Original Article
Protective effects of curcumin on hepatocytes in cecal ligation and puncture-induced sepsis in rats

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Abstract: We aim to observe the protective effects of curcumin in hepatocytes in septic rat. Sprague-Dawley rats were randomly divided into six groups of 20 rats each: sham group; model group; xuebijing group; low dose group (50 mg/kg), middle dose group (100 mg/kg) and high dose group (150 mg/kg). Another 60 rats were randomly divided into the six above-mentioned experimental groups of 10 rats each to determine the survival rate. Our results showed that, treatment with curcumin and Xuebijing significantly attenuated the CLP-induced hepatocyte edema and inflammation indicators, especially in the Xuebijing and 100-mg/kg curcumin groups. This result was also supported by the histopathologic examination findings. Additionally, curcumin improved the survival rate of rats with CLP. Curcumin has a protective effect on CLP-induced liver dysfunction and that the effect varies at different doses; the 100-mg/kg dose was optimal for protection of hepatocytes in septic rats. This protective effect can be attributed to the ability of curcumin to counteract inflammatory cell infiltration and regulate cytokines.

Keywords: Curcumin, sepsis, hepatocyte, cecal ligation, puncture

Introduction

Sepsis is a systemic, deleterious host response to infection and a common complication of burns, trauma, shock, and major surgery. It may lead to septic shock, multiple organ dysfunction syndrome, and other serious conditions. The mortality rate associated with sepsis is extremely high; the overall hospital mortality rate is reportedly 28.6%, while the mortality rate associated with severe sepsis and septic shock are higher at 25% to 30% and 40% to 70%, respectively [1, 2]. Sepsis has become the leading cause of death in the intensive care unit, causing more deaths than acute myocardial infarction, lung cancer, and breast cancer and killing 1,400 people worldwide each day [3-5]. Moreover, the cost associated with sepsis is remarkably high. According to an epidemiological survey, more than 370,000 people die of sepsis in Europe and the United States each year, and the cost of treatment reach up to $25 billion [6]. Sepsis has thus become a serious problem and challenge to human health [7]. The development of effective treatments

and drugs for sepsis is significant and emergent.

As the central organ of metabolism, the liver is one of the most important organs participating in the elimination of bacteria and endotoxins from the body [8-10]. Numerous studies have identified a close relationship between the development of liver injury and the occurrence and aggravation of sepsis and septic shock [11]. Liver dysfunction plays a critical role in the disease course [9]. Patients with sepsis have significantly high levels of oxygen free radicals and release of inflammatory mediators, which occurs secondary to increased membrane permeability caused by damage to the liver cell structures and mitochondrial membranes. Sodium pump dysfunction leads to sodium retention, hepatocyte swelling, and finally hepatocyte apoptosis [12-15].

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural compound with extensive physiologic and pharmacologic activities, including anti-tumor, anti-oxi-

dation, and anti-inflammation effects [16]. Curcumin also exhibits relatively low toxicity, has a simple extraction process, and is low in cost. Curcumin has been confirmed to be extremely safe in various animal models and clinical studies, even at the maximum daily dose of 12 g, and can be administered topically, orally, and by inhalation. Therefore, curcumin shows great potential for clinical applications [17]. Curcumin has a dual role in apoptosis, showing different reactions in different cells. Previous studies have revealed that curcumin exerts a protective effect against liver cell injury caused by multiple factors including ethanol-induced and carbon tetrachloride-induced liver cell damage [18-20]. The underlying mechanism involves increased superoxide dismutase vitality, regulation of inflammatory and anti-inflammatory cytokine expression, inhibition of cytochrome C activity, and other processes. Although curcumin has been shown to be beneficial in sepsis, its potential value in protection of sepsis-induced hepatocyte dysfunction has not been evaluated [20, 21].

Therefore, the present study was designed to demonstrate the protective effect of curcumin and its possible mechanism on hepatocytes in septic rats. We also explored the potential clinical use of curcumin for treatment of sepsis-induced liver dysfunction.

Materials and methods

Experimental animals

Male 3-month-old specific-pathogen-free Sprague-Dawley rats weighing 250 to 350 g were obtained from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China). The rats were maintained in cages in a temperature-controlled room at 25°C±1°C, under an artificial 12-h light-dark cycle, and on a standard diet and water. Animal housing and all experimental procedures were approved by the Institute of Laboratory Animal Science, Jinan University.

Chemicals and reagents

Curcumin of analytical grade was purchased from Sigma-Aldrich (St. Louis, MO, USA). Xuebijing (XBJ) (batch number 1308221) was supplied by Tianjin Chase Sun Pharmaceutical Co., Ltd. (Tianjin, China). All other chemicals and biochemicals used in this study were of high analytical grade.

Animal groups

Sprague-Dawley rats were randomly divided into six groups of 20 rats each: animals undergoing sham CLP (Sham group), animals undergoing CLP (CLP group), animals undergoing CLP and treated with XBJ (XBJ group), and animals undergoing CLP and treated with curcumin at 50 mg/kg (low-dose curcumin [L-Cur] group), 100 mg/kg (middle-dose curcumin [M-Cur] group), or 150 mg/kg (high-dose curcumin [H-Cur] group). Curcumin was diluted with normal saline solution to 10 ml/kg and freshly prepared on the day of the experiment. It was administered intraperitoneally after CLP. The rats received an additional dose of curcumin at 8, 16, and 24 h post-CLP.

Another 60 experimental rates were observed to determine the survival rate. The rats were randomly divided into the six above-described experimental groups of 10 rats each. XBJ and curcumin were intraperitoneally injected three times a day beginning after surgery and continuing for 3 days. The survival rates were recorded at 12, 24, 36, 48, and 72 h after surgery.

Establishment of animal model

The rat models of sepsis in this study were established by CLP, which is generally recognized as a reliable and clinically relevant animal model of the human septic condition. The animal models began to be established after 1 week of adaptive feeding. After fasting for 12 h, the rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (35-40 mg/kg) and placed in a dorsal position. After shaving and iodine disinfection, a 3-cm midline abdominal incision was made to expose the cecum. The cecum was then removed from the abdominal cavity, taking care to maintain the integrity of the intestinal mesentery. The cecum was ligated at a distance of 1.5 cm from the end using 4-0 silk, and then punctured three times with an 18-gauge needle. A small amount of intestinal excreta was expressed through the puncture wound. Indwelling rubber drainage strips were placed to prevent the pinhole from closing, and the cecum was placed back into the abdominal cavity. The abdomen was closed
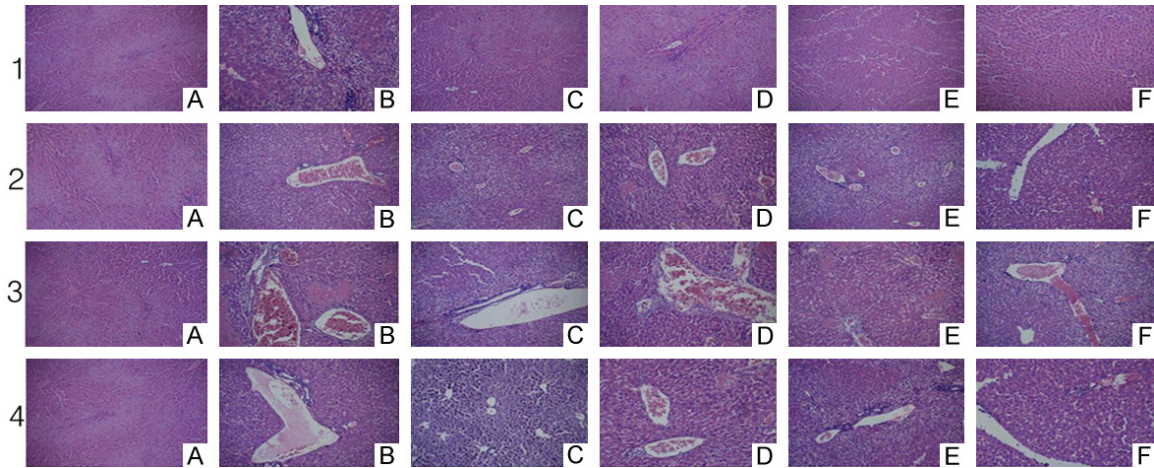
Figure 1. 1: 2 h after surgery. 2: 6 h after surgery. 3: 12 h after surgery. 4: 24 h after surgery. A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p.

layer by layer [22]. After the surgery, sterile normal saline solution (100 mL/kg of body weight) was administered for fluid resuscitation.

In the Sham group, the abdominal cavity was opened and the cecum was flipped over and placed back into the abdominal cavity without ligation or puncture; the remaining steps were the same.

Specimen collection

The animals were anesthetized with 2% sodium pentobarbital at 2, 6, 12, and 24 h post-CLP (n = 5 per time point), and the abdominal cavity was opened to collect liver tissue specimens and portal venous and peripheral venous blood samples. Some samples were stored at -80°C until analysis.

Histological evaluation

The harvested liver specimens were fixed in 10% paraformaldehyde for 24 h, dehydrated in a graded alcohol series, embedded in paraffin, and serially sectioned. Some of the specimens were stained with hematoxylin and eosin to assess morphological changes. The remaining specimens underwent terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining using an In Situ Cell Death Detection Kit (Hoffmann-La Roche, Ltd., Basel, Switzerland). The occurrence of tissue damage was evaluated independently by a pathologist and histologist blinded to the experiment.

Serological evaluation

The serum concentrations of procalcitonin (PCT), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) were analyzed by enzyme-linked immunosorbent assay (Boster Biological Engineering Co., Ltd., Wuhan, China). The plasma samples were centrifuged to separate the serum. All experimental procedures were carried out according to the manufacturer's instructions.

Statistical analysis

All experimental data were statistically processed using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). Measurement data are expressed as mean ± standard error, and a *t*-test was performed. Multiple comparisons were analyzed for significant differences using one-way analysis of variance with Tukey's *post hoc* test for multiple comparisons. A *P* level of < 0.05 was considered statistically significant.

Results

*Histopathologic findings (**Figure 1**)*

In the Sham group, the liver tissues exhibited normal histological features at all postoperative time points, including normal hepatocytes, portal areas, and parenchyma.

Two hours after surgery in the CLP group, obvious and severe histological changes were

Table 1. The changes of apototic index (%) in different time phase

Group	Different Time Phase			
	2 H	6 H	12 H	24 H
A	2.02±0.13	2.10±0.13	2.20±0.05	2.14±0.06
B	23.59±2.00[*]	30.92±1.69[*]	50.18±2.11[*]	52.05±1.31[*]
C	11.89±1.34[*,#]	17.76±1.73[*,#]	27.08±1.64[*,#]	28.95±1.40[*,#]
D	17.59±1.43[*,#,▲]	23.07±1.18[*,#,▲]	33.76±1.58[*,#,▲]	34.89±1.76[*,#,▲]
E	11.56±0.96[*,#]	18.36±1.10[*,#]	28.25±1.20[*,#]	30.35±1.20[*,#]
F	17.48±1.94[*,#,▲]	23.45±2.01[*,#,▲]	34.25±1.41[*,#,▲]	35.92±1.79[*,#,▲]

A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p. Data are presented as the mean ± standard error (n = 5). [*]P < 0.05 vs. Sham group; [#]P < 0.05 vs. CLP group; [▲]P < 0.05 vs. XBJ group.
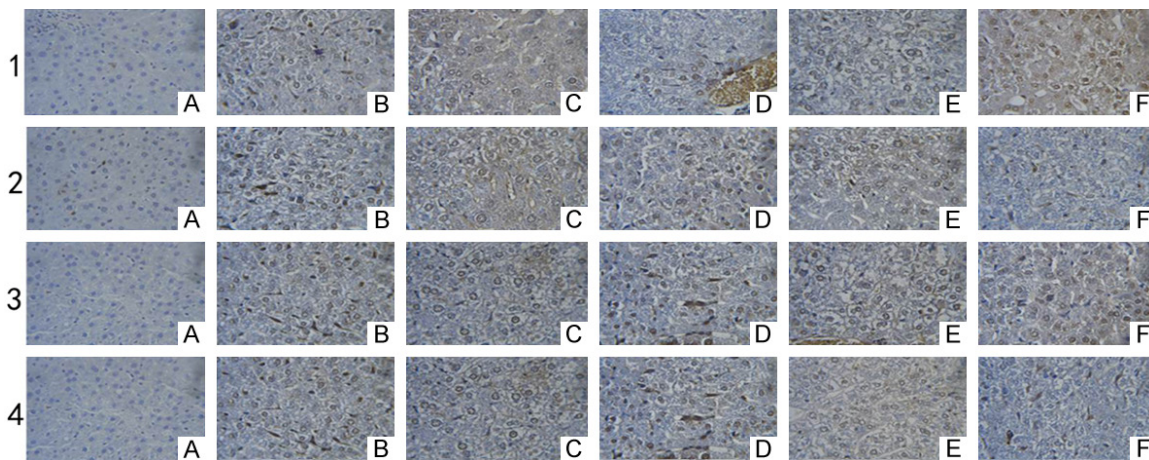

Figure 2. 1: 2 h after surgery. 2: 6 h after surgery. 3: 12 h after surgery. 4: 24 h after surgery. A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p.

observed. The central vein and hepatic sinus were conspicuous, sinusoids were mildly dilated, and ballooning degeneration of hepatocytes was partly visible among periportal inflammatory cell infiltration. The XBJ and curcumin groups also showed varying degrees of histological changes, central vein and hepatic sinus congestion, and sinusoidal dilation. Nevertheless, this congestion and dilation were significantly less severe than those in the CLP group.

Six hours after surgery in the CLP group, central vein and hepatic sinus congestion and sinusoidal dilation were widespread. Hepatocyte hypertrophy and infiltration of inflammatory cells were more visible. A small amount of liver cell degeneration and punctate eosinophilic necrosis could be seen at this time point. In the XBJ and curcumin groups, the degrees of central vein and hepatic sinus congestion and sinusoidal dilation were aggravated, but still less severe than those in the CLP group.

Twelve hours after surgery, the range of damage continued to expand in all groups. Hepatocytes showed varying degrees of irregular arrangements. Fragmentation of hepatic cell cords and multiple spotty hepatocyte necroses were widespread in the CLP group. Lesions in the XBJ and curcumin groups were still significantly less severe than those in the CLP group.

Twenty-four hours after surgery in the CLP group, the injury was further aggravated, showing a large area of hepatocyte edema and necrosis, accumulation of swollen hepatocytes, and inflammatory cell infiltration. No worsening was noted in the XBJ group or curcumin groups.

TUNEL results

Stained apoptotic nuclei appeared as blue-black granule deposits [23]. The apoptotic index was calculated with a formula, the ratio between the number of aptptotic cells and the
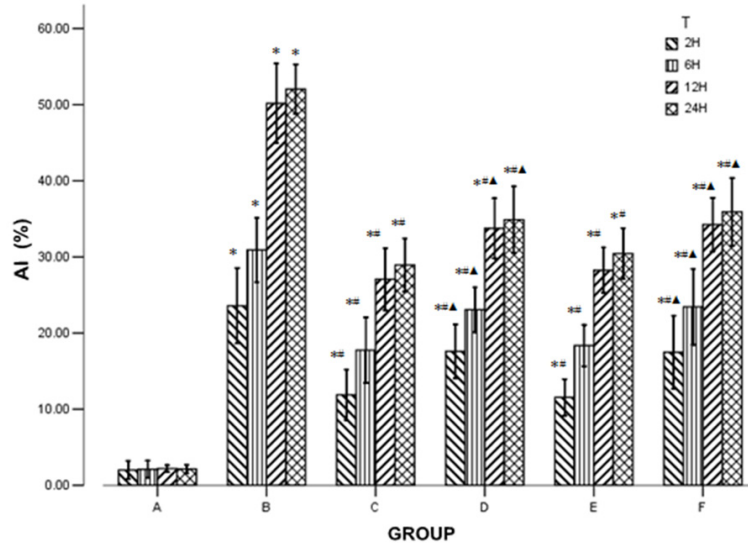
Figure 3. Effects of curcumin on the apoptotic index at 6, 12, and 24 h after surgery. A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p. Data are presented as the mean ± standard error (n = 5). *$P < 0.05$ vs. Sham group; #$P < 0.05$ vs. CLP group; ▲$P < 0.05$ vs. XBJ group.
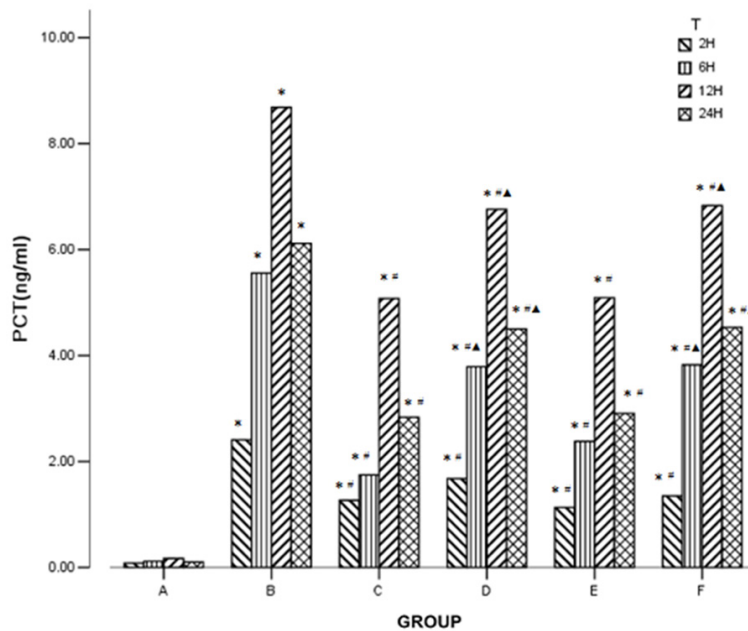

Figure 4. Effects of curcumin on PCT level in peripheral blood at 6, 12, and 24 h after surgery. A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p. Data are presented as the mean ± standard error (n = 5). *$P < 0.05$ vs. Sham group; #$P < 0.05$ vs. CLP group; ▲$P < 0.05$ vs. XBJ group.

apoptotic cells were found at each time point. More apoptotic cells were observed in the other groups, with varying degrees of cytoplasm concentration and karyopyknosis (**Figure 2**).

Precise information was revealed by quantitative analysis. The apoptotic index was significantly higher in the CLP group than in the XBJ and curcumin groups ($P < 0.05$). The index continued to rise with time in the CLP group ($P < 0.05$), while the upward trend ended between the 12- and 24-h time point in the XBJ and curcumin groups ($P > 0.05$). Additionally, the index was not significantly different between the XBJ and M-curcumin groups ($P > 0.05$), whereas it was significantly lower than in the L-curcumin and H-curcumin groups ($P < 0.05$) (**Figure 3**).

Serological evaluation

The PCT, TNF-α, and IL-1β levels were elevated in all groups from 2 h post-CLP and were significantly higher in all groups than in the Sham group ($P < 0.05$); the most obvious increase was in the CLP group ($P < 0.05$). The PCT, TNF-α, and IL-1β levels changed only slightly at each time point in the Sham group ($P > 0.05$), while they continued to rise in the CLP group ($P < 0.05$). The levels of each parameter peaked at 12 h post-CLP in the XBJ group and curcumin groups and were significantly lower than those in the CLP group ($P < 0.05$). Additionally, the difference between the XBJ and M-Cur groups was not statistically significant ($P > 0.05$), but all levels were significantly lower than those in the L-Cur and H-Cur groups ($P < 0.05$) (**Figures 4**, **5**).

total number of cells analyzed multiplied by 100 (**Table 1**). In the Sham group, few to no
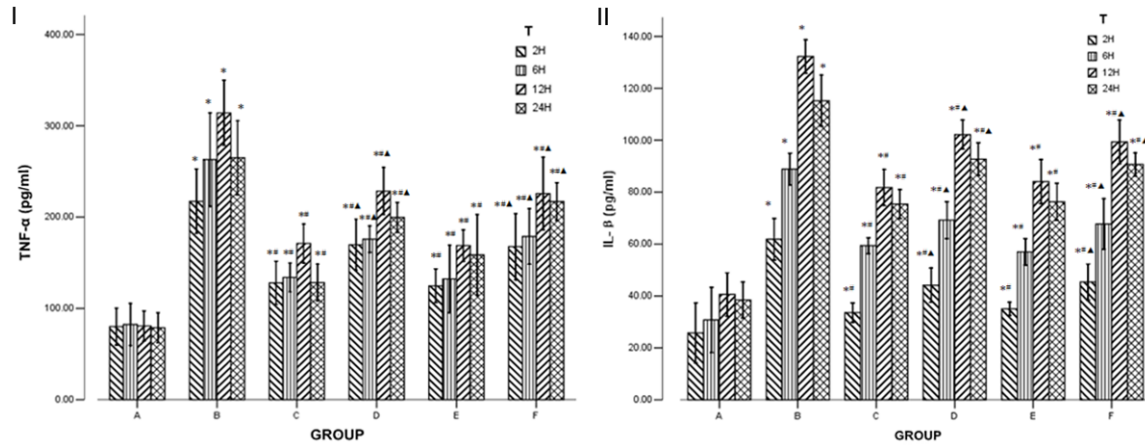
Figure 5. Effects of curcumin on cytokine production in peripheral blood at 6, 12, and 24 h after surgery. A: Sham group. B: CLP group. C: XBJ group. D: L-Cur group. E: M-Cur group. F: H-Cur group. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p. I: TNF-α. II: IL-1β. Data are presented as the mean ± standard error (n = 5). *P < 0.05 vs. Sham group; #P < 0.05 vs. CLP group; ▲P < 0.05 vs. XBJ group.
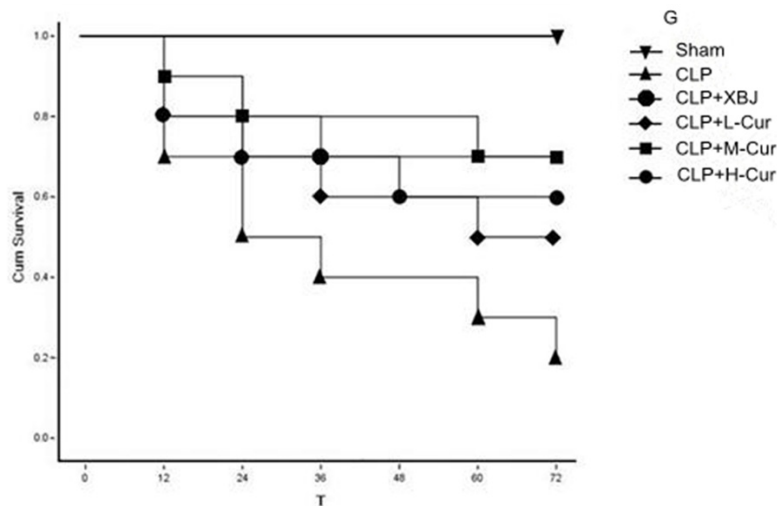

Figure 6. Effects of curcumin on survival of rats with CLP-induced liver dysfunction. L-Cur, low-dose curcumin, 50 mg/kg i.p.; M-Cur, middle-dose curcumin, 100 mg/kg i.p.; H-Cur, high-dose curcumin, 150 mg/kg i.p. The survival rates were observed within 72 h. The results are expressed as cumulative survival, n = 10. The survival rate was estimated by the Kaplan-Meier method and compared by the log-rank test.

Survival study results

The rats in the CLP group of the survival study exhibited a 72-h survival rate of only 20%. Conversely, only rats in the XBJ group and curcumin groups showed 30% to 50% mortality, which was significantly lower than that in the CLP group (*P* < 0.05). All rats in the sham group survived to the 72-h time point (**Figure 6**).

Discussion

Sepsis is a disease syndrome that involves many different aspects of the host immune system. The initial trigger of the disease may be multifaceted, but the most common source is bacterial infection, especially from the abdominal cavity. Consequently, various animal models of abdominal infection have been developed to mimic the disease progression in sepsis, among which the CLP model of peritoneal polymicrobial sepsis is the gold standard.

The presence of necrotic tissue combined with intestinal contents containing a variety of bacterial species can easily cause abdominal infection. This disease process is similar to that of peritonitis secondary to perforating appendicitis in clinical practice. Studies have shown that the animal exhibits a high-power cycle and metabolic state in the early stage, and then enters a low-power cycle later. Additionally, compared with other animal models both the endotoxin detection rate in peripheral blood and bacterial culture-

positive rate are higher. The serum cytokine elevation is relatively flat and longer in duration. Therefore, CLP animal models have strong clinical relevance [24, 25]. We thus chose to perform CLP in the present experiment.

At each time point in this study, animals were sacrificed by laparotomy. The rats that had undergone CLP exhibited a black, edematous cecum with purulent exudates in the abdominal cavity. These findings combined with the histological changes indicated that the septic shock model was successfully established. After treated by XBJ and curcumin, all the parameters and histological changes are better than those of CLP only group, which showed that XBJ and curcumin were effective.

Liver, as the center of metabolism, is one of the most important organs of toxins clearance. Numerous studies indicate that liver damage significantly correlates with the occurrence and the progression of septic shock. Previous studies suggest that curcumin performs a protective role in relieving hepatic cell damage caused by a variety of factors. This protective effect carried out via the power of increasing superoxide dismutase (SOD) vitality and inhibiting cytochrome C activity [18-20]. Whereas, the effect of curcumin to liver cell in sepsis is still unclear, which is also the aim of the present study.

XBJ injection is a traditional Chinese medicine used to treat sepsis and has been approved by the State Food and Drug Administration of China. The injection combines five traditional Chinese medicines, including Flos Carthami (the corolla of *Carthamus tinctorius* L.), Radix Paeoniae Rubra (the root of *Paeonia veitchii* Lynch), Rhizoma Ligustici Chuanxiong (the root of *Ligusticum chuanxiong* Hort.), Salviae Miltiorrhizae (the root of S*alvia miltiorrhiza* Bge.), and Angelicae Sinensis Radix (the root of *Angelica sinensis* (Oliv.) Diels.) [26]. XBJ is widely used in the treatment of sepsis and multiple organ dysfunction syndrome [27-29]. Therefore, we choose the XBJ group as the positive control group to identify the effect of curcumin in sepsis.

PCT, a precursor of calcitonin, is a 116-amino-acid protein secreted in the neuroendocrine cells of the thyroid, lung, and pancreas. PCT can be broken into three distinct molecules with different enzymatic functions: calcitonin (32 amino acids), katacalcin (21 amino acids), and an N-terminal fragment called aminoprocalcitonin (57 amino acids) [30].

The circulating PCT levels in healthy individuals are very low (usually < 0.1 ng/ml), but rapidly and significantly increase under conditions of bacterial infection (> 1000 ng/ml). In sepsis, a rise in the PCT level can be detected at 3 to 4 h, and serial measurements are useful to monitor the response to therapy, embodying good clinical acumen [31]. PCT is therefore considered to be a valuable adjunct in the diagnosis and management of sepsis. In the present study, the PCT level significantly increased in the rats that underwent CLP, whereas it decreased after treatment with XBJ and curcumin [32]. Therefore, curcumin may effectively control infection, and middle-dose curcumin could work as well as XBJ.

TNF-α is a cytokine with a wide range of biological effects. It is secreted by macrophages, monocytes, T lymphocytes, and other cells and is synthesized in many tissues and organs including the heart, liver, and lungs. TNF-α is mainly secreted by macrophages and plays critical roles in many biological processes, such as host resistance to infection and the inflammatory response [33-35].

In one animal experiment, sepsis initiated extensive apoptosis of gastrointestinal epithelial and liver cells. This phenomenon then occurred in other organs, resulting in multiple organ failure [36]. Numerous studies have shown that the major promoters of sepsis are lipopolysaccharides, which finally induce apoptosis. TNF-α, as a proinflammatory factor, is the first multifunctional cytokine produced from lipopolysaccharide-stimulated monocytes and macrophages [37]. It activates inflammatory cells, which then release IL-1, IL-6, and other inflammatory mediators to extend and expand the inflammatory response, eventually inducing a systemic inflammatory response. IL-1β, a member of the IL-1 cytokine family, is a pleiotropic cytokine. It plays a role in the regulation of systemic and local inflammatory responses and functions in almost all cells of the human body [38]. Previous studies have suggested that IL-1β is involved in the apoptosis pathway of the c-Jun N-terminal kinase pathway, mitogen-activated protein kinase pathway, and p38 kinase pathway, thereby regulating physiologi-

cal processes such as cell proliferation, differentiation, apoptosis, and necrosis [39-41]. TNF-α and IL-1β may therefore elicit the inflammatory cascade and contribute to the severity of liver injury in sepsis. In the present study, significantly greater increases in the expression of TNF-α and IL-1β in the peripheral blood were seen in the CLP group than in the Sham group, as expected. After treatment with XBJ and curcumin, the expression of both TNF-α and IL-1β significantly decreased, also as expected. Furthermore, XBJ and M-Cur showed the best treatment effect.

This study also demonstrated that the protective effect of curcumin varies with the dose used; the middle concentration of curcumin was most effective for septic rat hepatocytes. This might be correlated with the pharmacokinetics of curcumin. Curcumin is metabolized mainly by the liver, and the first pass effect is obvious. After enterohepatic circulation, most ingredients are transformed and cleared [42, 43]. For this reason, the low concentration of curcumin lacked effective protection. Therefore, in severe sepsis, when large numbers of liver cells are damaged by inflammatory cytokines and mediators, a high concentration of curcumin could increase the burden of liver metabolism. Thus, the middle concentration of curcumin was most effective for protection of septic rat hepatocytes.

In conclusion, the present study has shown that curcumin can protect against CLP-induced liver dysfunction by decreasing the TNF-α and IL-1β levels. The pathological examination findings and changes in the PCT level confirmed this conclusion. Furthermore, the present study found that the protective effect of curcumin may be related to the drug concentration; neither a low nor high dose of curcumin will achieve the optimal effect. Although we confirmed the beneficial effect of curcumin on hepatocytes in septic rats, the precise mechanisms remain to be fully elucidated. Clinical trials are also necessary to fully realize the potential use of curcumin in patients with sepsis.

Disclosure of conflict of interest

None.

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