## Original Article (-)-Epigallocatechin-3-gallate (EGCG), a polyphenol, protects against sepsis-induced acute liver injury in rats

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**Abstract:** Sepsis is a significant public health problem and is one of the leading causes of death in critically ill patients admitted to the intensive care unit. Liver dysfunction occurs frequently in cases of sepsis. This study aims to investigate the protective effects of (–)-epigallocatechin-3-gallate (EGCG) on polymicrobial sepsis-induced acute liver injury and to explore the underlying mechanism. Sepsis was induced by cecal ligation and puncture (CLP) method in Sprague Dawley rats. Rats were randomly divided into four groups: sham group, CLP group, low-dose EGCG group and high-dose EGCG group. The results demonstrated that EGCG significantly improved the survival of septic rats. Administration of EGCG attenuated the CLP-induced acute liver injury, as indicated by the lower serum ALT and AST levels and the fewer histopathologic abnormalities. EGCG could suppress the CLP-induced oxidative stress via upregulation of SOD and GSH levels and downregulation of MDA level. Treatment with EGCG reduced neutrophil infiltration and hepatic inflammatory cytokine TNF- $\alpha$  and IL-1 $\beta$  release. Meanwhile, EGCG significantly inhibited the transcriptional activity NF- $\kappa$ B. In conclusion, EGCG treatment could protect the sepsis-induced liver injury and the possible mechanism seems to involve its ability to inhibit oxidative stress, to reduce inflammatory response, and to suppress NF- $\kappa$ B activation.

Keywords: (-)-Epigallocatechin-3-gallate, sepsis, acute liver injury, inflammation, oxidative stress, NF-KB

#### Introduction

Sepsis is defined as the acute systemic inflammatory response to infection with a clinical spectrum ranging from hemodynamic changes to multiple organ dysfunction syndrome and even death [1]. Liver dysfunction occurs frequently in cases of sepsis. The incidences of sepsis-associated liver dysfunction and liver failure range from 34% to 46% and from 1.3% to 22% in patients with sepsis, respectively [2, 3]. Despite great progress was achieved in the understanding of the molecular basis of sepsis, most of its complications remain refractory to treatment, and mortality rates have not changed effectively [4, 5]. Therefore, it is urgent to develop an effective treatment for sepsis.

The mechanisms of sepsis-induced acute liver injury are complicated, including uncontrolled systemic inflammatory activation, coagulopathy, hepatic ischemia, and deregulated cell apoptosis [6-9]. Liver plays a crucial role in bacterial phagocytosis and clearance in sepsis. Inflammatory response is triggered during bacterial clearance in sepsis. Proinflammatory cytokines including tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , high mobility group box1 (HMGB1) and macrophage migration inhibitory factor (MIF) [10, 11] are implicated in sepsis, which individually, or in combination, contribute to the recruitment of leukocyte and subsequent organ dysfunction [12]. Furthermore, sepsis is also associated with the enhanced production of reactive oxygen metabolites, leading to multiple organ dysfunctions.

(-)-Epigallocatechin-3-gallate (EGCG), a natural polyphenol, is the major component of green tea and is believed to be primarily responsible for biological activities of green tea extracts [13]. EGCG has shown a variety of physiologic and pharmacologic activities including antiinflammatory, anti-oxidant, anti-tumor, anti-carcinogen, anti-obesity, anti-bacterial, and antiviral activities [14-16]. Furthermore, EGCG has also been demonstrated to exhibit hepatoprotective effects against liver ischemia/reperfusion injury [17], carbon tetrachloride ( $CCl_4$ )induced liver injury [18], alcoholic liver disease [19], and non-alcoholic fatty liver disease [20]. However, the role of EGCG in polymicrobial sepsis-induced acute liver injury and its regulatory mechanisms remains unknown. This study aims to evaluate the protective effect of EGCG on the polymicrobial sepsis-induced acute liver injury by cecal ligation and puncture (CLP) model and to explore the potential mechanism.

## Materials and methods

### Animals

Specific pathogen-free Sprague Dawley rats  $(200 \pm 20 \text{ g})$  were purchased from the Experimental Animal Center of Suzhou Aiermaite Technology Co. Ltd. (SPF grade, Certificate No. SCXK20140007). All rats were housed in groups of five with free access to food and water and kept in a regulated environment with a 12 h light/dark cycle at  $23 \pm 3^{\circ}$ C and 40-70% humidity. Rats were fasted for 12 h before experiments, but were allowed free access to water. All procedures involving the animals were conducted in accordance with the protocol approved by the committee on animal experimentation of Yuhuangding Hospital of Yantai.

## Drugs

Epigallocatechin-3-gallate (EGCG, **Figure 1**) was purchased from Sigma (St. Louis, MO, USA). All other chemicals and biochemical agents used in this study are of high analytical grade.

## CLP model of sepsis

Sepsis was introduced by CLP technique as described previously [21]. Under brief chloral hydrate anesthesia (40 mg/kg intraperitoneally), a 20 mm midline abdominal incision was made to expose the cecum, before being ligated below the ileocecal junction; so intestinal continuity was maintained. The cecum was punctured twice with an 18 gauge needle, and a small amount of cecal contents was expressed through the puncture wound. The incision was closed, and sterile saline solution (0.9% intraperitoneally, 24 mL/kg of body weight) was administered for fluid resuscitation. Sham group rats underwent laparotomy and bowel manipulation without ligation or perforation. All rats had free access to food and water after recovery from anesthesia.

### Animal groups

Rats were randomly divided into four groups (n = 10 per group): sham group (rats undergoing a sham CLP); CLP group (rats undergoing CLP and treated with vehicle); low-dose EGCG group (L-EGCG, rats undergoing CLP and treated with 30 mg/kg EGCG) and high-dose EGCG group (H-EGCG, rats undergoing CLP and treated with 100 mg/kg EGCG). To investigate whether EGCG treatment could protect CLP-induced acute liver injury, rats were administrated intraperitoneally with different dosages of EGCG or saline at 2 h and 12 h following CLP.

### Liver damage assessment

To evaluate the hepatocellular injury following CLP, the levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined in serum using an Automated Chemical Analyzer (Hitachi Co, Tokyo, Japan).

## Histopathological analysis

Liver tissue was fixed in 10% neutral-buffered formalin and processed routinely by embedding in paraffin. Sections of tissue were cut at 4 to 5  $\mu$ m, mounted on slides, and stained with hematoxylin-eosin (HE) before examination under light microscopy (Olympus BH-2; Olympus, Tokyo, Japan).

### Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) and myeloperoxidase (MPO) levels assay

Liver tissues were homogenized in cold PBS (4°C), and the homogenates were collected for measurement of MDA, SOD, GSH and MPO contents. The assays were performed using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's recommended protocol.

## Inflammatory markers assay

The level of inflammatory markers TNF- $\alpha$  and IL-1 $\beta$  in serum were determined using commercially available enzyme-linked immunosorbent



Figure 1. Chemical structure of EGCG.



**Figure 2.** Effect of EGCG on ALT and AST levels in serum samples of different groups. Serum ALT and AST levels were analyzed as a measure of hepatocellular injury. Values are present as means  $\pm$  SD for 10 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group; \**P* < 0.05, \*\**P* < 0.01 compared with the CLP group.

assay (ELISA) kits according to the manufacturer's instructions (R&D Systems, Minneapolis, USA).

### Western blot assay

Cytoplasmic and nuclear proteins from the liver tissues were isolated using a nuclear and cytoplasmic protein extraction kit (Beyotime Institute of Biotechnology, Haimen, China). Protein concentrations were determined by the bicinchoninic acid assay. Proteins were denatured, separated by SDS-PAGE electrophoresis and transferred to a PVDF membrane (Millipore, Bedford, MA, USA). After blocking, the membranes were incubated with primary antibodies: anti-HMGB1 (1:1000, Abcam Inc., Cambridge, USA), anti-MIF (1:1000, Abcam Inc., Cambridge, USA), anti-NF-KB (1:1000, Cell Signaling Technology, Boston, USA) and anti-IkBα (1:1000, Cell Signaling Technology, Boston, USA) overnight at 4°C. After incubation with the corresponding secondary antibodies at room temperature for 2 h, the blots were visualized with an enhanced chemiluminescence (ECL) detection system (GE, Healthcare Life Sciences), the results were analyzed by LabImage version 2.7.1 (Kapelan GmbH, Halle, Germany).

#### Survival rate detection

To investigate whether EGCG was benefit for septic rat, rats were randomly divided into four groups (n = 20 per groups) as mentioned above, and then survival rates were assessed at 48 h following CLP.

### Statistical analysis

The data were expressed as means  $\pm$  SD. Multiple comparisons were analyzed for significant differences by using the one-way analysis of variance (ANOVA) with Tukey post hoc test for multiple comparisons. The statistical analysis was preformed using SPSS 13.0 software (Chicago, IL, USA). A *p*-value blow 0.05 was considered statistically.

### Results

## EGCG protects against the acute liver injury from sepsis

Serum ALT and AST activities were measured as markers of liver injury. To investigate whether EGCG treatment could alleviate acute liver injury during sepsis, the level of ALT and AST was determined. As demonstrated in **Figure 2**,



L-EGCG

H-EGCG

Figure 3. Histopathologic examination of liver tissue in different groups. Liver tissues were stained with H&E, and histological assessment was performed under a microscope.

EGCG treatment did significantly decrease acute liver injury as indicated by the lower level of liver enzyme. The levels of ALT and AST were significantly higher in CLP group compared with sham group (P < 0.01). However, EGCG treatment decreased both of these values significantly. Meanwhile, the histologic examination further demonstrated liver tissue injury after CLP. As shown in **Figure 3**, the liver tissue of in CLP group was characterized by swollen hepatocyte, leukocyte infiltration and hepatocellular necrosis. Administration of EGCG resulted in a significant alleviation of these changes.

## EGCG inhibits the oxidative stress induced by CLP

MDA, an indicator of lipid peroxidation level, was significantly increased in the CLP group compared with the sham group (P < 0.01), whereas the increase was significantly alleviat-

ed in H-EGCG group (P < 0.01), suggesting that lipid peroxidation level was suppressed by EGCG (**Figure 4A**). SOD and GSH are essential components in protecting against the deleterious effects of ROS. As demonstrated in **Figure 4B** and **4C**, the levels of SOD and GSH were significantly decreased in CLP group compared with the sham group (P < 0.01). However, both of EGCG intervention groups increased the levels of SOD and GSH compared with CLP group, indicating that EGCG could inhibit the oxidative stress induced by CLP via upregulation of SOD and GSH activities in rats.

# EGCG decreases the inflammatory mediator production and neutrophil infiltration

As shown in **Figure 5**, the levels of TNF- $\alpha$  and IL-1 $\beta$  in CLP group were significantly increased compared with the sham animals (P < 0.01). Treatment with EGCG significantly inhibited the

## EGCG protects against acute liver injury



**Figure 4.** Effects of EGCG on lipid peroxide and antioxidant parameters in liver tissue of different groups. Values are presented as means  $\pm$  SD for 10 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group; \**P* < 0.05, ##*P* < 0.01 compared with the CLP group.

CLP-induced production of TNF- $\alpha$  and IL-1 $\beta$  levels. In addition, MPO, an index of netrophil infiltration, was significantly unregulated in the CLP group compared with sham group (P < 0.01), while EGCG treatment reversed these elevations in liver tissue (**Figure 6**).

#### EGCG decrease HMGB1 and MIF expression

HMGB1 and MIF acts as the important mediator of systemic inflammation. The hepatic



**Figure 5.** Effect of EGCG on TNF- $\alpha$  and IL-1 $\beta$  levels in liver tissue of different groups. Values are presented as means ± SD for 10 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group; \**P* < 0.05, \*\**P* < 0.01 compared with the CLP group.

HMGB1 and MIF expressions were determined by western blot analysis at 20 h after CLP. As demonstrated in **Figure 7**, the expressions of hepatic HMGB1 and MIF were significantly increased in CLP group compared with the sham group (P < 0.01). However, the EGCGtreated rats showed lower hepatic HMGB1 and MIF expression when compared with the CLP group.

## EGCG modulates the inflammatory signaling pathway

The influence of EGCG on the activity of NF- $\kappa$ B signaling pathway was assessed at 6 h following CLP. The CLP group resulted in the upregulated level of NF- $\kappa$ B in the nucleus and the downregulated level of I $\kappa$ B $\alpha$  in the cytoplasm during sepsis compared with the sham group (*P* < 0.01). However, EGCG intervention significantly suppressed the elevated levels of nuclear NK- $\kappa$ B and decreased levels of cytoplasmic I $\kappa$ B $\alpha$  (**Figure 8**).



**Figure 6.** Effect of EGCG on MPO levels in liver tissue of different groups. Values are presented as means  $\pm$  SD for 10 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group; \**P* < 0.05, \*\**P* < 0.01 compared with the CLP group.



**Figure 7.** Effect of EGCG on the expression of HMGB1 and MIF in liver tissue of different groups. The hepatic HMGB1 and MIF expression were determined by western bolt analysis at 20 h after CLP. Representative blots are presented and GAPDH is served as a loading control. Values are presented as means  $\pm$  SD for 6 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group; \**P* < 0.05, \*\**P* < 0.01 compared with the CLP group.

### EGCG improve the survival of CLP-induced sepsis

To explore whether EGCG was benefit for septic rats, survival was evaluated at 48 h following CLP. As demonstrated in **Figure 9**, EGCG treatment significantly increased the survival rate of rats. CLP group had a 48 h survival rate of 20%, while the survival rate of rats in L-EGCG and H-EGCG group was 45% and 60%, respectively.

#### Discussion

Sepsis is a significant public health problem and is one of the leading causes of death in critically ill patients admitted to the intensive care unit [22]. Liver is site responsible for the elimination of endotoxins and proinflammatory cytokine, which are released during sepsis, can directly or indirectly damage the liver and hepatic damage is an important event that leads to the damage of other organs [23]. Despite the treatment of sepsis has steadily improved, most of its complications remain refractory to treatment and mortality rates have not changed much over the past few decades [24]. Therefore, the development of new drugs to decrease the incidence and mortality associated with this devastating condition would be valuable. Natural products have long been used to prevent and treat disease, including inflammatory and immune-related disease. Epidemiological data showed that natural products in the human diet may have lower toxicity and less possibility of drug resistance and have long lasting beneficial effects on human health [25]. EGCG is a major component of green tea and has been used in traditional medicine in China. It has been shown to possess anti-inflammatory and antioxidative properties [16]. This study aims to investigate the ability of EGCG to alleviate pathophysiologic change in CLP-induced acute liver injury and whether EGCG could be used in the therapy of the acute live injury associated with sepsis.

ALT and AST are normally localized in cytoplasm and the increase of serum ALT and AST activities indicated hepatic damage [26]. In this study, ALT and AST activities were significantly evaluated in CLP group compared with the sham group, indicating CLP-induced sepsis could induce considerable hepatocellular inju-



**Figure 8.** Effect of EGCG on the NF-κB activation in liver tissue of different groups. Liver tissues were harvested at 6 h after CLP. The nuclear NF-κB and the cytoplasmic IκBα were determined by western blot analysis. Representative blots are presented and lamin B1 and GAPDH are served as a loading control for the nuclear and cytoplasm fractions, respectively. Values are presented as means ± SD for 6 rats in each group. \**P* < 0.05, \*\**P* < 0.01 compared with the sham group, #*P* < 0.05, ##*P* < 0.01 compared with the CLP group.

ry. This finding is further confirmed by the histological results that the liver tissue of in CLP group was characterized by swollen hepatocyte, leukocyte infiltration and hepatocellular necrosis. However, EGCG treatment significantly reversed these changes, indicated that EGCG could attenuate CLP-induced sepsis hepatic damage.

Oxidative stress, defined as a persistent imbalance between excessive reactive oxygen species (ROS) production and limited antioxidant defense, is thought to be a key contributor to various hepatic damages [27]. The endogenous antioxidants SOD and GSH peroxidase inhibit



**Figure 9.** Effect of EGCG on the survival of rats with CLP-induced acute liver injury. The survival was observed within 48 h and was analyzed using Kaplan-Meier analysis and the long-rank test. Results are expressed as percent survival, n = 20.

oxidative stress by quickly removing superoxide radicals [28]. GSH can clear reactive oxygen species, such as free radicals and peroxides, and plays an important role in clearing intracellular hydrogen peroxide and lipid peroxides [29]. MDA is a lipid peroxidation by-product that has been frequently used as an indicator of cellular oxidation status [30]. In this study, CLP operation triggered accumulation of MDA levels and restored GSH level and SOD activity in the liver tissue, while EGCG treatment reversed this alteration. These findings are accordance with the fact that EGCG functions as a powerful antioxidant and thus prevents oxidative damage in different cells [14].

Sepsis was associated with overproductions of proinflammatory cytokines, which can lead to the recruitment of leukocytes and tissue damage [31]. A number of inflammatory cytokines, including IL-6, IL-10, IL-1 $\beta$  and TNF- $\alpha$  were involved in sepsis, which contributes to tissue and organ damage. TNF- $\alpha$  is secreted by hepatic macrophages and activated T cells, and as a major mediator in inflammation-induced hepatocyte apoptosis by binding to its receptors [32]. The IL-1 $\beta$  cytokine plays an important role in mediating the inflammatory and immune responses [33]. In the present study, CLP induced significantly increase TNF- $\alpha$  and IL-1 $\beta$ level in rat liver in CLP group. However, EGCG downregulated the expression of TNF- $\alpha$  and IL-1ß compared with CLP group. In many inflammatory processes, important components of pathological processes are linked to the ability of neutrophils to release a complex assortment of agents that can destroy normal cells and dissolve connective tissue [34]. MPO, an essential enzyme for normal polymorphonuclear leukocyte function, is released as a response to various stimulatory substances [35]. This study showed that the presence of increased MPO activity in hepatic tissues indicate the contribution of polymorphonuclear leukocyte infiltration in sepsis-induced tissue damage.

Previous study showed that HMGB1 and MIF play a pivotal role in the pathogenesis of sepsis. HMGB1 can bind DNA and regulate transcription. HMGB1 also acts as a mediator of inflammation when passively released by damaged cells or actively secreted by immune cells [36]. MIF regulates both the set-point and the direction of the inflammatory response by counter-regulating the anti-inflammatory and immunosuppressive effects of glucocorticoids [37, 38]. For example, MIF directly increases IL-8 and TNF- $\alpha$  and over rides glucocorticoid mediated inhibition of cytokine secretion [39]. Therapeutic antagonists of MIF effectively attenuate the inflammatory response and improve survival in experimental sepsis [40]. In this study, HMGB1 and MIF expression was significantly upregulated in sepsis, while EGCG treatment led to a lower HMGB1 and MIF expression, indicating that reducing HMGB1 and MIF expression may be involved in the protective effects of EGCG during sepsis.

NF-kB is a crucial transcription factor that regulates the production of proinflammatory cytokine and chemokine and plays an important role in the pathophysiology of sepsis. Blockade of NF-KB activity using genetic or pharmacologic approaches successfully reduces NF-KBmediated cytokine production, and suppresses multiple organ inflammation and injury during sepsis and/or endotoxemia, suggesting that the inhibition of NF-kB could be a promising therapeutic target in sepsis [41, 42]. In the present study, EGCG could effectively disrupt the activation of NF-kB signaling pathway by showing its activity on inhibiting the degradation of IkBa and the nuclear translocation of NF-kB following CLP. This result was consistent with the previous the study of Xiao et al that EGCG is able to inhibit NF-kB signaling in various liver diseases in vivo [43].

## Conclusion

EGCG could improve survival of rats with polymicrobial sepsis and ameliorated acute liver injury, characterized by alleviating liver pathological injury, reducing ALT and AST release. The underlying mechanisms appear to be attributed to reducing sepsis-induced oxidative tissue damage, depressing tissue neutrophil infiltration, modulating the release of inflammatory cytokines, inhibiting NF- $\kappa$ B activation. Therefore, EGCG may be a promising novel agent for the prevention and the treatment of CLP-induced acute injury.

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### Disclosure conflict of interest

### None.

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