

YiQiFuMai Lyophilized Injection Attenuates Sepsis-Induced Acute Lung Injury via TLR4/Src/VE-cadherin/p120-catenin Signaling Pathway

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Research

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Abstract

Background: Sepsis is a severe disorder leading to a clinically critical syndrome of multiple organ dysfunction syndrome. Most patients with sepsis will be associated with acute lung injury (ALI), which is an independent risk factors of organ failure and death in patients with sepsis at the same time. YiQiFuMai Lyophilized Injection (YQFM) is a modern traditional Chinese prescription preparation, which could ameliorate ALI induced by lipopolysaccharide (LPS) or fine particulate matter. The current study aimed to investigate the effect of YQFM on sepsis-induced ALI and the underlying mechanism.

Methods: Male C57BL/6J mice were treated with cecal ligation and puncture (CLP) after tail intravenous injected with YQFM (1, 2 and 4 g/kg). The measurements of lung edema, Evans blue leakage, myeloperoxidase content, inflammatory cells in bronchoalveolar lavage fluid, histopathological assay and expression of associated proteins were performed at 18 h after CLP.

Results: The results illustrated that YQFM inhibited pulmonary edema and inflammatory response, thus ameliorated ALI in sepsis mice. Furthermore, the expression of TLR4 and phosphorylated Src was down-regulated, and the expression of p120-catenin and VE-cadherin was restored by YQFM administration.

Conclusion: Our study suggested the therapeutic potential of YQFM on treating sepsis-induced ALI via regulating TLR4/Src/VE-cadherin/p120-catenin signaling pathway.

Background

Acute lung injury (ALI) is a serious clinical illness in the intensive care unit (ICU) worldwide with a mortality rate of 35–40%, which is characterized by acute, diffuse, and inflammatory lung injury[1]. The clinical manifestation of ALI including increased alveolar capillary permeability and vascular leakage, collapsed alveolar, reduced vital capacity and increased respiratory frequency, thus lead to pulmonary edema or severe hypoxemia[2, 3]. A variety of direct or indirect factors contribute to ALI, including sepsis, pneumonia, trauma, acute pancreatitis and inhalation of gastric contents[4]. Moreover, ALI combined with other critical illnesses is an important cause of death[2].

Sepsis is a clinically critical syndrome caused by imbalanced host response to infection, leading to multiple organ dysfunction syndrome (MODS) with high fatality rate[5]. Epidemiological studies reported that more than 30 million people worldwide are threatened by sepsis each year[6]. The complicated pathogenetic process of sepsis begin with the invasion of pathogenic microorganisms and their toxins into the body and the activation of immune response, thus causing imbalance of anti-inflammatory and pro-inflammatory response[7]. Then, the release of inflammatory factors and excessive reactive oxygen and nitrogen would cause oxidation stress, tissue factor expression and microthrombus formation[8]. The uncontrollable inflammatory responses further caused the destruction of endothelial integrity characterized by increased permeability, coagulation microcirculation disorders and MODS[9, 10]. The most vulnerable sites of sepsis infection are lung (64%), abdominal cavity (20%), blood (15%), kidney and urogenital tract (14%)[8]. Studies have shown that the first organ to be attacked in the progress of MODS

is lung, and 50% of sepsis patients have combined symptoms of ALI with poor prognosis[11]. At the same time, ALI is also an independent risk factor for organ failure and death in patients with sepsis[12]. Therefore, ameliorating ALI is an important orientation for the treatment of sepsis. At present, the main clinical treatments for ALI are low-tidal volume mechanical ventilation, bronchodilator drugs and corticosteroids. However, these methods cannot reduce the mortality of sepsis-associated ALI due to large side effects and poor prognosis[13]. Therefore, it is urgent to explore effective drugs for improving sepsis-induced ALI.

The destruction of pulmonary capillary endothelial barrier function is the most fundamental pathological feature of sepsis-induced ALI. Adherent junctions (AJs) in pulmonary endothelium, especially vascular endothelial cadherin (VE-cadherin) play a major role in maintaining the integrity of the lung endothelial barrier[14]. The combination of p120-catenin, a substrate of tyrosine kinase (Src), and VE-cadherin in the near-membrane region inhibits VE-cadherin endocytosis and maintains the stability of AJs[15]. Under pathological conditions, the activation of Toll-like receptor 4 (TLR4) promotes Src phosphorylation, thus accelerates the degradation and dissociation of p120-catenin and VE-cadherin, resulting in AJs destruction and increasement of pulmonary microvascular endothelial permeability[16]. Therefore, inhibition of TLR4/Src/VE-cadherin/p120-catenin signaling pathway is an important therapeutic strategy to prevent damage on pulmonary microvascular endothelial barrier function caused by ALI. However, whether this signaling pathway participates in sepsis-induced ALI is unknown.

There were few researches on the therapeutic effect of Traditional Chinese medicines (TCMs) on sepsis-induced ALI. YiQiFuMai Lyophilized Injection (YQFM) is a modified preparation derived from Sheng-mai San (SMS), a well-known TCM formula, composed of three medicinal plants: *Panax ginseng* C.A. Mey., *Ophiopogon japonicus* (Linn. f.) Ker-Gawl. and *Schisandra chinensis* (Turcz.) Baill.[17], which is widely used for the treatment of cardiovascular and cerebrovascular diseases, chronic obstructive pulmonary disease (COPD) and septic shock[18–20]. It has been illustrated that YQFM could ameliorate ischemic stroke, brain microvascular endothelial barrier dysfunction, myocardial oxidative damage, heart failure and mesenteric microcirculation disorders[21–25]. Besides, clinical researches indicated that YQFM showed significant effects on septic shock by improving microcirculation disorder, increasing the central venous oxygen saturation and decreasing mortality of patients[26, 27]. In previous studies, YQFM could improve ALI caused by intratracheal infusion of lipopolysaccharide (LPS) or fine particles linked with TLR4-mTOR-autophagy pathway[28, 29]. However, whether YQFM could ameliorate sepsis-induced ALI is unknown. Therefore, in the current study, we investigated the protective effect of YQFM on sepsis-induced ALI in cecal ligation and puncture (CLP)-induced mice and explored its potential mechanism based on TLR4/Src/VE-cadherin/p120-catenin signaling pathway.

Materials And Methods

Materials

YQFM was obtained from Tasly Pharmaceutical Co., Ltd. (China), which is composed of the ethanol extract (78°C) of three herbals (*Panax ginseng*: *Ophiopogon japonicus*: *Schisandra chinensis* = 1: 3: 1.5). After extraction, filtration and lyophilization, aseptic packaging and strict quality control were done before appearing on the market[24]. Dexamethasone sodium phosphate (lyophilized) was purchased from Biosharp biotechnology company (China).

Animal model of CLP and treatment of drugs

C57BL/6J male mice (18-22g) were obtained from the Experimental Animal Center of Yangzhou University (Yangzhou, China). All mice were housed for 3-5 days to adapt to the environment with controlled temperature (23±1°C), humidity (30%-40%), light (12 h light-dark cycle) and a commercial standard solid rodent chow and water ad libitum. All experiments were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Animal Ethics Committee of China Pharmaceutical University.

Animals were randomly divided into six groups: Sham group, Model group, Dexamethasone group (Dex) and three YQFM groups of different dosages. Mice were injected with 5 mg/kg dexamethasone or multi-doses of YQFM (1, 2, 4 g/kg) from tail vein before surgery. Sepsis in mice was induced by the CLP surgery as described previously within 1 min after drug administration[30]. The mice were anesthetized by intraperitoneal injection of 4% chloral hydrate (400 mg/kg) and then fixed on the operating table in supine position. After shaved the abdominal hair of mice, a 1 cm incision was made along the ventral white line. The cecum was isolated through blunt separation and ligated with a 5-0 nylon monofilament suture. The length from the free end of cecum to the ligated position accounts for 75% of the total length of cecum. The cecum was punctured in twice with a 16-gauge needle from the mesenteric side without blood vessels to the non-mesenteral side, avoiding damaging the cecum vessels. A small amount of feces was extruded from the two ends of the pinhole. Then the cecum was put back into the abdominal cavity. The incision of mice was sutured layer by layer and iodine was used to prevent infection. Mice in the sham group were injected with same volume of saline and performed with the same surgical procedures without ligation and perforation of the cecum. The mice were sacrificed and relevant examinations were carried out at 18 h after surgery.

Determination of wet weight to dry weight (W/D) ratio of lung tissues

The mice were sacrificed at 18 h after CLP. The lungs were taken out and the wet weight of tissue was accurately measured. After dried in an oven at 120°C for 48 h, the lung tissues were weighed again, recording as dry weight. The wet weight to dry weight ratio of lung tissues were calculated to determine the degree of lung edema.

Lung hematoxylin-eosin (H&E) staining

Histomorphological analysis was measured by H&E staining. At 18 h after CLP, the mice were euthanized. The lungs were rapidly taken out and dipped in 4% paraformaldehyde. The examination was finished in

the pathology department of Jiangsu Center for Safety Evaluation of drugs (Jiangsu, China).

Determination of cell contents in bronchoalveolar lavage fluid (BALF)

The contents of white blood cells, neutrophil and lymphocyte in BALF were measured as previous[31]. At 18 h after CLP, the mice were sacrificed and the trachea was peeled off. The alveoli were lavaged with 500 μ L phosphate buffer saline (PBS) for 3 times. The BALF were collected and centrifuged (1500 rpm, 5 min, 4°C). The precipitation was resuspended in 800 μ L PBS and the cell contents determinations were finished in the Center for New Drug Safety Evaluation and Research in China Pharmaceutical University (Jiangsu, China).

Determination of myeloperoxidase (MPO) content in lung, BALF and plasma

The content of MPO in lung, BALF and plasma was determined by Myeloperoxidase kit (Nanjing Jiancheng Biotechnology Research Institute, China). At 18 h after CLP, the blood, BALF and lung tissues of mice were collected. The determination of MPO was performed according to the manufacturer's instructions[32]. The absorbance at 450 nm was measured spectrophotometrically using an Infinite M200 Pro plate reader (Tecan, NC, USA). The MPO content in lung homogenate was expressed as Unit per gram of wet lung tissue, while that in BALF or plasma was expressed as Unit per litre.

Measurement of evans blue (EB) leakage into the lung tissue

EB extravasation was used to determine pulmonary microvascular permeability. At 16 h after CLP, EB dye (50 mg/kg, Sigma, USA) was injected via the tail vein. The mice were anesthetized at 2 h after injection of EB and then perfused with saline. The lungs were rapidly taken out and imaged. The lung tissues were weighed and homogenized in formamide (1 mL/100 mg tissue) and centrifuged (5000 g, 30 min). The supernatants were collected to determine the quantity of EB, the absorbance at 620 nm was measured spectrophotometrically using an Infinite M200 Pro plate reader (Tecan, NC, USA). EB leakage was assessed with a standard curve and expressed as micrograms per gram of wet lung tissue.

Western blotting analysis

Western blotting analysis was performed as previously described[33]. The lung tissues and were lysed and centrifuged. The protein concentration was calculated using a bicinchoninic acid protein assay kit (Beyotime Biotechnology, China). Equal amounts of proteins were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, USA) using electrophoresis. The membranes were blocked with 5% bovine serum albumin (BSA) for 2 h and incubated overnight at 4°C with primary antibodies against VE-cadherin (1:1000, Santa Cruz, USA), p120-catenin (1:1000, Abcam, USA), phospho-Src (Y416) (1:1000, Life, USA), Src (1:1000, Life, USA), TLR4 (1:1000, Santa Cruz, USA) and β -actin (1:5000, Bioworld, USA), followed by incubation with the HRP-conjugated secondary antibodies (1:8000, Bioworld, USA) and visualization using enhanced chemiluminescence (ECL, Vazyme Biotech, P.R. China). The bolts were quantified by the Image Lab™ software (version 5.2, Bio-Rad, USA) and the relative values were expressed relative to GAPDH signals.

Immunofluorescence (IF) analysis

Immunofluorescence analysis were performed as previously described[21]. The lung tissues were sectioned at 8 µm thicknesses to adhesive slides. The specimens were treated with blocking buffer (5% BSA, 0.1% Triton-100) at 4°C for 1 h and then incubated overnight at 4°C with primary antibody against CD31 (1:100 dilution, R&D Systems, USA), VE-cadherin (1:200 dilution, Abcam, UK), p120-catenin (1:100 dilution, Santa Cruz Biotechnology, USA), followed by incubation with an Alexa Fluors 488-conjugated donkey anti-rabbit IgG (H_pL) antibody (Invitrogen, USA) and 4',6-Diamidino-2-phenylindole (DAPI, Beyotime Biotechnology, P.R.China). Fluorescent images were observed with confocal laser scanning microscope (LSM700, Zeiss, Germany) and processed using the ZEN imaging software.

Statistical analysis

All analyses were performed using GraphPad Prism Version 5.01 (GraphPad Software Inc., USA). Results are expressed as the means ± SEM. Statistical analyses were performed using Student's two-tailed t test for comparison between two groups and one-way analysis of variance (ANOVA) followed by Dunnett's test when the data involved three or more groups. The tests were considered statistically significant at $P < 0.05$.

Results

YQFM attenuated CLP-induced lung histopathological changes and edema in mice

H&E staining was used to investigate whether YQFM could reduce the extent of sepsis-induced pulmonary histopathological changes. The results showed that the structure of lungs in Sham group mice were clear without exudate in alveolar cavity and bronchial cavity. However, the lung of mice in Model group exerted obvious alveolar collapse, congestion of the alveolar wall accompanied by inflammatory cell infiltration, pulmonary edema and mild inflammation around the trachea. YQFM and dexamethasone could alleviate these lung histopathological changes to varying degrees (Fig. 1A). Besides, by measuring the weight of wet and dry lung tissues, we found that the ratio of wet to dry weight of lung tissues in CLP mice was significantly increased compared with the Sham group ($P < 0.01$), which indicates pulmonary edema caused by sepsis. However, the degree of pulmonary edema in the mice of YQFM groups were all reduced. According to statistical analysis, YQFM of medium-dose (2 g/kg) showed the most significant inhibition rate (89.61%, $P < 0.01$), but dexamethasone showed little effect (Fig. 1B). These results indicated that YQFM could significantly ameliorate sepsis-induced lung histopathological changes and edema in CLP-mice.

YQFM reduced CLP-induced pulmonary and systemic inflammation in mice

The inflammatory response in lungs is a typical pathological manifestation of sepsis-induced ALI[34]. To investigate the effects of YQFM on pulmonary inflammation in CLP-induced mice, the number of leukocytes, neutrophils and lymphocytes in BALF of mice were measured. Compared with the Sham

group, the number of leukocytes, neutrophils and lymphocytes in BALF of CLP-induced mice were significantly increased ($P<0.05$ or $P<0.01$). However, compared with CLP group, YQFM of medium dosage (2 g/kg) could significantly inhibit the leakage of leukocytes, neutrophils and lymphocytes from pulmonary microvascular into the alveolar cavity ($P<0.05$ or $P<0.01$). Besides, 4 g/kg YQFM could also reduce the white blood cells in the BALF ($P<0.01$, Fig. 2A-C). Interestingly, the effect of YQFM (2 g/kg) was even better than that of dexamethasone. Moreover, to determine the degree of neutrophil infiltration in lungs of sepsis mice, the MPO activity in lung tissue homogenate, BALF and plasma of mice were examined. Compared with the Sham group, the MPO activity of the CLP mice were significantly enhanced ($P<0.01$), while YQFM and dexamethasone significantly inhibited the infiltration and activation of neutrophils ($P<0.05$ or $P<0.01$). Among them, YQFM of medium dosage showed the best effect on inhibiting MPO in lung homogenate and BALF ($P<0.01$, Fig. 2D-F). Collectively, these results showed that YQFM could significantly inhibited the pulmonary infiltration and activation of inflammatory cells, including leukocytes, lymphocytes and neutrophils, and plasma MPO activity induced by sepsis.

YQFM ameliorated CLP-induced lung vascular hyperpermeability in mice

The leakage of inflammatory cells into the alveolar cavity is mainly associated by the destruction of the pulmonary microvascular endothelial barrier function, which plays an important role in ALI caused by sepsis[35]. The degree of lung vascular permeability was examined by EB dye. The lung vascular hyperpermeability were found in sepsis-induced ALI mice as indicated by enhanced lung EB concentrations, which was dramatically greater than that of sham group ($P<0.01$). However, three dosages of YQFM and dexamethasone could significantly inhibited EB leakage of pulmonary microvascular in sepsis-induced ALI mice with inhibition rate as 54.79%, 59.03%, 49.31% and 34.09% respectively ($P<0.05$ or $P<0.01$, Fig. 3). This result indicated that YQFM could significantly maintain the integrity of pulmonary microvascular endothelial barrier in mice and improve CLP-induced ALI.

YQFM attenuated CLP-induced pulmonary endothelial barrier destruction in mice

The interaction of VE-cadherin and p120-catenin is the basis for maintaining the endothelial barrier function[36]. The protein expressions of VE-cadherin and p120-catenin in the lung tissue and the fluorescence intensity on the lung endothelium were detected by western blotting and immunofluorescence analysis respectively. The expression of VE-cadherin and p120-catenin significantly decreased in the lung of sepsis mice compared with the Sham group ($P<0.01$). While YQFM (2 g/kg) administration significantly restored the VE-cadherin and p120-catenin content ($P<0.05$ or $P<0.01$, Fig. 4). These data suggested that YQFM could ameliorate the destruction of AJs and improve the pulmonary microvascular endothelial barrier function in CLP-induced ALI mice.

YQFM inhibited CLP-induced activation of TLR4/Src signaling pathway in lung tissue of mice.

It has been confirmed that under pathological conditions of ALI, the activation of TLR4 and the phosphorylation of Src downstream in pulmonary microvascular endothelium accelerated AJs degradation and endothelial barrier destruction[37]. The current study examined whether the TLR4/Src

signaling pathway participate in CLP-induced ALI by analyzing the protein expressions of TLR4 and p-Src. Compared with the Sham group, Src phosphorylation level and TLR4 expression in the lung tissue of CLP mice were significantly increased ($P<0.01$). In contrast, YQFM (2 g/kg) significantly decreased the high expression of TLR4 and p-Src in the lung tissue of sepsis mice ($P<0.05$, Fig. 5). These results demonstrated that YQFM might restore the AJs by suppressing the activation of TLR4/Src signaling pathway, thus improving ALI induced by sepsis.

Discussion

The key finding of the present study is that YQFM ameliorated sepsis-induced ALI. We demonstrated that YQFM could suppress sepsis-associated pulmonary capillary endothelial barrier destruction via regulating TLR4/Src/VE-cadherin/p120-catenin signaling pathway. These findings indicate that YQFM might be a promising potential drug to ameliorate ALI in patients with sepsis.

In the progress of sepsis, the intestinal pathogenic microorganisms and their toxins could enter the body circulation after the damage of intestinal barrier, thus causing systemic infections and MODS[38]. Studies have shown that the first organ to be attacked is lung, and about half of sepsis patients have combined symptoms of ALI[11]. ALI is a serious respiratory disease with diffuse alveolar damage as the main pathological feature and 31.5% of patients with ALI are caused by non-pulmonary sepsis[39]. In addition, the increase of systemic endothelial permeability in patients with sepsis combined with ALI will also increase the mortality[40]. Therefore, improving ALI in patients with sepsis is an important direction for the treatment of sepsis. However, there are still no clinically effective and safe drugs for sepsis treatment[41]. Therefore, the development of new drugs for the treatment of sepsis-associated ALI is imminent.

YQFM is a modern preparation deriving from a TCM compound prescription SMS and is widely used in clinical practice in China, principally for the treatment of microcirculatory disturbance-related diseases[19, 42]. Previous investigations indicated that YQFM has strong antioxidative and anti-inflammatory effects and could protect the microvascular endothelial integrity[21, 22, 43]. Moreover, studies have shown that YQFM could attenuate ALI in LPS or fine particles-induced mice[28, 29]. Hence, we hypothesized that YQFM could ameliorate sepsis-induced ALI and investigated the underlying mechanism.

There are many animal models for studying sepsis, including bacterial attack model, endotoxin attack model, CLP model and ascending colon support tube peritonitis model[44]. To simulate sepsis caused by appendicitis, diverticulum perforation-induced peritonitis or multiple pathogenic microorganisms-induced infections, CLP model was chosen in our study. As the gold standard model for polymicrobial sepsis, CLP model has the advantages of simple operation and high reproducibility[30]. Besides, the systemic infection caused by CLP is similar to the pathological changes of clinically sepsis patients.

Excessive inflammatory response, nonspecific diffuse alveolar injury and increased alveolar epithelium and pulmonary capillary endothelial permeability are the most fundamental pathological features of ALI[45]. In the course of sepsis, bacteria and endotoxins invaded lung tissues. Infection inside and

outside the blood vessels will lead to pulmonary capillary endothelial barrier dysfunction. The increased permeability of the pulmonary microvascular endothelium give rise to leakage of a large amount of plasma proteins and inflammatory cells into the alveolar cavity, which eventually leads to declining pulmonary ventilation and respiratory failure[46]. As shown in our investigations, distinct lung injury was found in CLP-induced mice, evidenced by remarkable alveolar collapse, pulmonary edema, congestion of the alveolar wall, inflammatory cell activation and neutrophil infiltration and EB leakage. Whereas, YQFM could significantly ameliorated sepsis-induced pulmonary inflammation and edema and protected the integrity of the pulmonary microvascular endothelial barrier (Figs. 1 and 3).

Besides, neutrophils play an important role in the occurrence and development of ALI. MPO levels are related to viability and infiltration and activation of neutrophils. When the body is stimulated by infection, MPO will be released accompanied with neutrophil infiltration to the site of inflammation[47]. The damaged capillary endothelial barrier would cause leakage of inflammatory cells from the blood vessels into the alveolar cavity. Studies have found that inhibition of neutrophil viability can inhibit inflammatory responses in the lung[48, 49]. Therefore, the MPO activity and the number of white blood cells, neutrophils and lymphocyte in BALF were measured. We found that YQFM administration could significantly inhibit the pulmonary inflammatory responses caused by CLP-induced sepsis in mice (Fig. 2).

Intercellular junctions, especially AJs, play a major role in maintaining the integrity of the lung endothelial barrier. As an endothelium-specific adhesion junction molecule, VE-cadherin plays a key role in maintaining vascular integrity by regulating cytoskeleton rearrangement, establishing cell polarity, and remodeling tight junctions[50]. VE-cadherin of adjacent cells binds with each other in the extracellular region homologously to form a zipper-like structure. Meanwhile, VE-cadherin binds to β -catenin and plakoglobin in the cytoplasmic region. In the near-membrane region, the combination of VE-cadherin and p120-catenin, a substrate of tyrosine kinase (Src), inhibits VE-cadherin endocytosis and maintains the stability of AJs[51, 52]. Src kinase is expressed in mammalian tissues and is involved in endothelial signaling. Src phosphorylation caused by the stimulation of vascular endothelial growth factor (VEGF), tumor necrosis factor- α (TNF- α), reactive oxygen species (ROS) or activated neutrophils participates in the regulation of adhesion between endothelial cells and intercellular matrix. Studies have found that the activation of Src can promote tyrosine phosphorylation of VE-cadherin and subsequent endocytosis degradation, thus lead to increased endothelial permeability[37]. TLR4 is a type of transmembrane protein that could recognize highly conserved pathogen-associated molecules and initiate the immune response and inflammation[53]. The extracellular region of TLR4 can recognize a variety of LPS-based ligands and initiate intracellular signal cascades. The abnormal activation of TLR4 is related to many inflammatory diseases[54]. During infection, the activation of TLR4 owing to pathogens and their toxin can promote Src phosphorylation, thus causing destruction of AJs, manifested by elevated TLR4 and p-Src expressions and reduced p120-catenin and VE-cadherin levels in lungs of sepsis mice (Fig. 4–5). Therefore, inhibition of TLR4 and its downstream pathway activation is the key to protecting the lung endothelial barrier function and improving sepsis accompanied with ALI. Our study verified that YQFM (2 g/kg) intervention could suppress the abnormal activation of TLR4 and Src phosphorylation, thus restored the expressions of p120-catenin and VE-cadherin in lung tissues significantly, suggesting that

YQFM could attenuate sepsis-induced ALI via protecting pulmonary endothelial barrier function (Fig. 4–5).

There are still some limitations in this study. First, we investigated the effect of YQFM on ALI in acute sepsis model. In addition to ALI, sepsis might also lead to MODS clinically. The effect of YQFM on chronic sepsis model and sepsis-induced injury of other organs needs further investigation. Second, as a TCM compound preparation, the active ingredients in YQFM are complicated. But what kind of ingredients play a key role in ameliorating lung injury is worthy to be further explored. It was reported that ruscogenin (RUS), a main active ingredient of *Ophiopogon japonicus* (Linn. f.) Ker-Gawl., could protect against LPS-induced endothelial cell apoptosis via regulating TLR4 signaling, and may be a promising agent in the management of ALI[55]. The therapeutic effect of RUS on CLP-induced ALI will also be investigated in our further research. Besides, major components in *Panax ginseng* C. A. Mey. also showed effects on sepsis treatment. Ginsenoside Rg1 could ameliorate sepsis-associated encephalopathy through regulating cerebral inflammation and apoptosis.[56] Ginsenoside Rg3 could improve mitochondrial dysfunction by regulating autophagy in mitochondria via activating the AMPK signal pathway, thus protecting organ injuries caused by sepsis[57].

Additionally, it was reported that the activation of TLR4 signaling pathways play a vital role in virus induced inflammatory response and subsequent cytokine storm, thus leading to severe ALI, ARDS (acute respiratory distress syndrome), MODS and even death[58–60]. Currently, the eruptible corona virus disease-19 (COVID-19), as a new coronavirus pneumonia with rapid infectiousness and great hazard, is also an infectious disease of the lungs[61]. Besides, patients with fatal disease develop ALI or ARDS and worsened in a short period of time and died of multiple organ failure[62, 63]. The appearance of ALI was mainly caused by diffuse damage of pulmonary capillary endothelial cells and alveolar epithelial cells due to cytokine storms correlated with TLR4 signaling pathways[64]. Therefore, it is urgent to develop more potential effective drugs to inhibiting cytokine storms and treating pulmonary diseases. TCM compounds have the advantages of comprehensive treatment of multi-path and multi-target, and has a unique effect in the treatment of diseases arise from cytokine storm[65, 66]. According to the previous and current studies, YQFM was widely used in treating coronary heart disease, angina pectoris, COPD as well as various shocks (septic shock, infectious shock and Qi-yin deficiency shock)[19, 26, 28]. Hence, YQFM may be a potential drug for treating COVID-19 by relieving pulmonary inflammation, preventing cardiogenic shock, thus avoiding exacerbation of the disease.

Conclusion

In conclusion, the present study demonstrated that YQFM could attenuate sepsis-induced ALI via TLR4/Src/VE-cadherin/p120-catenin signaling pathway in mice. Our findings provide a pharmacological basis for its treatment of sepsis-induced ALI and guiding significance for clinical application in treating lung diseases caused by pathogenic microorganism infections such as bacteria and viruses.

Abbreviations

ALI, acute lung injury; ICU, intensive care unit; MODS, multiple organ dysfunction syndrome; AJs, adherent junctions; VE-cadherin, vascular endothelial cadherin; TLR4, toll-like receptor 4; YQFM, YiQiFuMai Lyophilized Injection; SMS, Sheng-mai San; TCMs, traditional Chinese medicines; COPD, chronic obstructive pulmonary disease; LPS, lipopolysaccharide; CLP, cecal ligation puncture; H&E, hematoxylin-eosin; BALF, bronchoalveolar lavage fluid; PBS, phosphate buffer saline; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MPO, myeloperoxidase; EB, evans blue; BSA, bovine serum albumin; VEGF, vascular endothelial growth factor; TNF- α , tumor necrosis factor- α ; ROS, reactive oxygen species; RUS, Ruscogenin; ARDS, acute respiratory distress syndrome; COVID-19, Corona Virus Disease-19.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LXX, YYZ and JPK conceived and designed the study. XWP, LXX, QLZ, JZZ and YJD carried out the study and acquired the data. XWP, LXX and QLZ analyzed and interpreted the data. XWP, YYZ and JPK wrote and revised the manuscript. FL, YYZ and JPK provided technical and material support. All authors read and approved the final version of the manuscript.

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Figures

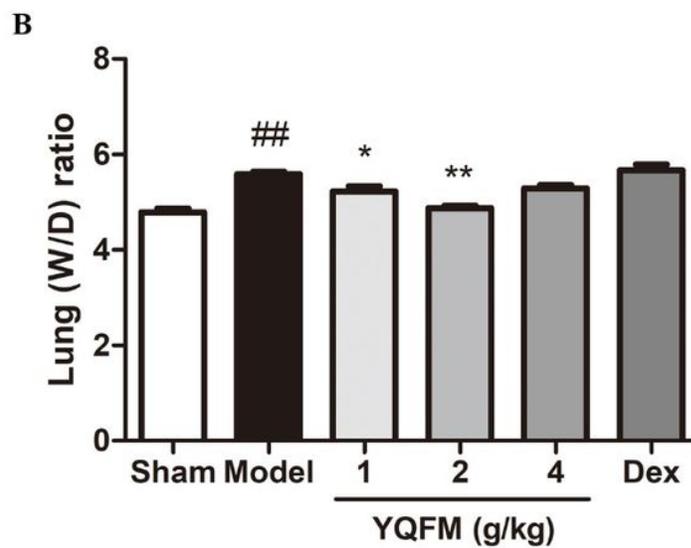
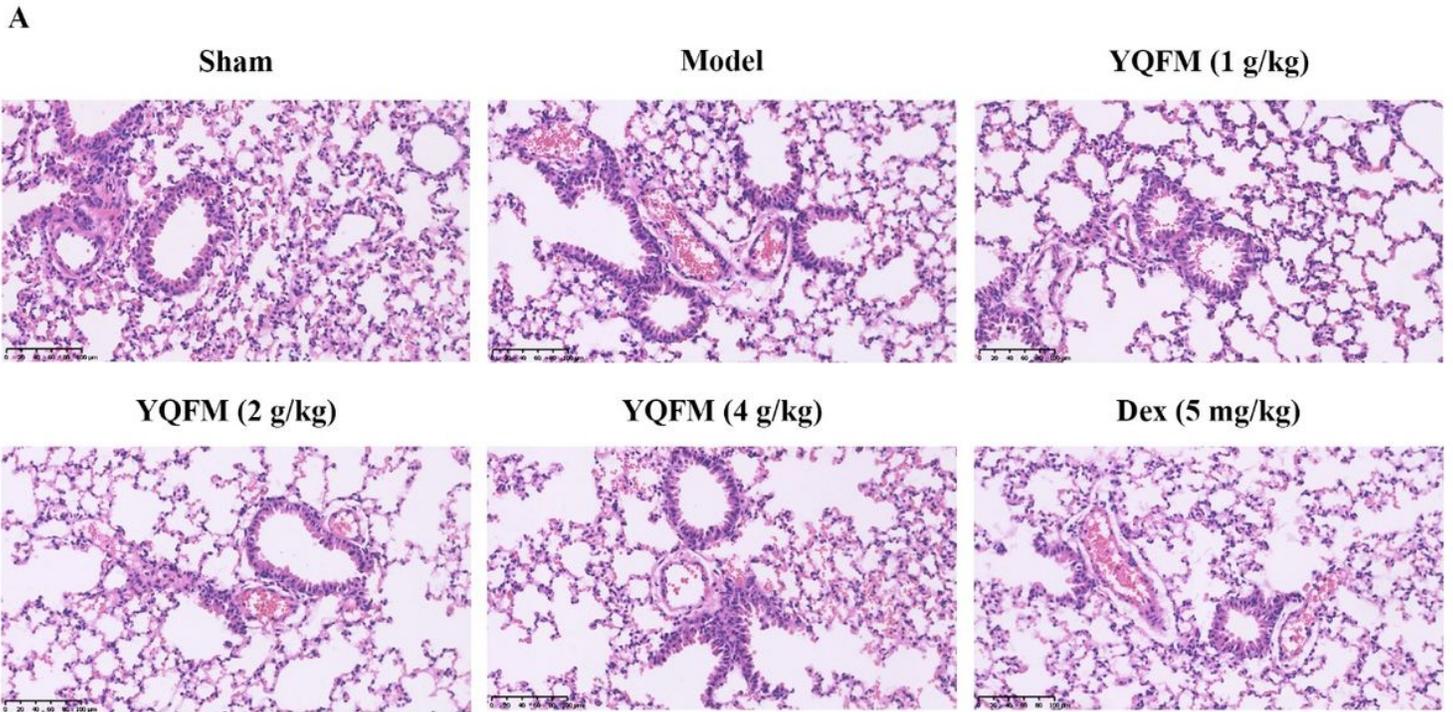


Figure 1

Effects of YQFM on CLP-induced lung histopathological changes and edema in mice. (A) Slides of lung tissues stained by hematoxylin-eosin (200×magnification, n=3) (B) The wet/dry ratio of lungs (n=8). Data are represented as means ± SEM. ##P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. CLP group.

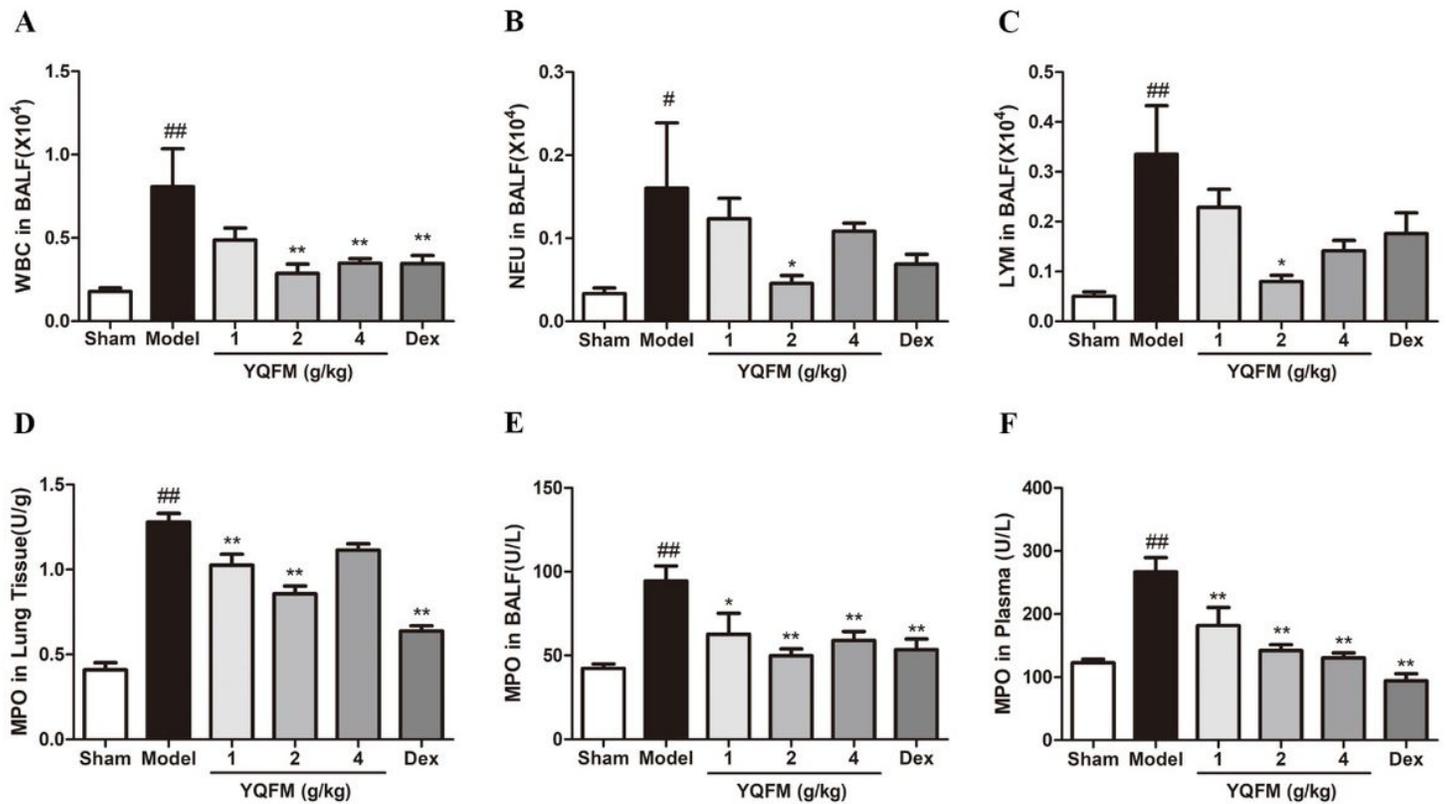


Figure 2

Effect of YQFM on CLP-induced pulmonary inflammation in mice. The quantity of white blood cell (A), neutrophil (B) and lymphocyte (C) in BALF. Activities of MPO in lung tissue (D), BALF (E) and plasma (F). Data are represented as means \pm SEM, n=8. ##P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. CLP group.

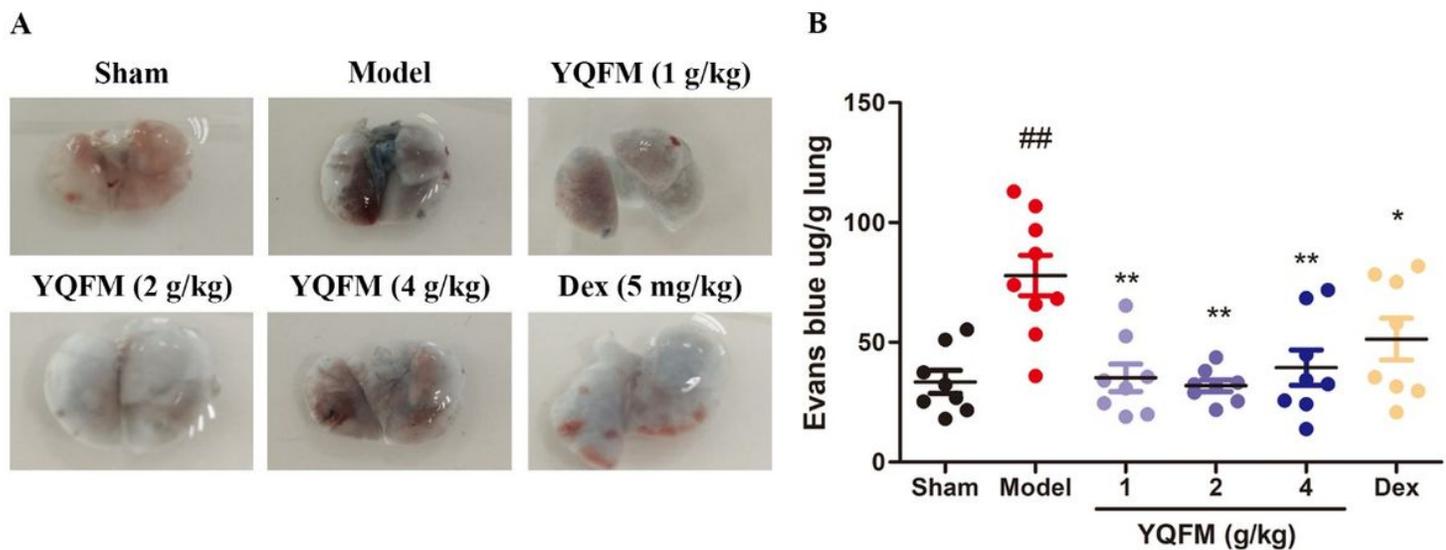


Figure 3

Effects of YQFM on CLP-induced lung vascular EB-leakage in mice. (A) Appearance of EB-stained lung of mice. (B) Quantity analysis of EB leakage by spectrophotometry. Data are represented as means \pm SEM,

n=8. ##P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. CLP group.

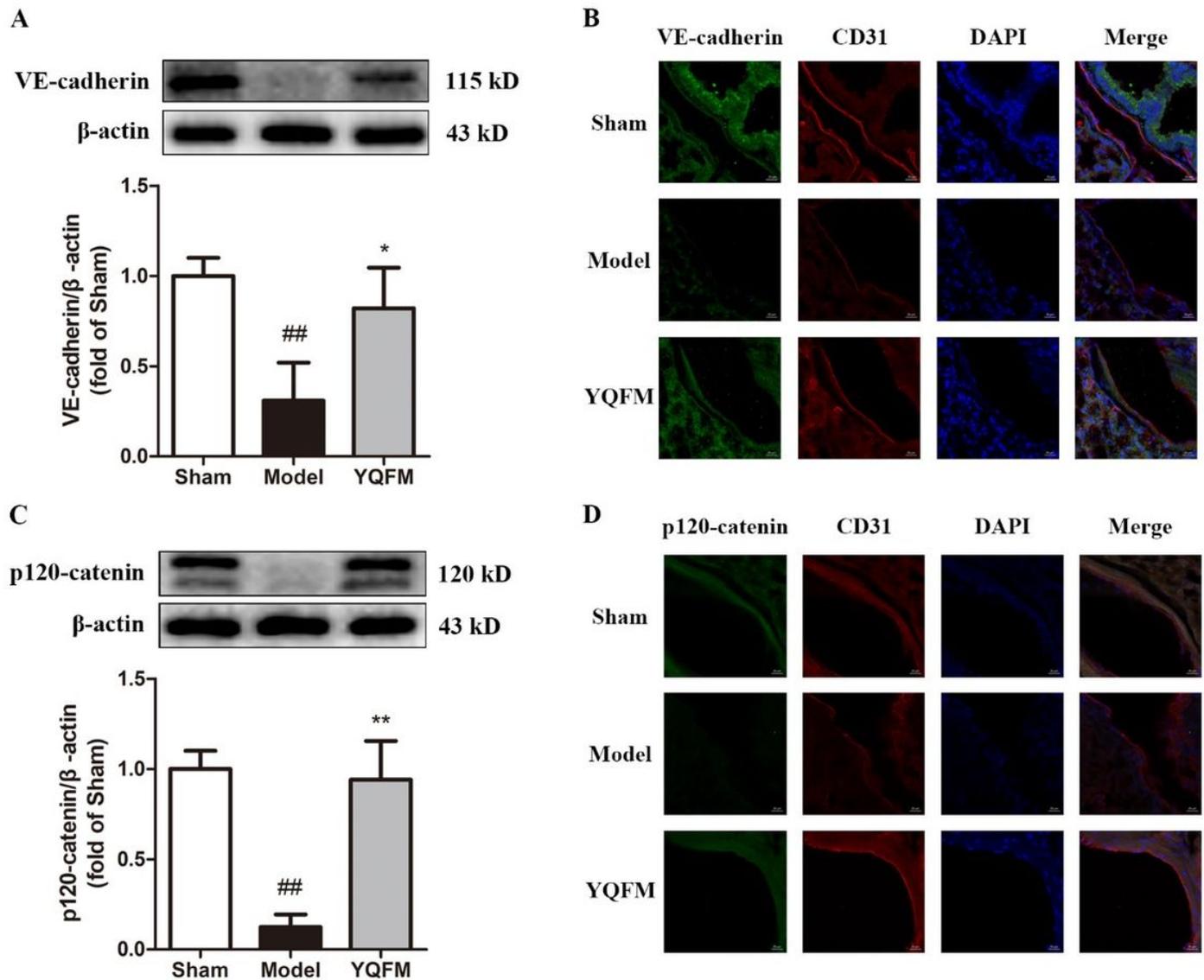


Figure 4

Effect of YQFM on CLP-induced damage of pulmonary endothelial barrier in mice. Representative western blots and quantitative analysis of VE-cadherin (A) and p120-catenin (C). Representative immunofluorescent microscope image of VE-cadherin (B) and p120-catenin (D) in lung tissue (200 \times magnification). Data are represented as means \pm SEM, n=3. ##P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. CLP group.

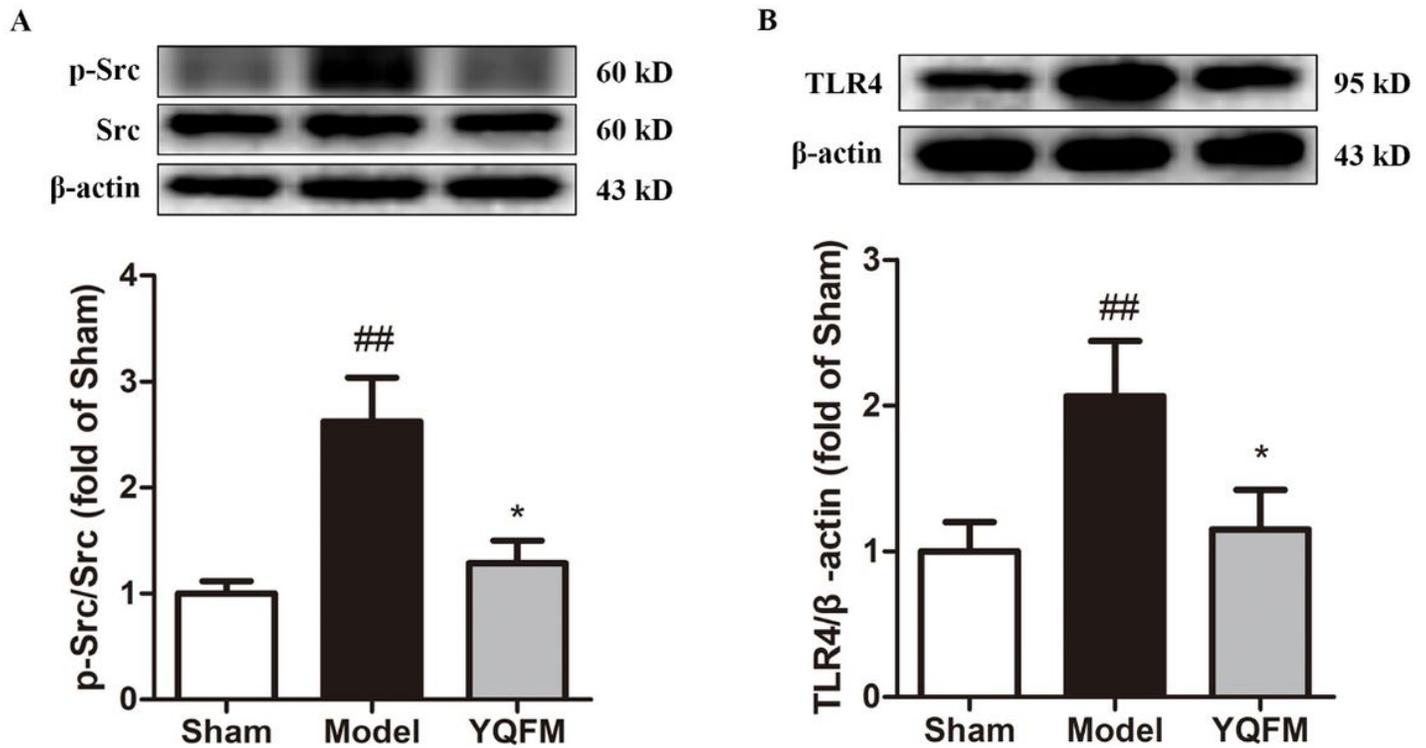


Figure 5

Effect of YQFM on CLP-induced TLR4/Src signaling pathway in lung tissue of sepsis-induced mice. (A) Representative western blots and quantitative analysis of phospho-Src. (B) Representative western blots and quantitative analysis of TLR4. Data are shown as Mean \pm SD, n=3. ^{##}P<0.01 vs. Sham group; ^{*}P<0.05 vs. CLP group.

Supplementary Files

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