiliary Surgery, The First Affiliated Hospital of Wenzhou Medical University).

To prepare LCD freeze-dried powder preparation, select authentic medicinal materials: rhubarb (12g), Magnolia officinalis (24g), citrus aurantium (12g), and Glauber's salt (9g). Wash, chop or grind and set aside. Place the ground medicinal materials into five times the mass of water, bring to a boil and then simmer over low heat for 2 hours. The decoction liquid is drip-filtered, and the filtrate is placed in a water bath and concentrated over low heat to a relative density of 1.1~1.2. The concentrated liquid is subjected to freeze crystallization filtration, and the filter cake is placed in an oven and dried continuously at 60°C for 24 hours to prepare freeze-dried powder. Mix the resulting freeze-dried powder evenly in a mortar, sieve and seal it in a humidity-free polyethylene bag or aluminum foil bag. Perform specification control on finished products: color, odor, stain inspection, etc. Specified storage conditions: cool and dry place, prohibited from direct sunlight. During the experiment, LCD freeze-dried powder preparation was used to prepare 0.1g/mL, 1g/mL, and 10g/mL drug-containing liquids for later use.

Preparation of Sepsis-Related Intestinal Injury Model

Except for the sham operation group, rat sepsis-related intestinal injury models were established. Cecal puncture was used to construct a sepsis-related intestinal injury model. SD rats were starved for 12 hours, given anesthesia and then shaved and disinfected. A straight abdominal incision was made in the midline to expose the small intestine and cecum. Use forceps to create two holes with a diameter of 1–2 mm in the small curvature of the cecum through the wall to allow the intestinal contents to leak out.

Locally perfuse 0.1mL TcdB solution (10 ng/mL), and use forceps to gently press the surrounding tissue of the necrotic area. After restoring intestinal patency, intestinal anastomosis and abdominal wall suture are performed to close the abdomen. Sham operation group: only laparotomy without exposing the cecum and small intestine. The LCD-containing medicinal solution was fed to three rats in the LCD group by gavage 2 hours before surgery. The dosage of the intragastric administration was based on the equivalent dose formula for humans: $Y = 0.814\ g/kg \times 70kg \times 0.0018 / 0.2kg \approx 0.5g/kg$, combined with the dosage concentration of the medicinal liquid contained in LCD, it is estimated that the weight of the rat is about 2kg. 0.1g/mL, 1g/mL, and 10g/mL were administered to the three groups of LCD respectively medicinal solution, 1 mL each, 2 times/d. And the rats' food intake, excrement, and activity ability were collected.

Detection of Protein Expression of HMGB1-TLR4/NF-κB Signaling Pathway

Sample Collection

- Serum Samples: Blood was collected from the orbital sinus under anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 minutes at 4°C and stored at −80°C until analysis.
- Intestinal Tissue Samples: Fresh intestinal tissue was collected from 5 rats in each group on days 1, 3, and 5 post-modeling. Tissues were rinsed with ice-cold saline, quickly frozen in liquid nitrogen, ground into powder, homogenized, and centrifuged at 12,000 rpm for 20 minutes at 4°C to collect the supernatant.

ELISA Assay

- Use enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions to detect serum levels of HMGB1, TLR4, IL-6, and MCP-1.
- Each sample was tested in triplicate, and results were expressed as mean ± standard deviation (SD).

Western Blotting

- Protein Extraction: Total protein was extracted from intestinal tissue samples using RIPA buffer containing protease inhibitors. Protein concentration was determined using the BCA protein assay kit.
- Electrophoresis and Transfer: Equal amounts of protein (30 μg per lane) were separated by SDS-PAGE and transferred onto PVDF membranes.
- Membrane Blocking and Incubation: Membranes were blocked with 5% non-fat milk in TBST for 1 hour at room temperature, then incubated overnight at 4°C with primary antibodies against HMGB1, TLR4, NF-κB, and β-actin (internal control).
- Secondary Antibodies and Detection: Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Proteins were visualized using an enhanced chemiluminescence (ECL) detection system.
- Quantification: Band intensities were quantified using ImageJ software, and the relative expression levels were normalized to β-actin.

Histopathological Examination

- Tissue Processing: Rat ileum tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm sections.
- Staining and Microscopy: Sections were stained with hematoxylin and eosin (HE) for histopathological examination. Slides were observed under a light microscope, and representative images were captured for documentation and analysis.

Statistical Methods

Use Statistics 26.0 for statistical analysis. If the measurement data conforms to the normal distribution, use $x \pm s$ for statistical description. If the measurement data does not conform to the normal distribution, use M (P25, P75) to describe it. If the data conforms to a normal distribution, one-way ANOVA is used to compare multiple groups of data. First, Levene's method is used to check whether the variances are homogeneous. When the variances are homogeneous, LSD-t is used to compare the means between multiple groups. Test rows for pairwise comparison. Differences were considered statistically significant with $P<0.05$.

Results and Analysis

Changes in the General Condition, Weight and Activity of Rats in Each Group

- Sham Operation Group: These rats underwent surgery but did not develop sepsis. They remained in good health with stable or gradually increasing weight and normal activity levels.
- Model Group (Sepsis Induced): After inducing sepsis, these rats exhibited symptoms such as loss of appetite, reduced activity, weight loss, and varying degrees of diarrhea or soft stools.
- 0.1g/mL LCD Group: Compared to the model group, there was some improvement in this group, but it was less significant than in the higher-dose groups. By the 5th day, improvements became noticeable.
- 1g/mL and 10g/mL LCD Groups:

By the 3rd Day: Significant improvements in appetite and activity were observed.

By the 5th Day: No obvious disease symptoms were present, and clinical manifestations were similar between these two groups.

In summary, higher doses of LCD (1g/mL and 10g/mL) showed more rapid and significant improvements in the general condition, weight, and activity of the rats compared to the lower dose (0.1g/mL) and the model group.

Changes in the Expression Levels of Serological Indicators in Rats in Each Group

In this study, we measured serum levels of HMGB1, TLR4, IL-6, and MCP-1 at 1-day, 3-day, and 5-day time points to evaluate the effects of different treatments on the inflammatory response.

- Sham Group (Control): Normal levels of all indicators.
- Model Group (Sepsis Induced): Significantly higher levels of HMGB1, TLR4, IL-6, and MCP-1 at all time points compared to the Sham group ($P < 0.05$), confirming successful model establishment.
- LCD Treatment Groups (0.1g/mL, 1g/mL, and 10g/mL):

Key Findings:

- HMGB1:

LCD1 Group: Higher than Sham on days 3 and 5 ($P < 0.05$), no significant difference on day 1.
LCD10 Group: Lower than Sham on day 5 ($P < 0.05$).

- TLR4:

LCD1 Group: Significantly lower than Model at all time points ($P < 0.05$).
LCD10 Group: Significantly lower than Model on day 5 ($P < 0.05$).

- IL-6:

LCD1 Group: Significantly lower than Model on day 3 ($P < 0.05$), no significant difference compared to Sham.
LCD10 Group: No significant difference compared to Sham, but significantly lower than Model on day 3 ($P < 0.05$).

- MCP-1:

LCD1 Group: Significantly reduced compared to Sham on day 3 ($P < 0.05$).
LCD10 Group: No significant difference compared to Sham at any time point.

The LCD1 and LCD10 groups showed regulatory effects on inflammatory factors, particularly noticeable for HMGB1, TLR4, and IL-6 on days 3 and 5. These results suggest that LCD treatment may help alleviate inflammatory conditions by regulating these key inflammatory markers.

For detailed data, see Table 1.

Expression Levels of HMGB1, TLR4, NF-κB and MCP-1 in Intestinal Tissue Fluid of Rats in Each Group

In this study, the effects of different doses of LCD on the expression levels of HMGB1, TLR4, MCP-1, and NF-κB in intestinal tissues were evaluated by establishing a rat model of septic intestinal injury. The experiment was divided into several groups, including the control group (Sham), the model group (Model), and the LCD groups with different doses [LCD0.1, LCD1, LCD10].

To assess the protein expression levels of these markers, we employed both ELISA for serum samples and Western blotting (WB) for intestinal tissue samples. The results showed that during the observation periods of 1, 3, and 5 days, the model group exhibited a significant increase in the expression levels of HMGB1, TLR4, MCP-1, and NF-κB compared with the control group ($P < 0.05$), indicating that the sepsis model was successfully established.

Western blotting analysis revealed that the LCD treatment groups exhibited different degrees of modulation on the expression levels of these inflammatory markers at different doses. Specifically, the LCD1 and LCD10 groups showed particularly significant effects. At day 3, the LCD1 group demonstrated significant changes in HMGB1 and TLR4 expression compared to the Model group, reaching their highest values. By day 5, MCP-1 and NF-κB levels in the LCD1 group had significantly decreased compared to the Model group, suggesting a strong inhibitory effect on sepsis-induced intestinal inflammatory response.

Table 1 Changes in the Expression Levels of Serum HMGB1, TLR4, IL-6 and MCP-1 in Rats in Each Group (n=5)

Groups	HMGB1(μg/L)			TLR4(ng/L)		
	1d	3d	5d	1d	3d	5d
Sham	79.99±6.62^{bcde}	70.51±9.54^{bcde}	86.04±12.49^{bcde}	2.42±0.12^{bcde}	0.36±0.03^{bcde}	0.36±0.03^{bcde}
Model	10.59±2.42^{a}	9.76±3.17^{acde}	7.22±1.69^{acde}	5.13±0.27^{a}	5.20±0.35^{acde}	4.94±0.07^{acde}
LCD0.1	11.18±3.13^{a}	30.61±7.08^{abe}	39.97±4.67^{abe}	5.00±0.27^{a}	3.05±0.43^{ab}	2.66±0.03abde
LCD1	9.71±1.89^{a}	47.45±1.48^{abc}	64.61±6.07^{abc}	4.82±0.08^{a}	2.53±0.37^{ab}	1.57±0.03abce
LCD10	10.52±1.38^{a}	39.75±8.27^{ab}	56.38±10.27^{ab}	4.77±0.11^{a}	3.13±0.29^{ab}	2.49±0.04abcd
Groups	**IL-6(pg/mL)**			**MCP-1(pg/mL)**		
	1d	3d	5d	1d	3d	5d
Sham	38.59±6.67	35.53±2.56^{bc}	42.67±4.93	429.58±49.18	436.38±16.49^{bc}	433.26±44.28
Model	41.64±4.69	54.97±4.97^{ade}	47.12±6.60^{d}	498.64±59.38	634.76±40.14^{a}	620.32±56.83
LCD0.1	40.03±5.56	49.16±2.96^{ad}	42.90±3.43	487.26±58.72	626.55±71.85^{a}	522.51±51.00
LCD1	39.04±4.51	38.15±7.60^{bc}	37.19±5.27^{b}	505.15±64.38	517.28±65.95	572.46±69.57
LCD10	40.37±4.51	44.03±4.54^{b}	38.34±1.07	486.26±62.35	542.77±90.09	546.09±65.09

Notes: $^{a}P < 0.05$ compared with Sham group; $^{b}P < 0.05$ compared with Model group; $^{c}P < 0.05$ compared with LCD0.1 group; $^{d}P < 0.05$ compared with Sham; $^{e}P < 0.05$ compared with Sham.

Furthermore, the WB results provided additional evidence supporting the potential therapeutic effect of LCD on sepsis-related intestinal injury by regulating the HMGB1-TLR4/NF-κB signaling pathway. For instance:

- HMGB1: In the LCD1 group, HMGB1 expression peaked at day 3 and then decreased by day 5.
- TLR4: Similar trends were observed for TLR4, with peak expression at day 3 followed by a decline by day 5.
- MCP-1 and NF-κB: Both markers showed significant reductions in the LCD1 group by day 5, indicating effective suppression of the inflammatory response.

These findings are summarized in Table 2 and further support the hypothesis that LCD can effectively mitigate sepsis-induced intestinal damage through modulation of the HMGB1-TLR4/NF-κB signaling pathway.

Histopathological Results of Ileum

In the same field of view of the sham-operated group, the number of small intestinal villi was high, the morphology was complete and neatly arranged; in the model group, the small intestinal villi were sharpened, severely broken, misarranged and severely damaged. In the LCD0.1 group, LCD1 group and LCD10 group, compared with the model group, the damage and edema of the ileum in the three Daxingqi Tang groups were significantly improved, the breakage was reduced, and the arrangement was closer to neatness, as detailed in Figure 1.

Discussion

This study systematically evaluated the therapeutic effect of LCD pairs by establishing a rat model of septic intestinal injury. In the study, the expression levels of key inflammatory markers HMGB1, TLR4, IL-6 and MCP-1 in serum and intestinal tissue were detected by ELISA and Western blotting, and the pathological changes in the rat ileum were observed by HE staining. The results showed that the LCD1 and LCD10 groups (ie, 1g/mL and 10g/mL doses of LCD) showed significant effects in regulating inflammatory markers and improving intestinal pathological damage, especially on the 3rd and 5th days of the experiment. The study revealed that LCD effectively inhibited the inflammatory response in septic rats and promoted intestinal repair by targeting the HMGB1-TLR4/NF-κB signaling pathway. These findings provide new strategies for the clinical treatment of sepsis-related intestinal injury and provide scientific basis for the potential of traditional Chinese medicine compounds in the treatment of sepsis, which is expected to promote the

Table 2 Changes in the Expression Levels of HMGB1, TLR4, NF-κB and MCP-1 in Rat Intestinal Tissues in Each Group (n=5)

Groups	HMGB1			TLR4		
	1d	3d	5d	1d	3d	5d
Sham	1.00±0.03	1.00±0.03^{bce}	1.00±0.03^{bcde}	1.00±0.03^{bcde}	1.00±0.03^{bcde}	1.00± 0.03^{bcde}
Model	0.98±0.07	0.73±0.07^{acd}	0.75±0.07^{ad}	1.12±0.07^{a}	1.65±0.07^{acde}	1.94 ± 0.07^{acde}
LCD0.1	0.96±0.03	0.86±0.03^{ab}	0.76±0.03^{a}	1.14±0.03^{a}	1.19±0.03^{abd}	1.24±0.03^{abde}
LCD1	0.95±0.03	0.95±0.03^{be}	0.85±0.03^{ab}	1.13±0.03^{a}	1.33±0.03^{abc}	1.53±0.03^{abce}
LCD10	0.99±0.04	0.80±0.04^{ad}	0.79±0.04^{a}	1.14±0.04^{a}	1.25±0.04^{ab}	1.34±0.04^{abcd}
Groups	**MCP-1**			**NF-κB**		
	1d	3d	5d	1d	3d	5d
Sham	429.58±49.18	431.25±44.35^{bcde}	433.26±44.28^{bd}	1.00±0.03^{bcde}	1.00±0.03^{bcde}	1.00±0.03^{bcde}
Model	498.64±59.38	609.26±64.38^{a}	620.32±56.83^{a}	1.25±0.07^{a}	1.69±0.07^{acde}	1.97±0.07^{acde}
LCD0.1	496.79±60.34	561.17±60.59^{a}	522.51±51.00	1.24±0.02^{a}	1.21±0.02^{ab}	1.25±0.02^{ab}
LCD1	505.15±64.38	597.86±66.23^{a}	472.46±69.57^{ab}	1.22±0.03^{a}	1.26±0.03^{ab}	1.12±0.03^{ace}
LCD10	503.28±67.19	564.23±63.28^{a}	516.09±65.09	1.28±0.02^{a}	1.20±0.02^{ab}	1.22±0.02^{abd}

Notes: $^{a}P < 0.05$ compared with Sham group; $^{b}P < 0.05$ compared with Model group; $^{c}P < 0.05$ compared with LCD0.1 group; $^{d}P < 0.05$ compared with Sham; $^{e}P < 0.05$ compared with Sham.

development of new treatments. Multiple studies have found that sepsis, as a severe clinical syndrome caused by infection, is characterized by systemic inflammatory response and multi-organ dysfunction, and that septic intestinal injury plays a key role in the pathological progression of sepsis. The disruption of intestinal barrier function not only aggravates inflammation, but may also lead to the translocation of bacteria and endotoxins, thereby triggering or exacerbating multiple organ failure.[24–26] Therefore, the development of effective treatments is important to reduce inflammation and protect the intestinal barrier. Function has important clinical significance.

Animal experiments[27] found that increased HMGB1 levels coincide with excess pro-inflammatory factors/chemokines in sepsis. Inhibiting TLR4 can enhance autophagy and HMGB1 levels in the cerebral cortex of rats with sepsis

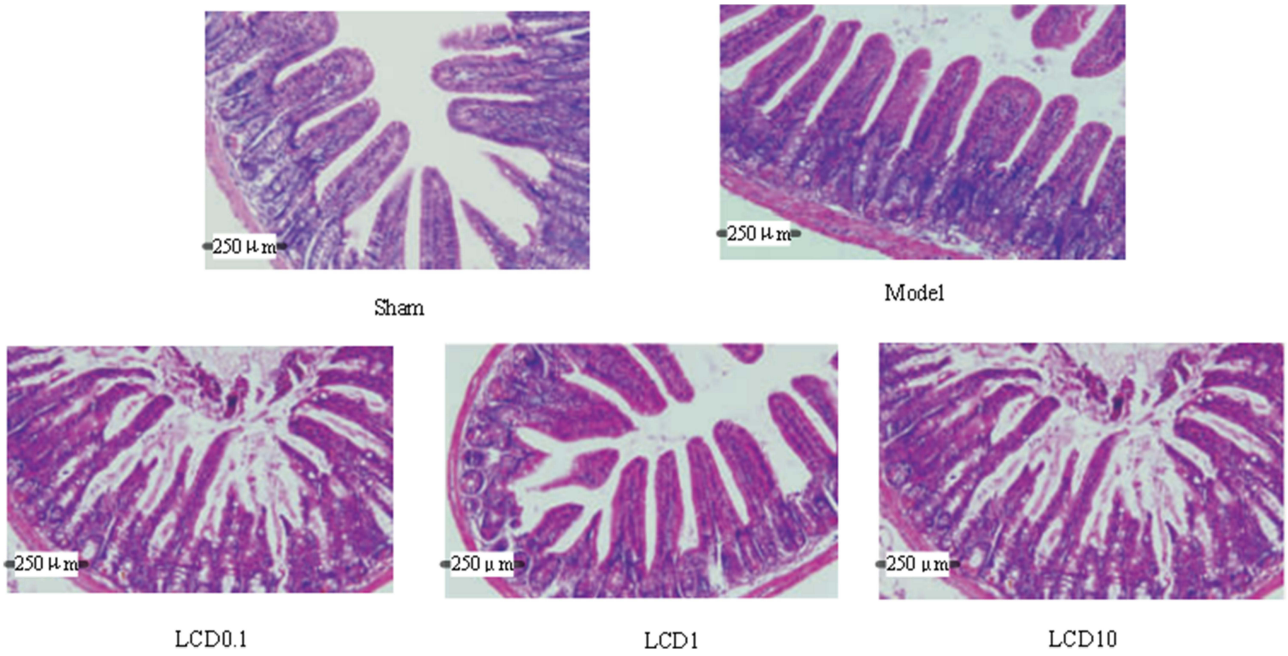

Figure 1 Histopathological changes of ileum in sepsis-associated intestinal injury by Da Cheng Qi Tang (HE×200).

https://doi.org/10.2147/JIR.S490679

injury.[28] The HMGB1-TLR4 signaling pathway is closely related to inflammation, oxidative stress, fibrosis, etc.[29] At the same time, the HMGB1-TLR4-MyD88 signaling pathway can regulate intestinal ischemia-reperfusion injury.[30] NF-κB is also involved in the HMGB1-TLR4 signaling pathway.[31] HMGB1-TLR4/NF-κB is closely related to inflammatory response.[32] The results of this study show that LCD has a significant protective effect on sepsis-related intestinal injury by regulating the HMGB1-TLR4 signaling pathway. In comparison with the existing literature and studies by others, we found that LCD has similar effects to some traditional Chinese medicine adjuvant treatments[33] and modern anti-inflammatory drugs in reducing the levels of inflammatory mediators in serum and intestinal tissue. A meta-analysis found that LCD is an effective treatment for patients with severe pancreatitis and can reduce mortality.[34] For example, the significant increase in HMGB1 levels and the decrease in TLR4 levels in the LCD1 group at 3 and 5 days echoed the regulatory effects of specific anti-inflammatory drugs on inflammatory mediators in previous studies.[35] In addition, the significant reduction in IL-6 levels in the LCD1 group at 3 days is consistent with the inhibitory effect of inflammatory factors observed in multiple studies.

However, as a traditional Chinese medicine compound, LCD's multi-component and multi-target characteristics may provide it with more comprehensive anti-inflammatory and intestinal protective effects. Compared to traditional single-target drugs, LCD can exert synergistic effects by influencing multiple inflammatory pathways, an advantage that single-ingredient drugs find difficult to achieve. Our study further strengthens the potential value of traditional Chinese medicine in regulating intestinal inflammation and protecting intestinal barrier function, consistent with the growing body of research emphasizing the importance of intestinal health in anti-inflammatory processes during sepsis treatment.[36–38] Notably, LCD showed an ameliorative effect on intestinal epithelial tissue damage in immunohistochemical analysis, which has not been commonly reported in previous studies. The significant improvement in ileal damage and edema in rats in the LCD1 and LCD10 groups, along with the regularization of villus arrangement, suggests that LCD may directly act on intestinal tissue, enhance intestinal barrier function, and reduce the infiltration of inflammatory cells.[39] To further validate these findings, future experiments should focus on assessing the impact of LCD on intestinal barrier integrity. Techniques such as trans-epithelial electrical resistance (TEER) measurements and histological examinations could provide concrete evidence of LCD's ability to restore and protect the intestinal barrier. This is particularly important because maintaining intestinal barrier function is key to preventing bacterial and endotoxin translocation and avoiding multi-organ failure,[40] which can effectively enhance the relative abundance of anti-inflammatory intestinal microorganisms.[41] In conclusion, our findings are not only consistent with existing literature on the regulation of inflammatory mediators but also highlight the potential advantages of traditional Chinese medicine compounds in multi-target anti-inflammation and intestinal protection. Future studies should investigate LCD's efficacy across various sepsis models and consider conducting clinical trials to verify its safety and efficacy in human patients. Additionally, in-depth study of the specific mechanisms of action of LCD, including its impact on intestinal microecology, will provide more insights and therapeutic strategies for comprehensive treatment of sepsis.

Although this study reveals that LCD has a potential therapeutic effect on sepsis-related intestinal injury through the HMGB1-TLR4 signaling pathway, there are still several limitations that need to be addressed and improved in future studies. First, the experimental subjects of this study were SD rats, and the pathological mechanism of sepsis in human patients may differ significantly from that observed in animal models. Therefore, the clinical application of LCD's efficacy and safety requires further verification through human clinical trials. Secondly, while this study primarily focused on the effect of LCD on inflammatory mediators, the complex composition of LCD suggests that there may be additional mechanisms of action that have not been fully explored. Future research should aim to identify which specific ingredients in LCD—comprising rhubarb, Magnolia officinalis, citrus aurantium, and sodium salt—are responsible for modulating the HMGB1-TLR4 pathway. For instance, Rhubarb (Da Huang) is known for its purgative properties and may reduce HMGB1 release and subsequent TLR4 activation. Magnolia officinalis (Hou Po), with its antispasmodic and anti-inflammatory effects, could contribute to stabilizing the intestinal barrier. Citrus aurantium (Zhi Shi) promotes digestion and regulates qi flow, potentially aiding in the reduction of inflammation. Sodium salt (Mang Xiao), a potent laxative, helps clear endotoxins from the gut, indirectly affecting inflammatory pathways. In-depth analyses of these components will provide valuable insights into their specific roles and mechanisms of action, thereby enhancing our understanding of LCD's therapeutic potential. Moreover, it is important to consider other significant pathways such as

HIF-1α and TGF-β, which play critical roles in hypoxic adaptation and tissue repair during sepsis-induced intestinal injury. LCD might influence these pathways to promote recovery while preventing adverse effects like fibrosis. Additionally, the dosage selection of LCD in this study was based on previous literature and preliminary experiments; however, the optimal dosage and dosing regimen still need to be determined within a wider range to identify the most effective treatment strategy. Finally, the pathogenesis of sepsis is multifaceted, involving interactions at multiple levels such as genetics, immunity, and metabolism. The impact of LCD on overall patient prognosis, including survival rates, organ function recovery, and mitigation of intestinal ischemia-reperfusion injury, has not been evaluated in this study. Therefore, future research should consider conducting multi-center, large-sample clinical studies to comprehensively evaluate the clinical application value of LCD and its broader implications for treating sepsis-related intestinal injuries. To strengthen the evaluation of histological changes triggered by LCD decoction, a standardized method for calculating histology scores should be implemented. This involves objectively measuring and comparing the extent of tissue damage across different groups. Histology scores are numerical values assigned based on the severity and extent of specific pathological features observed under microscopy, allowing for a standardized and quantitative assessment of tissue damage. Clear criteria should be established for scoring various aspects of tissue damage, such as inflammation, villus height, crypt depth, epithelial integrity, and presence of edema or necrosis. Each criterion can be scored on a defined scale (eg, 0–3 or 0–5), where higher scores indicate more severe damage. Multiple pathologists should evaluate the sections independently and blindly to ensure objectivity. Statistical analysis of the collected scores will support the conclusions drawn from the histopathological examination, providing measurable evidence of LCD's protective effects on the intestinal tissue. By incorporating this rigorous approach, the study can offer robust and reliable data to substantiate its findings.

Conclusion

This study reveals for the first time that LCD has a significant ameliorative effect on sepsis-related intestinal injury through the HMGB1-TLR4 signaling pathway. LCD displays multi-target and multi-effect therapeutic characteristics, which may have a more comprehensive therapeutic effect compared with single-component drugs.

Data Sharing Statement

All the data related to this study can be obtained from the correspondent Professor Qiuqi Gao.

Ethics Approval and Consent

This study was conducted in accordance with the ethical guidelines and regulations for animal research. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of The First Affiliated Hospital of Wenzhou Medical University. The study adhered to the Decree on the Administration of Laboratory Animals and the Guiding Principles for the Care and Use of Laboratory Animals, ensuring the welfare and humane treatment of all laboratory animals used in this research.

Acknowledgments

The authors would like to show sincere thanks to those techniques who have contributed to this research.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by Research Project funded by the Science and Technology Project of Wenzhou (Y20210720, Y20210172).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the global burden of disease study. *Lancet*. 2020;395(10219):200–211. doi:10.1016/S0140-6736(19)32989-7
2. Pandey S. Sepsis, management & advances in metabolomics[J]. *Nanotheranostics*. 2024;8(3):270. doi:10.7150/ntno.94071
3. Tian WZ, Yue Q, Fei W, Yao PZ, Han RQ, Tang J. PE (0:0/14:0), an endogenous metabolite of the gut microbiota, exerts protective effects against sepsis-induced intestinal injury by modulating the AHR/CYP1A1 pathway. *Clin Sci*. 2023;137(22):1753–1769. doi:10.1042/CS20230704
4. Pandey S. Advances in metabolomics in critically ill patients with sepsis and septic shock. *Clin Experimental Emergency Med*. 2024;12(1):4–15. doi:10.15441/ceem.24.211
5. Hu Q, Ren H, Li G, et al. STING-mediated intestinal barrier dysfunction contributes to lethal sepsis. *EBioMedicine*. 2019;41:497–508. doi:10.1016/j.ebiom.2019.02.055
6. Liu YY, Bao DQ, Zhang ZS, Zhu Y, Liu LM, Li T. Radix sanguisorbae improves intestinal barrier in septic rats via HIF-1 α/HO-1/F(e2+) axis. *Chin J Integr Med*. 2024;30(12):1101–1112.
7. Chung HK, Xiao L, Han N, et al. Circular RNA Cdr1as inhibits proliferation and delays injury-induced regeneration of the intestinal epithelium. *JCI Insight*. 2024;9(4):e169716[pii]. doi:10.1172/jci.insight.169716
8. Maulydia M, Rehatta NM, Soedarmo SM. Effects of glutamine and arginine combination on pro- and anti-inflammatory cytokines. *Open Vet J*. 2023;13(5):613–619. doi:10.5455/OVJ.2023.v13.i5.14
9. Marr EE, Mulhern TJ, Welch M, et al. A platform to reproducibly evaluate human colon permeability and damage. *Sci Rep*. 2023;13(1):8922. doi:10.1038/s41598-023-36020-8
10. Wang G, Ma F, Xie K, et al. Liensinine alleviates mouse intestinal injury induced by sepsis through inhibition of oxidative stress, inflammation, and cell apoptosis. *Int Immunopharmacol*. 2024;127:111335. doi:10.1016/j.intimp.2023.111335
11. Du YC, Lai L, Zhang H, et al. Kaempferol from penthorum chinense Pursh suppresses HMGB1/TLR4/NF-κB signaling and NLRP3 inflammasome activation in acetaminophen-induced hepatotoxicity. *Food Funct*. 2020;11(9):7925–7934. doi:10.1039/D0FO00724B
12. Hu Y, Liu C, Yang J, et al. HMGB1 is involved in viral replication and the inflammatory response in coxsackievirus A16-infected 16HBE cells via proteomic analysis and identification. *Virol J*. 2023;20(1):178. doi:10.1186/s12985-023-02150-8
13. Zhou G, Xie D, Fan R, et al. Comparison of pulmonary and extrapulmonary models of sepsis-associated acute lung injury. *Physiol Res*. 2023;72(6):741–752. doi:10.33549/physiolres.935123
14. Wenying S, Jing H, Ying L, Hui D. The role of TLR4/MyD88/NF-κB in the protective effect of ulinastatin on the intestinal mucosal barrier in mice with sepsis. *BMC Anesthesiol*. 2023;23(1):414. doi:10.1186/s12871-023-02374-9
15. Lee SC, Hsu JS, Li CC, Chen KM, Liu CT. Protective effect of leaf essential oil from Cinnamomum osmophloeum Kanehira on endotoxin-induced intestinal injury in mice associated with suppressed local expression of molecules in the signaling pathways of TLR4 and NLRP3. *PLoS One*. 2015;10(3):e0120700. doi:10.1371/journal.pone.0120700
16. Li H, Qiu D, Gao Q, Wang H, Sun M. Selectively activating melanocortin 4 receptor acts against rat sepsis-induced acute liver injury via HMGB1/TLR4/NF-κB signaling pathway. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2016;32(8):1055–1059.
17. Li JQ, Cao HY, Xiao TG, Zhou H, Fu ZQ. Clinical observation of large Chengqi decoction on gastrointestinal dysfunction in patients with acute pancreatitis. *Chinese J Integrated Trad Western Med Digestion*. 2021;29(10):686–690.
18. Li Z-J, Liu -Q-Q, Shen H, Zhou R-B, Li Y-P, Ren X-S; Chinese Society of Integrative Medicine EMC, Editorial CoCJoIMaEM. Expert consensus on combined diagnosis and treatment of sepsis. *Chinese Crit Care Med*. 2013;25(4):194–197. doi:10.3760/cma.j.issn.2095-4352.2013.04.002
19. Wang DC, Gao X, Su XP, et al. Protective effect of Fuzheng septic granules on intestinal injury caused by abdominal infection in septic rats. *Trad Chin Med Res*. 2012;25(2):51–54.
20. Deng ZJ. Formulary. Beijing. Traditional Chinese Medicine. 2008; 59–61.
21. Lu XL, Wu MZ, Jia XL. Discussion of large Chengqi decoction. *J Tianjin University Trad Chin Med*. 2009;28(1):4–5.
22. Zhang CL, Liang C, Wang BA, Xu YT. Emodin regulates HMGB1/TLR4/NF-κB signaling pathway and affects inflammatory factors in rats with cerebral hemorrhage in elderly. *Chin J Immunol*. 2022;38(24):2992–2996.
23. Wang Y QWJ, Zeng YW GPY, Miao B. Mechanism of action of magnolol in the treatment of acute lung injury in a rat model of severe acute pancreatitis. *J Clin Hepatol*. 2020;36(12):2782–2787.
24. Aboyoussef AM, Mohammad MK, Abo-Saif AA, Messiha B. Granisetron attenuates liver injury and inflammation in a rat model of cecal ligation and puncture-induced sepsis. *J Pharmacol Sci*. 2021;147(4):358–366. doi:10.1016/j.jphs.2021.08.005
25. Liu J, Wang Z, Lin J, et al. Xuebijing injection in septic rats mitigates kidney injury, reduces cortical microcirculatory disorders, and suppresses activation of local inflammation. *J Ethnopharmacol*. 2021;276:114199. doi:10.1016/j.jep.2021.114199
26. Huang Z, Teng W, Yao L, et al. mTOR signaling pathway regulation HIF-1 α effects on LPS induced intestinal mucosal epithelial model damage. *BMC mol Cell Biol*. 2024;25(1):13. doi:10.1186/s12860-024-00509-5
27. Imbaby S, Matsuda N, Tomita K, et al. Beneficial effect of STAT3 decoy oligodeoxynucleotide transfection on organ injury and mortality in mice with cecal ligation and puncture-induced sepsis. *Sci Rep*. 2020;10(1):15316. doi:10.1038/s41598-020-72136-x
28. Li Y, Zhang L, Tang J, et al. Role of toll-like receptor 4 in the regulation of the cell death pathway and neuroinflammation. *Brain Res Bull*. 2019;148:79–90. doi:10.1016/j.brainresbull.2019.03.015
29. Jing X, Zhou G, Zhu A, Jin C, Li M, Ding K. RG-I pectin-like polysaccharide from Rosa chinensis inhibits inflammation and fibrosis associated to HMGB1/TLR4/NF-κB signaling pathway to improve non-alcoholic steatohepatitis. *Carbohydr Polym*. 2024;337:122139. doi:10.1016/j.carbpol.2024.122139
30. Rong Y, Xu M, Hu T, Zhang S, Fu J, Liu H. Effects of butyrate on intestinal ischemia-reperfusion injury via the HMGB1-TLR4-MyD88 signaling pathway. *Aging*. 2024;16(9):7961–7978. doi:10.18632/aging.205797

31. Jia Z, Yu W, Li X, et al. Du-moxibustion ameliorates depression-like behavior and neuroinflammation in chronic unpredictable mild stress-induced mice. *J Affect Disord*. 2024;358:211–221. doi:10.1016/j.jad.2024.05.025
32. Qi X, Chen X, An WB, et al. Ligusticum cycloprolactam inhibits IL-1β-induced apoptosis and inflammation of rat chondrocytes via HMGB1/TLR4/NF-κB signaling pathway. *Zhongguo Zhong Yao Za Zhi*. 2024;49(4):1007–1016. doi:10.19540/j.cnki.cjcmm.20230904.401
33. Xie K, Guan S, Jing H, et al. Efficacy and safety of traditional Chinese medicine adjuvant therapy for severe pneumonia: evidence mapping of the randomized controlled trials, systematic reviews, and meta-analyses. *Front Pharmacol*. 2023;14:1227436. doi:10.3389/fphar.2023.1227436
34. Lin J, Han C, Dai N, Bi S, Du D, Xia Q. Effectiveness of Chengqi-series decoctions in treating severe acute pancreatitis: a Systematic review and meta-analysis. *Phytomedicine*. 2023;113:154727. doi:10.1016/j.phymed.2023.154727
35. Wang ZC, Xue P, Huang ZW. Effect of an early application of chaiqin chengqi decoction in treating severe acute pancreatitis complicated with acute respiratory distress syndrome. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2009;29(4):322–324.
36. Xie L, Zhang G, Wu Y, et al. Protective effects of Wenqingyin on sepsis-induced acute lung injury through regulation of the receptor for advanced glycation end products pathway. *Phytomedicine*. 2024;129:155654. doi:10.1016/j.phymed.2024.155654
37. Pan G, Wu Y, Liu Y, Zhou F, Li S, Yang S. Large Chengqi decoction ameliorates sepsis-induced liver injury by inhibiting the TGF-β1/Smad3 pathways. *J Tradit Complement Med*. 2024;14(3):256–265. doi:10.1016/j.jtcme.2023.09.001
38. Zhang H, Dong N, Yao Y. Optimal strategy for treatment of sepsis based on the host inflammatory reaction and immune response. *J Intensive Med*. 2024;4(2):175–180. doi:10.1016/j.jointm.2023.10.002
39. Liu J, Song K, Lin B, et al. HMGB1 promotes neutrophil PD-L1 expression through TLR2 and mediates T cell apoptosis leading to immunosuppression in sepsis. *Int Immunopharmacol*. 2024;133:112130. doi:10.1016/j.intimp.2024.112130
40. Wang M, Liao J, Lin W, et al. YL-109 attenuates sepsis-associated multiple organ injury through inhibiting the ERK/AP-1 axis and pyroptosis by upregulating CHIP. *Biomed Pharmacother*. 2024;175:116633. doi:10.1016/j.biopha.2024.116633
41. Chen H, Yu Z, Qi Z, Huang X, Gao J. Tongfu Lifei decoction attenuated sepsis-related intestinal mucosal injury through regulating Th17/Treg balance and modulating gut microbiota. *J Interferon Cytokine Res*. 2024;44(5):208–220. doi:10.1089/jir.2024.0001