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Dachengqi decoction ameliorates sepsis-induced liver injury by inhibiting the TGF-β1/Smad3 pathways

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ABSTRACT

Background: Sepsis-induced acute liver injury (ALI) is a major contributor to mortality in septic patients. Exploring the pathogenesis and developing effective treatment strategies for sepsis-induced ALI is critical for improving patient outcomes. Dachengqi decoction (DCQD), which is a classic Chinese herbal medicine, has been shown to possess potent anti-inflammatory properties. However, the protective effects and underlying mechanisms of DCQD against sepsis-induced ALI remain unclear. This study aimed to investigate the protective effect of DCQD on sepsis-induced ALI and elucidate the involvement of the TGF-1β/Smad3 pathways.

Methods: A septic mouse model was established using caecal ligation and puncture (CLP) to evaluate the protective effect of DCQD on sepsis-induced ALI in vivo. An in vitro cellular inflammation model was established using LPS-stimulated LO2 cells to further investigate the underlying mechanism.

Results: DCQD (2.5, 5.0, and 10.0 g/kg body weight) was administered twice daily for 2 days and exerted a dose-dependent protective effect against sepsis-induced ALI. DCQD treatment significantly inhibited inappropriate inflammatory responses and oxidative stress in liver tissue. Moreover, DCQD maintained liver homeostasis by inhibiting hepatocyte apoptosis and improving sepsis-induced liver damage. In vivo and in vitro studies indicated that the TGF-β1/Smad3 signalling pathway played an important role in sepsis-induced ALI, and DCQD treatment significantly inhibited the activation of this pathway.

Conclusions: DCQD can effectively suppress excessive inflammatory responses and oxidative stress, leading to a substantial reduction in hepatocyte apoptosis in sepsis-induced ALI.

1. Introduction

Sepsis is a systemic multiorgan dysfunction caused by a dysregulated host response to infectionand is the leading cause of death in intensive care unit patients.[1,2] Although advanced antibiotic regimens have been applied in current clinical practice, the mortality of sepsis patients remains high.[3,4] The liver is an important organ that plays a fundamental physiological role in metabolism,[5] and sepsis-induced acute liver injury (ALI) is a strong independent predictor of intensive care unit mortality in patients with sepsis.[6,7] Exploring the pathogenesis and effective treatment strategies of septic liver injury is crucial for the treatment and prognosis of septic patients.

Sepsis is characterized by an overactivated inflammatory response,[2] and accumulating evidence demonstrates that oxidative stress plays an important role in the pathogenesis of sepsis.[8,9] The liver is one of the common organs associated with sepsis injury, and sepsis-induced ALI involves a series of complex pathological mechanisms, including inflammation, the immune response, cellular hypoxia, oxidative stress, and apoptosis.[6] Effectively inhibiting the excessive inflammatory response and oxidative stress and reducing hepatocyte apoptosis is an important strategy for the treatment of sepsis-induced ALI. Transforming growth factor-β1 (TGF-β1) is a crucial inflammatory cytokine involved in various pathophysiological processes. TGF-β1 has a

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List of abbreviations

DCQD	Dachengqi decoction
UPLC-MS/MS	ultra-high performance liquid chromatography-tandem mass spectrometry
ALI	acute liver injury
TGF-β1	transforming growth factor-β1
DMEM	Dulbecco's modified Eagle's medium
ELISA	enzyme-linked immunosorbent assay
AST	aspartate aminotransferase
ALT	alanine aminotransferase
IL-6	interleukin-6
IL-1β	interleukin-1β
TNF-a	tumor necrosis factor-a
MDA	malondialdehyde
SOD	superoxide dismutas
TUNEL	terminal deoxynucleotide transferase dUTP nick end labelling
SEM	standard error of the mean
ANOVA	one-way analysis of variance

Dachengqi decoction (DCQD), which is a representative Chinese traditional medicine prescription recorded in Shang-Han-Lun, consists of *Rheum palmatum* L., Mirabilite, *Magnolia officinalis* Rehd. and *Citrus aurantium* L (Fig. S1A). DCQD plays an important role in various inflammatory-related diseases,[14,15] effectively controlling disease progression and reducing mortality.[16,17] Previous studies have reported that DCQD exerts a positive regulatory effect on various inflammation-related diseases and protects against liver injury. Furthermore, DCQD has been shown to be actively involved in sepsis-induced organ damage.[18] However, the protective effects and underlying mechanisms of DCQD against sepsis-induced ALI have not been elucidated, and the relationship between the protective effect of DCQD on sepsis-induced liver injury and the TGF-1β/Smad3 pathways remains unexplored.

This study aimed to demonstrate the therapeutic effects of DCQD on sepsis-induced ALI using a septic mouse model established by caecal ligation and puncture (CLP) in vivo. Furthermore, we investigated the regulatory effects of DCQD on the TGF-β1/Smad3 signalling pathways using a cellular inflammation model in lipopolysaccharide-stimulated LO2 cells in vitro. This study not only establishes a foundation for clarifying the mechanism of DCQD in sepsis-induced ALI but also provides new strategies for the clinical treatment of ALI.

Table 1
The chromatographic and content data of 8 compounds identified in DCQD by UPLC–MS/MS.

No.	Compound	RT (min)	Formula	*m/z*	MS1/MS2	Content (μg/mL)
1	Emodin	8.83	$C_{15}H_{10}O_5$	271.0597 (+)	271.0596/229.0493	14.12
2	Rheic acid	7.69	$C_{15}H_8O_6$	283.0250 (−)	283.0249/257.0454	6.32
3	Chrysophanic acid	8.28	$C_{15}H_{10}O_4$	253.0495 (−)	253.0495/197.1172	4.33
4	Magnolol	8.57	$C_{18}H_{18}O_2$	265.1231 (−)	265.1231/247.1124	13.24
5	Honokiol	8.34	$C_{18}H_{18}O_2$	265.1231 (−)	265.1231/224.0836	9.11
6	Hesperidin	6.63	$C_{28}H_{34}O_{15}$	611.1965 (+)	611.1964/303.0858	90.39
7	Naringin	6.55	$C_{27}H_{32}O_{14}$	581.1866 (+)	581.1866/273.0749	97.13
8	Neohesperidin	6.64	$C_{28}H_{34}O_{15}$	609.1818 (−)	609.1818/301.0718	25.63

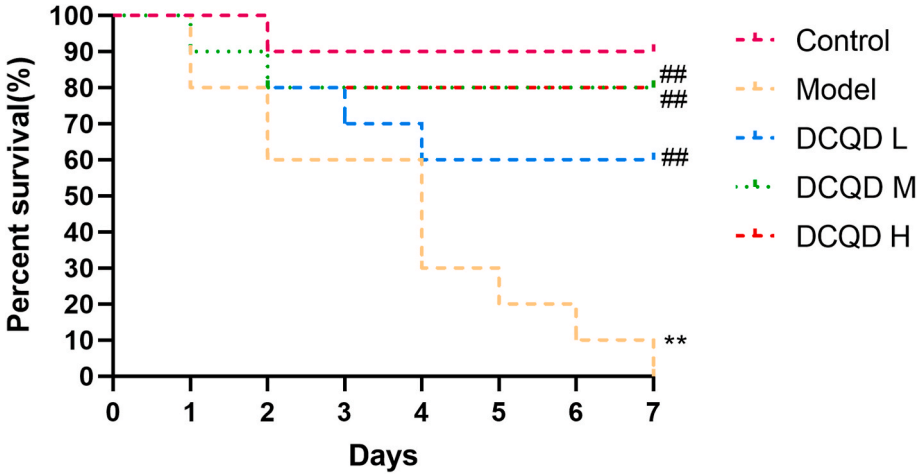

Fig. 1. Effect of DCQD on the survival rate of CLP-septic mice (n = 10 per group).
$^{**}P < 0.01$ versus the control group; $^{\#\#}P < 0.01$ versus the model group.

regulatory effect on hepatocyte proliferation and apoptosis and binds to its receptor after being activated to further activate downstream Smad phosphorylation.[10] The TGF-β1/Smad signalling pathway is one of the important pathways involved in liver injury,[11] and the TGF-1β/Smad3 pathway is closely related to apoptosis and organ damage in sepsis.[12,13] Inhibiting the activation of the TGF-1β/Smad3 pathway is an important strategy for the treatment of sepsis-induced ALI.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN, LC-MS/MS grade) and formic acid (≥98%, analytical grade) were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai,

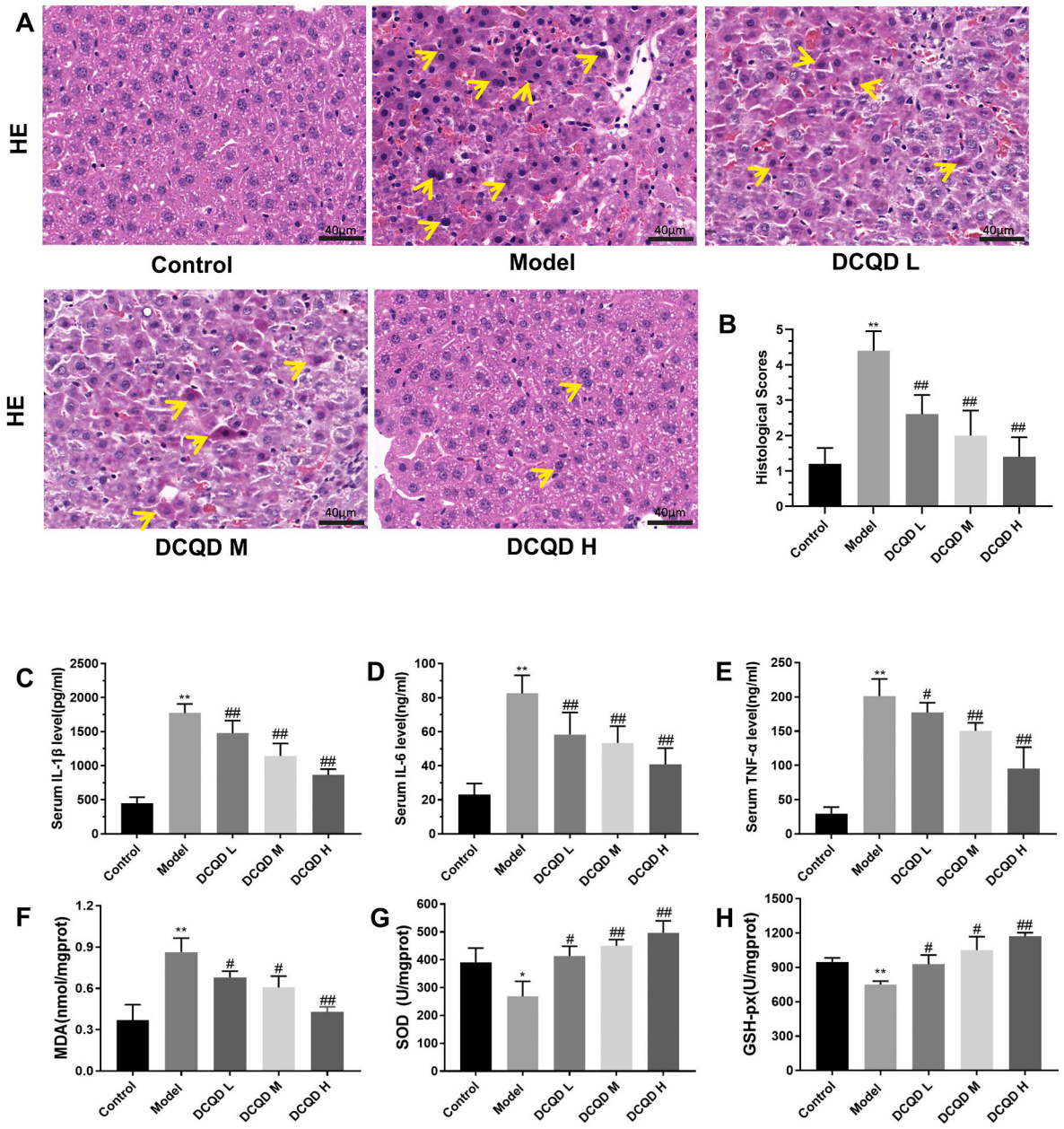

Fig. 2. DCQD significantly ameliorated sepsis-induced ALI. (A) H-E-stained liver tissues (magnification 200 ×). (B) The histological scores of liver sections from CLP mice treated with DCQD (n = 6). (C–H) The levels of IL-1β, IL-6, TNF-α, MDA, SOD and GSH-px.
The data are presented as the means ± SEMs. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control group; $^{\#\#}P < 0.01$ versus the model group.

China), respectively. Purified water was obtained from Millipore using a Milli-Q system (Bedford, MA). The reference standards (emodin, rheic acid, chrysophanic acid, magnolol, honokiol, hesperidin, naringin and neohesperidin) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. The purities of these standards were determined to be greater than 98% using HPLC coupled with a UV detector. *Rheum palmatum* L. (No. 190207), *Magnolia officinalis* Rehd. et Wils. (No. 190619), *Citrus aurantium* L. (No. 190128), and mirabilite (No. 190515) were purchased from Hubei Chenmei Herbal Pharmaceutical Co., Ltd. The samples were identified by Dr. Sen Li of Huazhong University of Science and Technology.

2.2. Sample preparation

DCQD was mixed in standard proportions of the four components (12:24:12:9, respectively) by weight. The preparation of the decoction was as follows: *Rheum palmatum* L., *Magnolia officinalis* Rehd. et Wils. and *Citrus aurantium* L. were soaked in 10 times distilled water (W/V) for 30 min. The samples were boiled until 1000 mL remained and were filtered. Subsequently, mirabilite was added to the medicinal solution and decocted with low heat. Finally, DCQD was concentrated under reduced pressure, and the residue was freeze-dried to yield DCQD powder. Each gram of DCQD powder was equivalent to 5.9 g of the raw herbs. When used, it was diluted with distilled water and administered by gavage.

2.3. Instruments and conditions

Qualitative and quantitative analyses of the main components of DCQD were performed by UPLC–MS/MS. Chromatographic analysis was performed on a Thermo Scientific Dionex Ultimate 3000 RS (Thermo Fisher Scientific, CA) composed of an online degasser, pump,

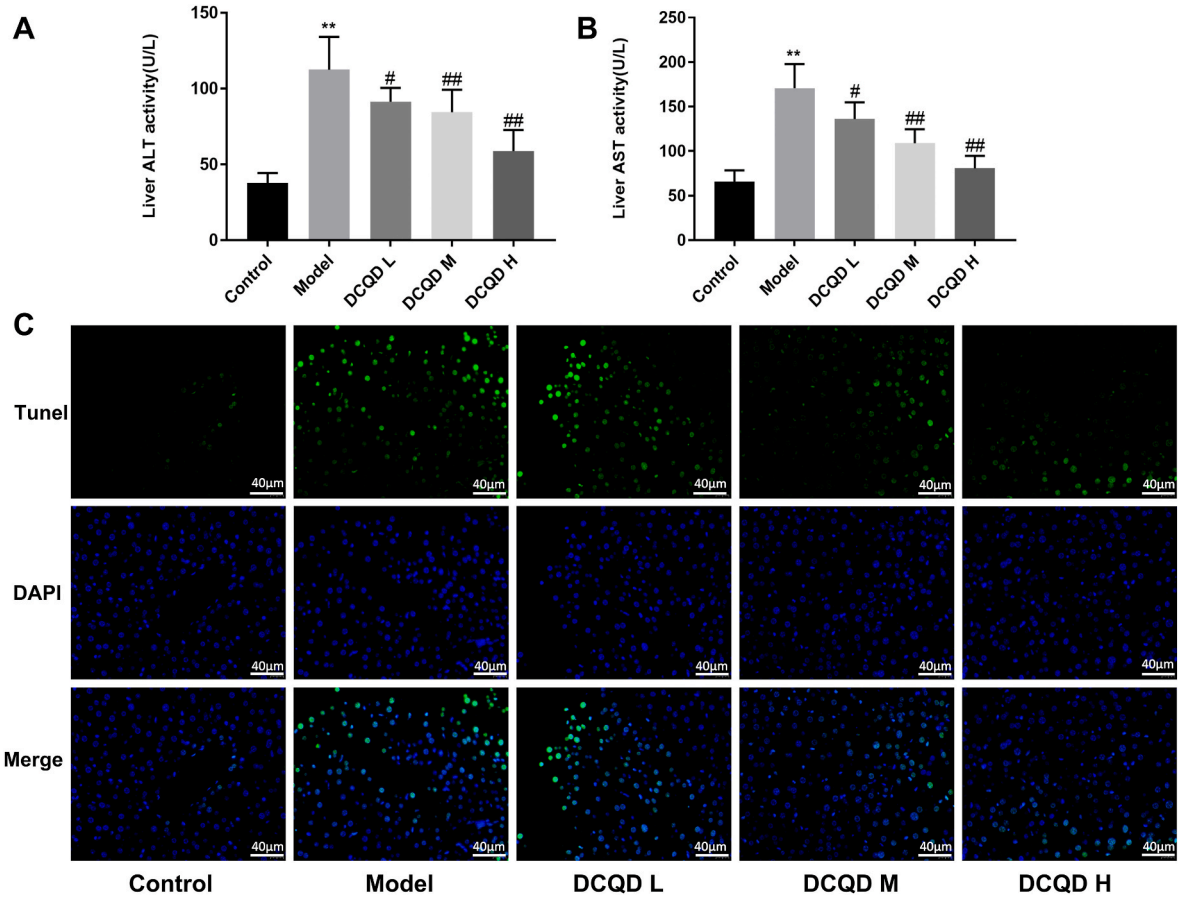

Fig. 3. DCQD improved liver function and inhibited hepatocyte apoptosis. (A–B) The liver activity of ALT and AST. (C) The results of TUNEL staining. The data are presented as the means ± SEMs. $^{**}P < 0.01$ versus the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus the model group.

autoinjector, column heater, and UV detector. Sample separation was carried out on a Waters C18 column (100 × 2.1 mm, 1.7 μm) using gradient elution at 40 °C. The mobile phases were solvent A (0.1% formic acid water, v/v) and solvent B (100% acetonitrile) at a flow rate of 0.3 mL/min. The gradient conditions of the mobile phases were optimized as follows: 0.01 min, 15% B; 4 min, 55% B; 7 min, 72% B; and 10 min, 95% B. The injection volume was 5 μL.

MS analysis was performed on a Thermo Scientific Qexactive Focus Orbitrap MS (Thermo Electron, Bremen, Germany) operated with heated electrospray ionization (HESI) in positive and negative ion modes. The mass spectra were acquired in full MS mode in a mass range from *m/z* 100−1200 at a resolution of 70,000, combined with the data-dependent scan (dd-MS^2) at a resolution of 35,000 and an isolation window of *m/z* 3.0. Other Q-Exactive general parameters were as follows: nebulizer pressure at 10 arb, sheath gas and auxiliary gas at a flow rate of 30 arb, capillary temperature at 320 °C, auxiliary gas heater temperature at 350 °C, spray voltage at 3.2 kV, and S-lens level at 50. The MS scan mode was detected in selected reaction monitoring (SIM) mode at a resolution of 35,000. The MS1 and MS2 parameters are shown in Table 1.

2.4. Animal ethics and maintenance

All animals were obtained from the Experimental Animal Center of Huazhong University of Science and Technology. All animals were adaptively fed for one week before the formal experiment at a temperature of 22 ± 2 °C, relative humidity of 65% ± 10%, and a 12-h light-dark cycle with a free diet.

Fifty BALB/c male mice (25–30 g) were divided into 5 groups (n = 10 per group) by the random number table method: the control group, model group, low concentration DCQD group (DCQD L), medium concentration DCQD group (DCQD M) and high concentration DCQD group (DCQD H). Except for the control group, the other 4 groups were subjected to caecal ligation and puncture (CLP) to establish the acute liver injury model as previously described.[19] Intraperitoneal injection of 2% sodium pentobarbital (80 mg/kg) was used to anaesthetize the mice. The mice were placed in the supine position on the laboratory bench, and skin preparation and disinfection of the operating area were performed. Next, a 1.5 cm incision in the midline of the abdomen was created to dissociate and fully expose the caecum. Then, sterile No. 4 sewing silk was used to ligate the caecum 1 cm away from the caecum tail, and a 20-gauge needle was used to perforate the blind end and squeeze a small amount of faeces. Finally, the caecum was replaced in the abdominal cavity, and the abdominal incision was sutured layer by layer. After the surgery, 1 mL of normal saline was injected subcutaneously into the back for liquid resuscitation, and the mice were placed on a thermostatic blanket for rewarming.

The drug dose was determined based on the literature and preliminary experiments.[20] One hour after being resuscitated, mice in the DCQD L, M and H groups were administered low, medium and high doses of DCQD (2.5, 5.0 and 10.0 g/kg, respectively) twice daily at 12-h intervals, and mice in the control and model groups were given the same volume of normal saline twice per day. All groups were treated for 2 consecutive days. The animals were sacrificed 48 h after surgery. Liver samples were collected for subsequent testing. Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) tubes and centrifuged at 1500 rpm for 10 min at 4 °C. The plasma was stored at −80 °C until analysis. To monitor the survival rate, 10 additional mice in each group were used to construct the acute liver injury model, and these mice were administered with the same procedure and maintained for 7 days. All experiments were performed with the approval of the

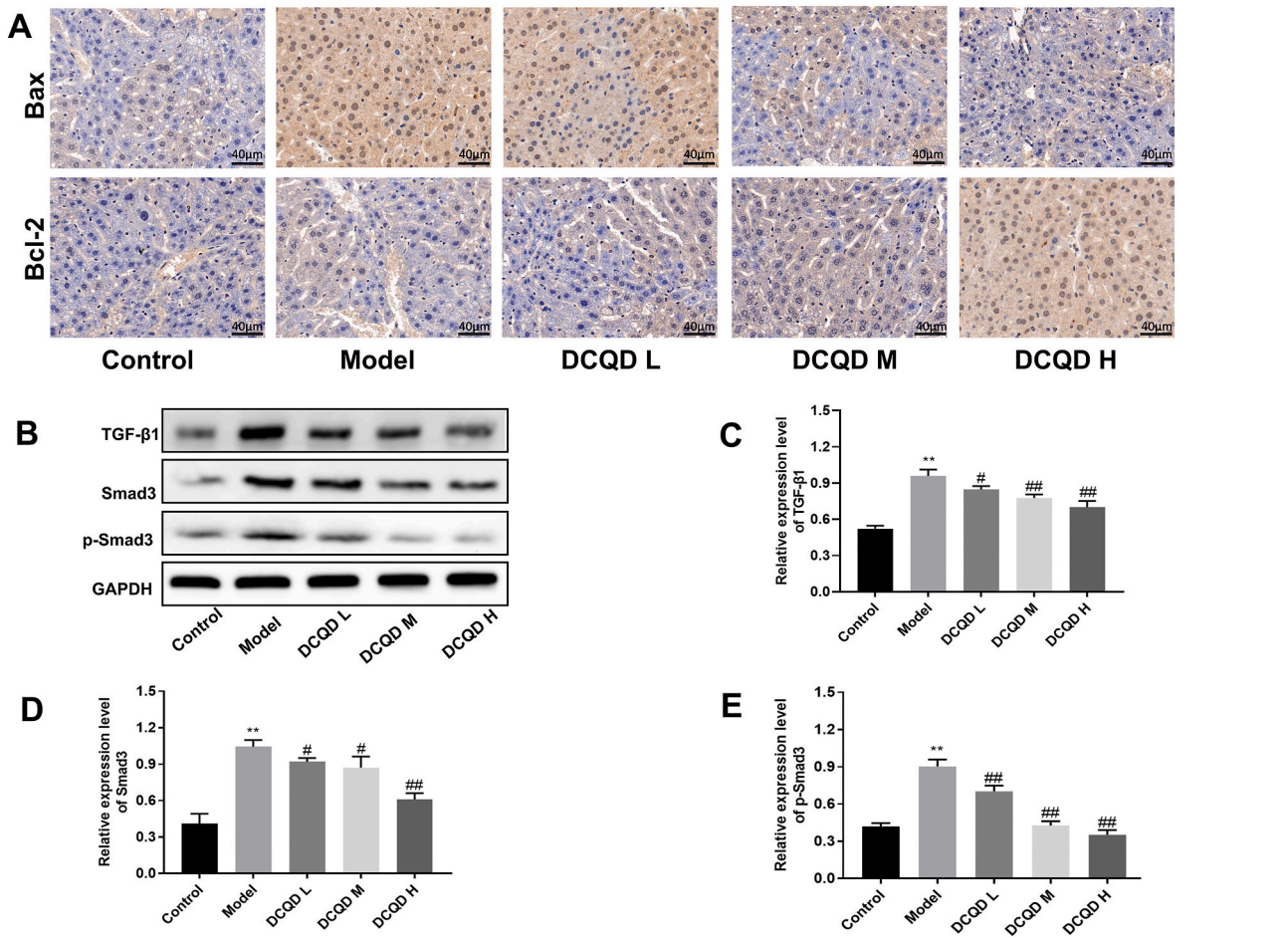

Fig. 4. DCQD inhibits apoptosis-related proteins and the TGF-B1/SMAD3 pathway in ALI. (A) The immunohistochemical results of Bcl-2 and Bax protein expression in liver tissue. (B) The levels of TGF-β1, Smad3, and p-Smad3 in liver tissues were analysed by Western blotting. (C–E) The statistical graphs corresponding to the related proteins.
The data are presented as the means ± SEMs. $^{**}P < 0.01$ versus the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus the model group.

Table 2
DCQD inhibits the inflammatory response and oxidative stress in LO2 cells.

Group	IL-1β (pg/mL)	IL-6 (ng/mL)	TNF-α (ng/mL)	MDA (nmol/mg prot)	SOD (U/mg prot)	GSH-px (U/mg prot)
LPS	1025.8 ± 61.38	49 ± 7.58	808.6 ± 53.21	0.40 ± 0.02	50 ± 4.74	74.2 ± 5.81
LPS + DCQD	898.6 ± 97.16 *	20.4 ± 2.70**	554.2 ± 43.27 **	0.29 ± 0.04 *	79.4 ± 2.30 **	83.1 ± 3.16 *
LPS + SRI-011381	1662.2 ± 61.45 **	79.4 ± 90.71 **	914.4 ± 52.92 *	0.69 ± 0.08 **	30.8 ± 2.17 **	65.6 ± 4.51 *
LPS + DCQD + SRI-011381	1040.4 ± 47.73$^{\#\#}$	48.4 ± 1.95$^{\#\#}$	826 ± 58.28$^{\#}$	0.42 ± 0.05$^{\#\#}$	51.8 ± 3.83$^{\#\#}$	74.8 ± 4.60$^{\#}$

Data are presented as the means ± SEMs.
$^{*}P < 0.05$, $^{**}P < 0.01$ versus the LPS group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus the LPS + SRI-011381 group.

Laboratory Animal Management Committee of Tongji Medical College of Huazhong University of Science and Technology (2017 IACUC Number: 2428). All operations involving animals were performed according to the Chinese Animal Research Guidelines.

2.5. Cell culture and maintenance

LO2 cells were used for the in vitro experiments. DMEM containing 10% foetal bovine serum (FBS, Gibco, South America origin) was used for cell culture. The culture environment was 37 °C and 5% CO_2. The cells were divided into 4 groups. In the lipopolysaccharide (LPS) group, LO2 cells were incubated with 1 μg/ml LPS for 24 h to simulate the sepsis-induced ALI model. In the LPS + DCQD group, 100 μg/ml DCQD solution was added and incubated for 24 h. In the LPS + SRI-011381 group, a TGF-β/Smad signalling pathway agonist (SRI-011381, 10 μM) and 1 μg/ml LPS were added and incubated for 24 h.[21–23] In the LPS + DCQD + SRI-011381 group, 10 μM SRI-011381 and 1 μg/ml LPS were added and incubated for 24 h.

2.6. Liver histology

The liver tissues were collected and fixed with 4% paraformaldehyde, embedded in paraffin, deparaffinized, and stained with haematoxylin-eosin. Then, liver pathological changes were observed under a light microscope. All sections were examined for hepatocellular cytoplasmatic color fading, vacuolization, nuclear condensation, nuclear fragmentation, and nuclear fading as well as erythrocyte stasis. Each phenomenon was scored according to the percentage of cells showing this phenomenon per 10 microscopic fields (200x): 0 = 0%, 1 = 0–10%, 3 = 10–50%, 5 = 50–100%.[24]

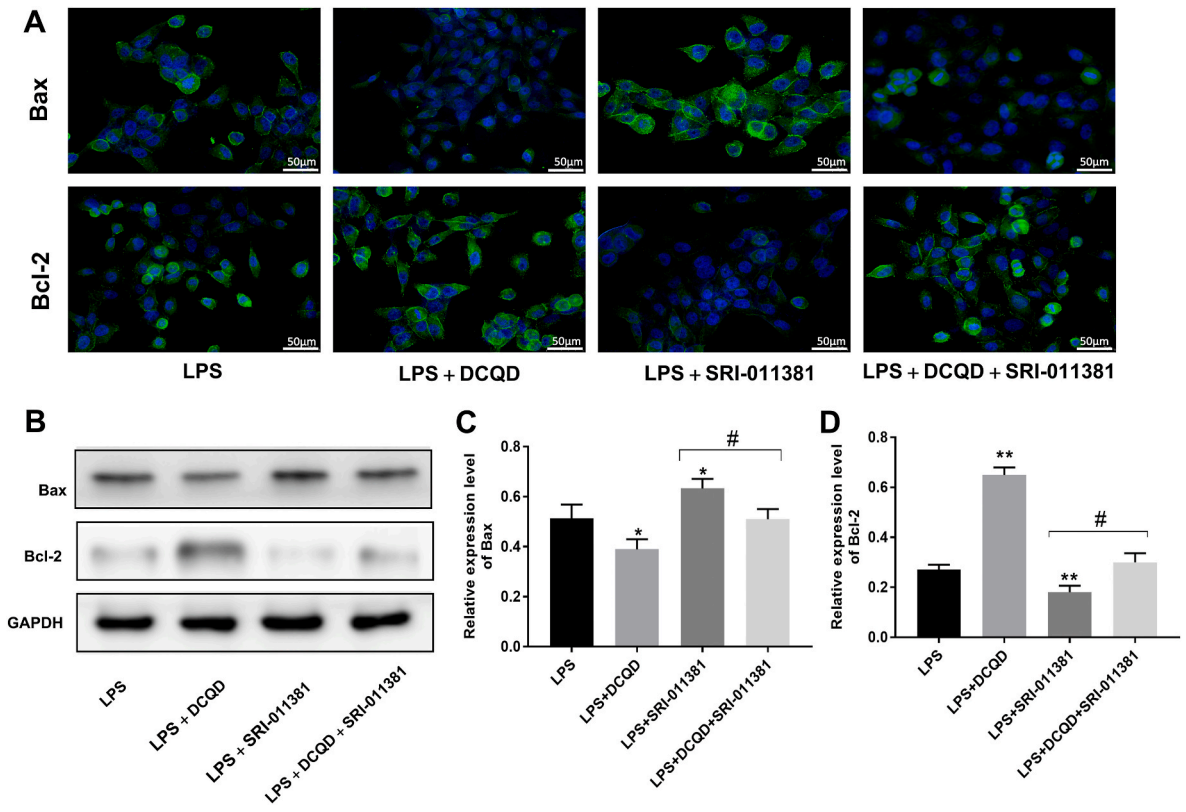

Fig. 5. DCQD regulated the expression of apoptosis-related proteins in LO2 cells. (A) Immunofluorescence staining of Bcl-2 and Bax in LO2 cells. (B) The protein levels of Bax and Bcl-2 in liver tissues were analysed by Western blotting. (C–D) The statistical graphs corresponding to Fig. 5B.
The data are presented as the means ± SEMs. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the LPS group; $^{\#}P < 0.05$ versus the LPS + SRI-011381 group.

2.7. Enzyme-linked immunosorbent assay

Mouse serum, LO2 cells, and supernatant were collected separately, and the levels of the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) were detected by enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, China) according to the manufacturer's protocol.

2.8. Liver function assessment

Biochemical kits (Elabscience, China) were used to evaluate the levels of alanine transaminase (ALT) and aspartate transaminase (AST) according to the manufacturer's instructions.

2.9. Oxidative stress assays

According to the manufacturer's instructions, the levels of oxidative stress-associated indicators, including malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px), were determined by kits (Elabscience, China).

2.10. TUNEL staining

Apoptotic hepatocytes were detected by terminal deoxynucleotide transferase dUTP nick end labelling (TUNEL) according to the manufacturer's protocol. Then, TUNEL-positive cells were observed under a fluorescence microscope.

2.11. Immunohistochemistry

Deparaffinized sections were incubated in 3% hydrogen peroxide for 30 min and then blocked with 5% goat serum albumin for 20 min. The sections were incubated with primary antibodies against target proteins and ChemMateTMEn Vision +/HRP overnight at 4 °C. Finally, the sections were stained with diaminobenzidine and haematoxylin.

2.12. Immunofluorescence assay

Paraffin sections were deparaffinized and permeabilized with PBS containing 1% Triton X-100 for 15 min. Primary antibodies against the target proteins were added after the samples were blocked with 10% goat serum for 1 h overnight at 4 °C. After 3 washes with PBS, the secondary antibodies were added and incubated for 1–2 h at room temperature in the dark. Then, positive cells were observed with an Olympus fluorescence microscope.

2.13. Western blot analysis

Total protein was extracted from liver tissue and LO2 cells. The protein concentration was determined by a BCA protein assay kit (Beyotime Biotechnology). After electrophoresis, the separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Biosciences). The membranes were blocked with 5% skimmed milk for 2 h at room temperature. The blocked membranes ere then incubated with primary antibodies overnight at 4 °C. The primary antibodies included anti-TGF-1β (1:2000, Proteintech, China), anti-Smad3 (1:2000, Proteintech, China), anti-p-Smad3 (1:2000, Proteintech, China), anti-Bax (1:2000, Proteintech, China), anti-Bcl2 (1:2000, Proteintech, China), and anti-GAPDH (1:40,000, Proteintech, China). After being washed 3 times, the membranes were incubated with secondary antibodies for 1 h. Then, the UVP imaging system (Upland, CA, USA) was used for exposure imaging. The Western blot images were then analysed with ImageJ software.

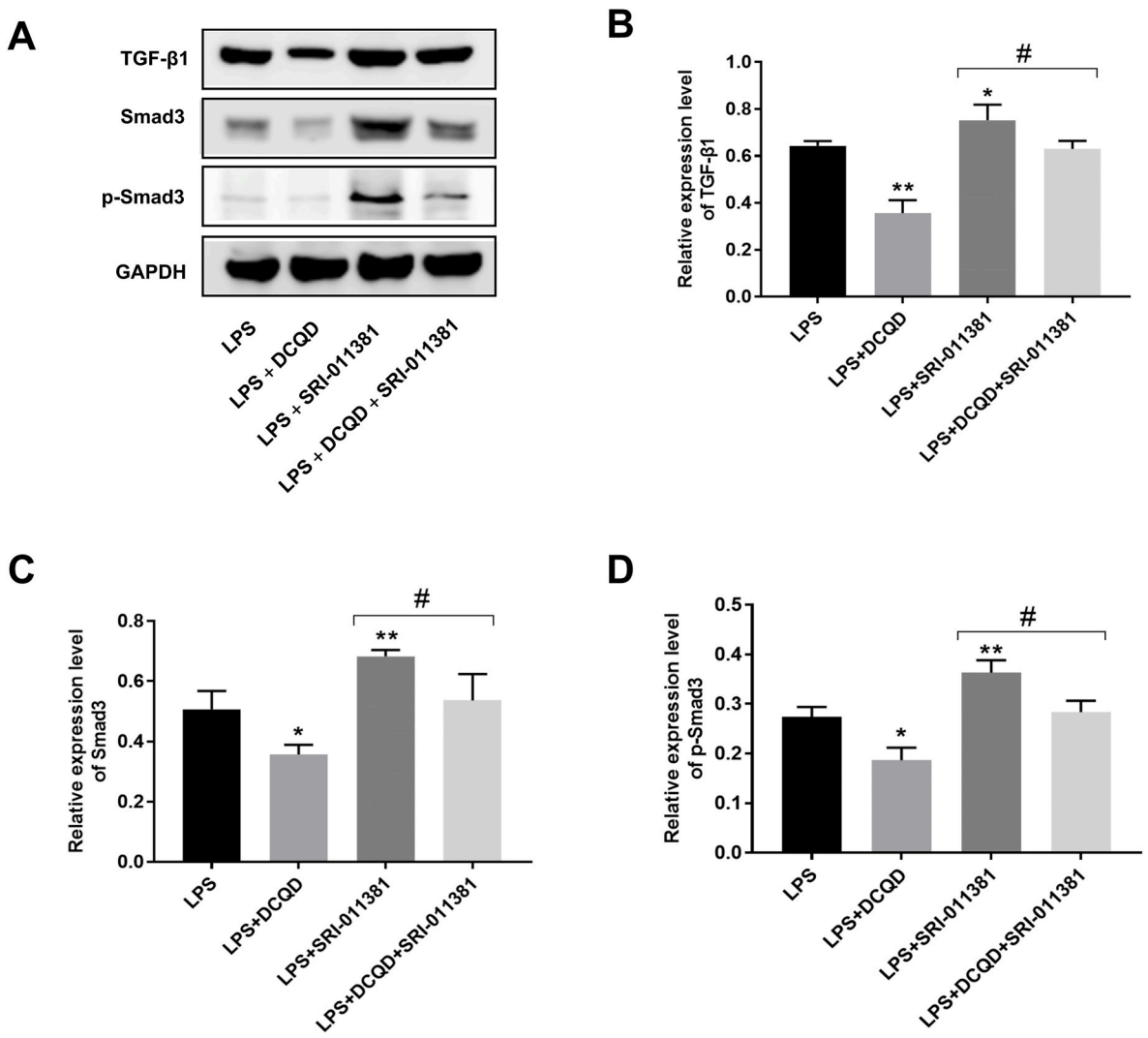

Fig. 6. DCQD inhibited the TGF-β1/Smad3 signalling pathway in LO2 cells.
The data are presented as the means ± SEMs. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the LPS group; $^{\#}P < 0.05$ versus the LPS + SRI-011381 group.
(A) The levels of TGF-β1, Smad3 and p-Smad3 in LO2 cells were analysed by Western blotting. (B–D) The statistical graphs corresponding to the related proteins.

2.14. Statistical analysis

All experiments were repeated at least three times. The data were analysed with GraphPad Prism 8 and are presented as the mean ± standard error of the mean (SEM). Survival rate was determined using the log-rank test. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to determine the statistical significance ($p < 0.05$) between different groups.

3. Results

3.1. Qualitative and quantitative analysis of the primary compounds in DCQD by UPLC–MS/MS

We conducted qualitative and quantitative analyses of the primary compounds present in DCQD using UPLC–MS/MS. Eight primary compounds in DCQD were identified by matching empirical molecular formulae, exact mass and MS/MS fragments with standard compounds using UPLC–MS/MS. These compounds were emodin, rheic acid and chrysophanic acid in *Rheum palmatum* L., magnolol and honokiol in *Magnolia officinalis* Rehd., and hesperidin, naringin and neohesperidin in *Citrus aurantium* L. (Fig. S1B). The contents of the 8 compounds are shown in Table 1.

3.2. DCOD improves survival rate of mice with sepsis-induced liver injury

The administration of DCQD significantly ($^{\#\#}P < 0.01$) improve the survival rate of septic mice. As shown in Fig. 1, in the model group, the survial rate on the second day of observation was 80%, and all mice eventually succumbed within 7 days after CLP surgery. However, by the 7th day, the survival rates in DCQD L, DCQD M and DCQD H groups were 60%, 80% and 80%, respectively. These results indicate that DCQD treatment led to a substantial increase in the survival rates of septic mice, particularly in the DCQD M and DCQD H groups, as compared to the model group.

3.3. DCQD alleviates pathological damage to liver tissue

The effects of DCQD on liver tissue damage in mice with liver injury were evaluated. As shown in Fig. 2A, compared with that in the control group, the liver tissue of mice in the model group exhibited significant pathological damage, which was characterized by severe inflammatory cell infiltration, congestion, nuclear fading and vacuolization. However, treatment with DCQD significantly reduced the pathological damage to the liver tissue and histological scores (Fig. 2B). The extent of liver histopathological damage was negatively correlated with the concentration of DCQD.

3.4. DCQD inhibits inflammatory responses and oxidative stress in septic liver injury

DCQD could alleviate inflammatory responses and oxidative stress in septic liver injury. As shown in Fig. 2C–H, the levels of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α) were significantly increased in the model group, but DCQD treatment significantly reduced their levels. The same trend was observed for the oxidative stress factor malondialdehyde (MDA). The inhibitory effect of DCQD on the inflammatory response and oxidative stress in ALI was dose dependent. The changes in the expression of SOD and GSH-px in each group were opposite to those of the inflammatory factors.

3.5. DCQD protects liver function in ALI

DCQD could protect liver function in ALI. The levels of the liver enzymes ALT and AST were significantly higher in the ALI model group than in the control group (Fig. 3A and B). However, DCQD significantly reduced the levels of ALT and AST, and this positive effect was dose dependent.

3.6. DCQD inhibits hepatocyte apoptosis in sepsis-induced ALI

DCQD could inhibit hepatocyte apoptosis in sepsis-induced ALI. TUNEL analysis of mouse liver tissue showed that the ALI model had significantly increased numbers of apoptotic hepatocytes, while DCQD treatment significantly reduced the number of apoptotic hepatocytes (Fig. 3C). DCQD also significantly promoted the expression of the anti-apoptotic protein Bcl-2 and inhibited the expression of the proapoptotic protein Bax (Fig. 4A).

3.7. DCQD inhibits the TGF-β1/Smad3 signalling pathway in ALI

The Western blot results showed that the ALI model had upregulated expression of TGF-β1/Smad3 pathway-related proteins, including TGF-β1, Smad3, and p-Smad3, but DCQD significantly inhibited the activation of the TGF-β1/Smad3 signalling pathway (Fig. 4B–E). The inhibitory effect of DCQD was positively correlated with its concentration.

3.8. DCQD inhibits the inflammatory response and oxidative stress in LO2 cells

In vitro studies using LO2 cells further supported our findings. As shown in Table 2, sepsis increased the levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α and MDA and decreased the levels of SOD and GSH-px in LO2 cells. SRI-011381 treatment further enhanced the effect of LPS, while DCQD had the opposite effect, significantly inhibiting the levels of inflammatory factors and oxidative stress in LO2 cells.

3.9. DCQD inhibits the expression of proapoptotic proteins in LO2 cells

As shown in Fig. 5, LPS significantly promoted the expression of the apoptosis-related protein Bax and inhibited the expression of Bcl-2, while LPS + SRI-011381 treatment significantly enhanced the effects of LPS. DCQD significantly inhibited the increase in the expression of proapoptotic proteins caused by LPS and SRI-011381 and promoted the expression of the antiapoptotic protein BCL-2.

3.10. DCQD inhibits the TGF-β1/Smad3 signalling pathway in LO2 cells

LPS stimulation increased the protein expression of TGF-β1, Smad3, and p-Smad3 in LO2 cells, while LPS + SRI-011381 treatment further aggravated this increase. DCQD significantly inhibited the activation of the TGF-β1/Smad3 pathway in LO2 cells (Fig. 6).

4. Discussion

The liver plays a key role in regulating metabolism and immune defence,[25,26] and liver dysfunction is of great prognostic importance in sepsis.[27] Although much research has been devoted to sepsis-induced ALI in recent years, the mechanism of sepsis-induced ALI has not been clearly elucidated, and effective treatment strategies are still lacking. Exploring the mechanisms and effective treatment strategies to improve sepsis-induced liver injury is an urgent task in critical care medicine research. Previous studies have shown that DCQD exerts positive effects, such as inhibiting the inflammatory response, apoptosis and oxidative stress and mediating immune homeostasis.[28] Therefore, DCQD may be a promising therapeutic alternative for treating ALI.

In the current study, we found that CLP treatment resulted in marked pathological damage to liver tissue and rapidly increased ALT and SAT levels. Serum ALT and AST levels are the most commonly used indicators of the degree of liver injury.[2] DCQD effectively alleviated pathological damage to liver tissue and reduced ALT and AST levels. The beneficial effects of DCQD were dose dependent, and higher doses induced greater improvements.

The mechanisms of sepsis-induced liver injury are diverse and include hyperactivated systemic inflammatory activation, coagulation disorders, liver ischaemic injury, excessive oxidative stress, and dysregulated apoptosis.[29] Excessive inflammation is one of the key factors in liver injury during sepsis.[30] The liver is a major source of proinflammatory cytokines, such as TNF, IL-1 and IL-6, and these proinflammatory cytokines can activate the cytokine cascade, leading to cytokine storms and exacerbating inflammation and immune responses. Excessive oxidative stress is thought to result from an imbalance between the production of oxidative stressors and their elimination by the antioxidant system.[31] The hyperactivation of the inflammatory response and oxidative stress are important mechanisms of organ damage in septic patients.[32,33] In addition, inflammation and oxidative stress are linked and have a synergistic effect on septic organ damage.[34] Therefore, we next assessed the effect of DCQD on inflammatory and oxidative stress responses in ALI. TNF-a, IL-1β, and IL-6 are important inflammatory factors in the host against infection and play important roles in sepsis-induced ALI.[7] This study showed that DCQD significantly inhibited the expression of these proinflammatory factors. We also assessed the regulatory effect of DCQD on oxidative factors in septic liver injury by examining MDA, SOD, and GSH-px. Our results suggest that DCQD can significantly inhibit the excessive inflammatory response and oxidative stress in ALI.

Under normal physiological conditions, cell apoptosis and proliferation maintain a dynamic balance, but under pathological conditions, cell apoptosis is significantly increased. Abnormal hepatocyte apoptosis is an important pathological mechanism of sepsis-induced ALI.[35] Bcl-2 can effectively inhibit apoptosis induced by various apoptosis-inducing factors,[36] and the inhibition of Bcl-2 and the promotion of Bax play important roles in apoptosis associated with liver injury.[37] Our results showed that DCQD promoted the expression of the antiapoptotic protein Bcl-2 and inhibited the expression of the proapoptotic protein Bax, thereby improving hepatocyte apoptosis induced by sepsis.

TGF-β1 is a pluripotent growth factor that has certain regulatory effects on liver inflammation, fibrosis, oxidative stress, and tissue repair and is involved in regulating cell proliferation, differentiation and apoptosis. The Smad3 protein plays a key role in TGF-β signalling. DCQD can play a regulatory role by inhibiting the TGF-β1/Smad3 signalling pathway, including regulating epithelial-mesenchymal transition (EMT), reducing lung inflammation and alveolar cell apoptosis,[38] and regulating the TGF-β1/Smad3 signalling pathway to reduce oxidative stress and the inflammatory response to prevent the occurrence of liver cancer.[39] Therefore, we verified DCQD-mediated regulation of the TGF-β1/Smad3 pathways in vitro and in vivo. We found that DCQD effectively inhibited the expression of TGF-β1 and p-Smad3 in the

animal model of septic liver injury simulated by CLP and in LPS-stimulated LO2 cells. The regulation of the TGF-β1/Smad3 pathway by DCQD was associated with protective effects against excessive inflammation, oxidative stress, and hepatocyte apoptosis in septic liver injury.

However, this study still has various limitations and requires further optimization. Glutamyl transpeptidase, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin and other indicators can show the level of liver function from different aspects. However, this study only examined serum levels of ALT and AST in mice, which could not completely reflect the changes in liver function. In addition, because mice with acute liver injury in the model group died easily, the measured liver function in the surviving mice may be lower than the actual value. The treatment of ALI in sepsis has made full use of the advantages of the multiple targets and multiple pathways of traditional Chinese medicine. Previous studies have proven that it this effect is associated with multiple signalling pathways, such as Toll-like receptor 4 (TLR4), NOD-like receptor thermal protein domain associated protein 3 (NLRP3), and nuclear factor-k-gene binding (NF-kB).[40] The present study focused on the TGF-β1/Smad3 pathway and did not explore other potential mechanisms of DCQD in ALI. Further research is needed to address these limitations and uncover additional mechanisms by which DCQD can treat sepsis-induced liver injury.

5. Conclusion

The findings of this study demonstrate that DCQD has significant therapeutic potential in sepsis-induced ALI. By inhibiting the excessive inflammatory response and oxidative stress, DCQD helps to maintain liver function and mitigate liver tissue damage. The protective effects of DCQD are attributed to its ability to inhibit liver cell apoptosis and regulate the TGF-β1/Smad3 signalling pathway.

This study highlights the value of DCQD as a potential treatment option for ALI and provides a new perspective on the use of traditional Chinese medicine prescriptions. Further research is warranted to explore additional mechanisms of action and optimize the treatment strategy for sepsis-induced ALI using DCQD. These findings contribute to the understanding of ALI pathogenesis and offer insights into novel therapeutic approaches for this condition.

Declaration of competing interest

We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. All authors have approved the manuscript and agree with its submission to *Journal of Traditional and Complementary Medicine*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.09.001.

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