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# 6-Gingerol attenuates sepsis-induced acute lung injury by suppressing NLRP3 inflammasome through Nrf2 activation

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**Abstract**

**Introduction.** Sepsis is characterized by an overactive inflammatory response. Acute lung injury (ALI) is the most common type of organ injury in sepsis, with high morbidity and mortality. 6-Gingerol is the main bioactive compound of ginger, and it possesses anti-inflammatory bioactivity in different diseases. This study is aimed to explore the specific function of 6-Gingerol in sepsis-induced ALI.

**Material and methods.** Lipopolysaccharide (LPS) was intraperitoneally injected into Sprague-Dawley rats for establishing the ALI models *in vivo*. The ALI rats were intraperitoneally injected with 20 mg/kg 6-Gingerol. The contents of oxidative stress markers malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) were detected in the lung tissues of ALI rats. The concentrations of inflammatory factors [Tumor Necrosis Factor alpha (TNF-α), interleukin (IL)-6, and IL-1β] were measured by ELISA. Inflammatory cell infiltration in bronchoalveolar lavage fluid (BALF) of rats was tested. Western blot was utilized to test the protein levels of nuclear factor erythroid 2-related factor (Nrf2) and heme oxygenase-1 (HO-1) in lung tissues. Furthermore, immunohistochemical staining was applied for testing the expression of NLRP3 inflammasome in lung tissues.

**Results.** The pathological changes in ALI rats were characterized by increased accumulation of inflammatory cells, alveolar hemorrhage, and pulmonary interstitial edema. However, the degree of pathological injury of lung tissues was significantly improved after 6-Gingerol treatment. Additionally, 6-Gingerol significantly attenuated the lung wet/dry ratio and protein permeability index (PPI) of LPS-induced rats. Furthermore, 6-Gingerol repressed oxidative stress and inflammatory reaction in LPS-induced rats by reducing the contents of MDA, GSH, SOD, TNF-α, IL-6, and IL-1β in the lung. LPS-induced infiltration of eosinophils, macrophages, neutrophils, and lymphocytes into lung was suppressed by 6-Gingerol administration. Moreover, 6-Gingerol activated Nrf2/HO-1 signaling and repressed LPS-induced-NLRP3 inflammasome expression in lung tissues of LPS-induced rats. Intraperitoneal injection of ML385 (Nrf2 inhibitor) treatment into rats reversed the effects of 6-Gingerol on lung injury, inflammation, and oxidative stress in LPS-subjected rats.

**Conclusions.** 6-Gingerol attenuates sepsis-induced ALI by suppressing NLRP3 inflammasome activation *via* stimulation of Nrf2. *(Folia Histochemica et Cytobiologica 2023, Vol. 61, No. 1, 68–80)*

**Keywords:** 6-Gingerol; acute lung injury; sepsis; NLRP3 inflammasome; Nrf2

## Introduction

Acute lung injury (ALI) is a severe multifactorial lung pathology with high incidence rate [1]. The clinical features of ALI include inflammatory cell infiltration, pulmonary edema, and arterial hypoxemia, which can damage the alveolar epithelium, thus weakening pulmonary function [2, 3]. Sepsis is a deadly syndrome characterized by the overactive systemic inflammatory response caused by the infection of bacteria, fungi, and viruses [4]. ALI is one of the most common complications of serious sepsis [5]. During the progression of sepsis-induced ALI, the overwhelming release of inflammatory factors leads to the disruption of alveolar epithelial cells, the increase of epithelial permeability, and the influx of edema fluid into the alveolar space [6,

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7]. However, at present, there is no effective treatment for ALI. Thus, it is essential to explore new approaches for the treatment of ALI.

Emerging studies have confirmed that inflammation plays a vital role in ALI pathogenesis [8, 9]. Lipopolysaccharide (LPS), a major biologically active component of the cell walls of Gram-negative bacteria, has been extensively utilized to establish the animal models of ALI [10, 11]. The LPS-induced animal model is similar to the pathological characteristics of ALI in humans [12]. For instance, NEAT1 downregulation represses LPS-induced ALI and inflammatory response by HMGB1-RAGE signaling [13]. Dehydrocostus lactone inhibits LPS-induced ALI and macrophage activation by modulating NF-κB pathway [14]. Furthermore, the NLRP3 inflammasome is a crucial signaling node that facilitates the maturation of proinflammatory factors such as IL-6 and IL-1β [15]. The NLRP3 inflammasome is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1β/ /IL-18 in response to microbial infection and cellular damage [16]. Some agents, *e.g.*, β-hydroxybutyrate can deactivate NLRP3 inflammasome to repress gout flares [17]. Glycine alleviates LPS-induced ALI by regulating NLRP3 inflammasome and Nrf2 signaling [18]. Thus, focusing on the potential targets of inflammatory processes is conducive to exploring new treatment strategies for ALI.

6-Gingerol is the main bioactive compound of ginger, and it has been confirmed to possess anti-inflammatory, anti-tumor, antioxidant and neuroprotective bioactivities [19–21]. Importantly, its protective effects on human diseases have also been confirmed by many studies [22, 23]. For example, 6-Gingerol inhibits sepsis-induced acute kidney injury by regulating methylsulfonylmethane and dimethylamine production [24]. 6-Gingerol exerts an anti-inflammatory effect and protective properties in LTA-induced mastitis [25]. Furthermore, 6-Gingerol has been recently reported to reduce pulmonary fibrosis by activating sirtuin 1 [26]. However, there are few studies on the protective effect of 6-Gingerol on pulmonary dysfunction caused by sepsis. The transcription factor nuclear factor erythrocyte-2 related factor 2 (Nrf2) is involved in the regulation of oxidative stress and inflammatory reaction [27]. Nrf2 translocates into the nucleus under oxidative stress and binds to antioxidant response elements, such as HO-1 [28]. The Nrf2/HO-1 axis can inhibit the activation of NLRP3 inflammasome in sepsis-induced ALI [29, 30]. Moreover, 6-Gingerol is reported to ameliorate sepsis-induced liver injury through the activation of Nrf2 [31]. However, whether 6-Gingerol regulates the progression of sepsis-induced ALI *via* Nrf2 signaling is unclear.

In this study, the main purpose was to explore the biological roles of 6-Gingerol in sepsis-induced ALI. We utilized LPS to establish the sepsis-induced ALI rat models and performed a series of assays. We hypothesized that 6-Gingerol could attenuate sepsis-induced ALI by suppressing NLRP3 inflammasome through Nrf2 activation, which may provide a novel therapeutic agent for ALI.

## Materials and methods

**Animal experiments.** A total of 32 male Sprague-Dawley rats (180–220 g) were obtained from Vital River Co. Ltd. (Beijing, China). The animal study was approved by the Ethics Committee of Wuhan Hospital of Traditional Chinese Medicine (Wuhan, China). All animals were maintained in cages under a specific pathogen-free (SPF) condition at 23°C with free access to food and water on a 12 h light/dark cycle.

The rats were divided into 4 groups (n = 8 each): control group, LPS group, LPS + 6-Gingerol (20 mg/kg) group, and LPS + 6-Gingerol + ML385 (30 mg/kg) group. Rats were intraperitoneally injected with 50 mg/kg sodium pentobarbital for anesthesia, and then they were subjected to intratracheal instillation of 5 mg/kg LPS in 50 $\mu$L PBS [32]. The control rats received an equal volume of PBS. After 30 min, rats in the LPS + 6-Gingerol group were intraperitoneally injected with 20 mg/kg 6-Gingerol dissolved in 0.5% 10 $\mu$L DMSO. Rats in the LPS + 6-Gingerol + ML385 group were intraperitoneally injected with 30 mg/kg ML385 [33] 30 min before LPS treatment followed by 20 mg/kg 6-Gingerol administration. After 24 h of LPS instillation, 100 mg/kg sodium pentobarbital in PBS was intraperitoneally injected into rats for euthanasia.

**Lung wet/dry (W/D) ratio analysis.** After the rats were euthanized, their lungs were harvested and weighed (W, wet) immediately. Then, the lung in each experimental group was put in an oven at 80°C for 24 h and weighed (D, dry). The lung W/D ratio was counted for evaluating the lung edema.

**BALF collection.** After rats were euthanized by an overdose of anesthesia, the right lungs were ligated. BALF was gathered by cannulating and lavaging the left lungs three times with 1.0 mL PBS. After centrifuging BALF for 10 min at 1500 rpm at 4°C, supernatants were collected and stored in a −80°C freezer until use. The right lower lobe of the lung was fixed in 10% formalin for histopathological analysis.

**Hematoxylin and eosin staining.** Lung tissue samples were fixed with 10% formalin and then embedded in paraffin. Next, tissues were cut into 5-$\mu$m-thick slices and stained with hematoxylin and eosin (H&E) according to the standard method [34]. After that, slices were dehydrated, sealed with a neutral gel, and observed by an optical light microscope (OLYMPUS IX51, Tokyo, Japan). Lung injury score was determined by


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4 categories: interstitial inflammation, neutrophil infiltration, edema, and congestion [35]. Those indexes were graded as follows: 0 means no injury; 1 means 25% injury; 2 means 50% injury; 3 means 75% injury; and 4 means 100% injury. Each specimen was analyzed in 10 randomly selected fields, and the severity of lung injury was evaluated by the average score.

**Immunohistochemical (IHC) analysis.** The paraffin-embedded lung tissues were cut into 5 $\mu$m-thick slices, deparaffinized in xylene, and rehydrated by placing in decreasing ethanol concentrations. Then, slices were placed in 0.01 mol/L citrate buffer for antigen retrieval and blocked with 5% bovine serum albumin. After that, slides were incubated with the primary antibody against NLRP3 (SC06-23, 1:200, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C overnight. Slices were then incubated with a secondary antibody (ab205718, Abcam, Cambridge, UK) for half an hour at room temperature. Next, DAB was used for color development for 5 min. Finally, a light microscope (OLYMPUS IX51) and Image-Pro Plus 6.0 software (National Institutes of Health, Bethesda, MD, USA) were utilized for analysis.

**Detection of lung protein permeability index (PPI).** Blood samples were obtained from the left ventricle and then subjected to centrifugation at 4° at 3500 rpm for 15 min. Then, the plasma was gathered for assays. Protein concentration in BALF supernatant and plasma was tested utilizing the Quick Start™ Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). The calculation formula is as follows: PPI (%) ¼ protein content in BALF/protein content in plasma ×100 [36].

**Detection of MDA, GSH, and SOD contents.** Lung tissues were homogenized in 0.3 g/mL (wet mass w/v) precooled 0.9% normal saline by a high-speed homogenizer (Heidolph, DIAX 900, Heidolph Instruments, Kelheim, Germany) five times for 10 seconds at 10,000 *g*. Following homogenization, the homogenates were subjected to centrifugation at 12000 *g* for 10 min at 4°. After that, the supernatant was collected for assays. MDA, GSH and SOD contents were detected in the supernatant by respective kits according to the manufacturer's instruction (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**ELISA.** The concentrations of IL-1β, TNF-α and IL-6 in tissue homogenates and BALF were estimated. The detection of absorbance values was calculated utilizing respective ELISA kits (MultiSciences Biotechnology, Hangzhou China). The absorbance at 450 nm was analyzed by a Multiskan MK3 microplate reader (Thermo Fisher Scientific, Inc.).

**Inflammatory cell counts.** Total cell count was measured in BALF utilizing a hemocytometer. Cell pellets were subjected to resuspension in saline and then centrifuged onto slides. After that, they were stained with Wright-Giemsa method for 8 min. The differential cell count was measured by counting a total of 200 cells/slide under a light microscope (Olympus).

**RT-qPCR.** Total RNAs were extracted from lung tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then, RNA was utilized for reverse transcription to synthesize cDNA using Reverse Transcription Kit (205313; Qiagen, Hilden, Germany). The qPCR was conducted using SYBR Green (Qiagen) according to the manufacturer's instruction on an ExicyclerTM 96 fluorescence quantitative assay system (Bioneer Corporation, Daejeon, Korea). Nrf2 and HO-1 expression at the mRNA level was calculated by the $2^{-\Delta\Delta Ct}$ method normalized to GAPDH. The sequences of primers used were as follows: Nrf2 forward, 5'-TCTGACTCCGGCATTTCACT-3'; Nrf2 reverse, 5'-TGTTGGCTGTGCTTTAGGTC-3'; .HO-1 forward, 5'-GCCACCAAGGAGGTACACAT-3'; HO-1 reverse, 5'-GGGGCATAGACTGGGTTCTG-3'; GAPDH forward, 5'-AACTCCCATTCTTCCACCT-3'; GAPDH reverse, 5'-TTGTCATACCAGGAAATGAGC-3'.

**Western blot.** Lung tissues were lysed in RIPA lysis buffer and the supernatant was collected by centrifugation at 7000 *g* for 10 min at 4°C. The protein concentrations were measured by BCA kit (Beyotime, Shanghai, China). Proteins were separated by 10% SDS–PAGE and transferred onto PVDF membranes (Millipore, MA, USA), followed by blocking with 5% skim milk for 1 h. Then, the membranes were incubated with primary antibodies (all from Abcam) against Nrf2 (ab92946, 1:1000), HO-1 (ab68477, 1:10000), NLRP3 (ab263899, 1:1000), ASC (ab180799, 1:1000), Caspase-1 (ab286125, 1:1000), and GAPDH (ab181602, 1:1000) as loading control overnight at 4°C. After that, membranes were rinsed and then incubated with secondary antibodies (ab205718, 1:2000) for 1 h. The bound antibodies were visualized with enhanced chemiluminescence (Advansta, Menlo Park, CA, USA). The relative densities of protein bands were analyzed by ImageJ (v. 8.0; National Institutes of Health).

**Statistical analyses.** Data are displayed as the means ± SD from three individual repeats. GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA) was applied for statistical analysis. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* analysis. $P < 0.05$ represented statistical significance.

## Results

### *6-Gingerol attenuates LPS-induced lung injury in the rat model*

To investigate the effects of 6-Gingerol in LPS-induced lung injury, we established the model of ALI rats by peritoneal injection of 5 mg/kg LPS. ML385 is the inhibitor of Nrf2, and because Nrf2 can regulate oxidative stress and inflammatory reaction in ALI [37], thus we also studied the effects of ML385 and 6-Gingerol in LPS-induced ALI. The groups of animals and time course of the experiment are shown in Fig. 1A. After 24 h of LPS administration, all rats were euthanized and lung tissue samples were isolated for experiments. Additionally, the pulmonary permeability index was increased by LPS, and 6-Gingerol attenuated this change, while ML385 treatment reversed the effect


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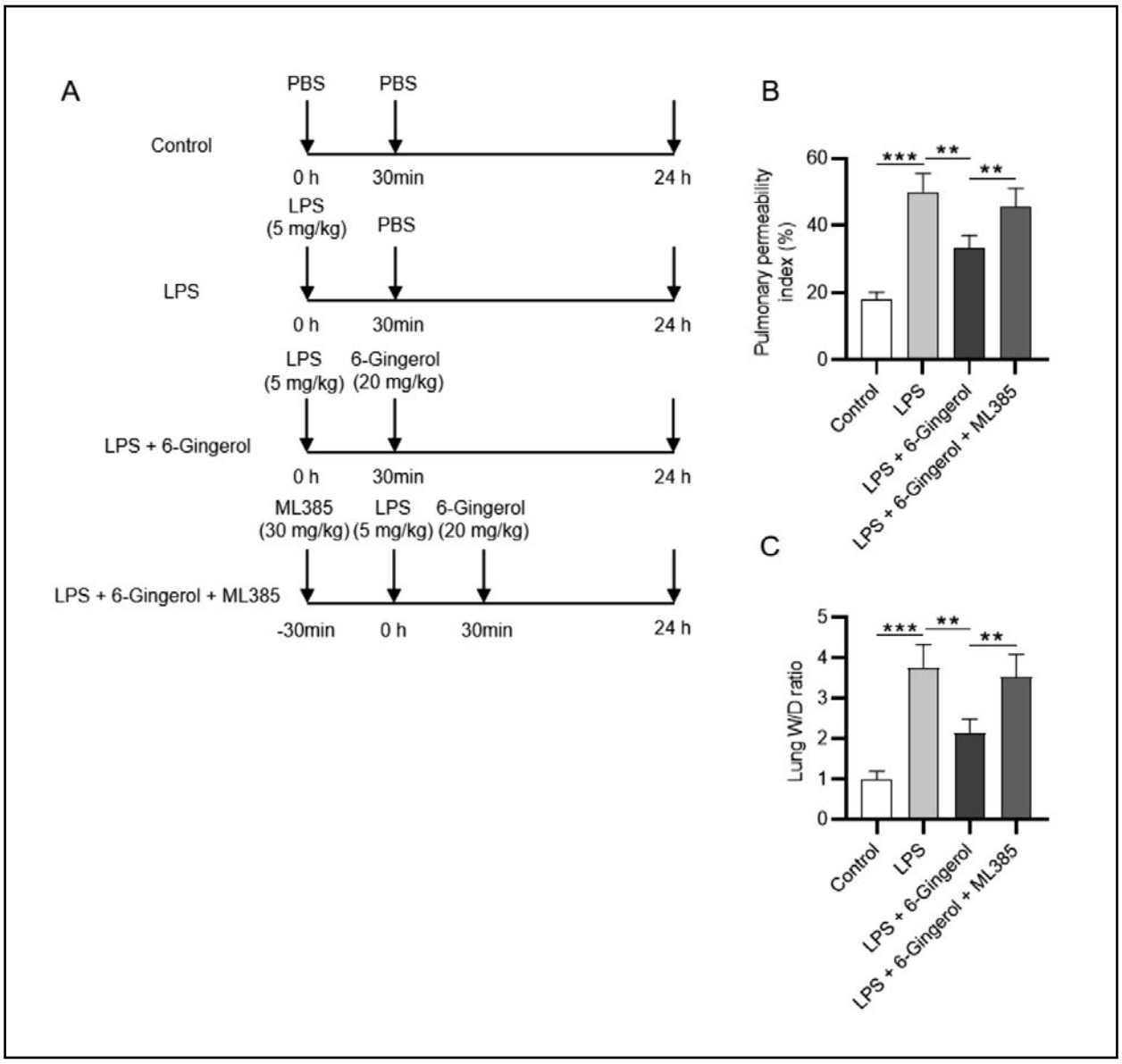


**Figure 1.** 6-Gingerol attenuates LPS-induced acute lung injury in rats. **A.** Rats were divided into four groups (the control group, the LPS group, the LPS + 6-Gingerol group, and the LPS + 6-gingerol + ML385 group). **B.** The detection of pulmonary permeability index. **C.** Lung W/D (wet/dry) ratio in different groups. $^{**}P < 0.01$, $^{***}P < 0.001$.

of 6-Gingerol (Fig. 1B). Lung wet/dry (W/D) weight ratio was determined to semi-quantitatively evaluate the extent of lung edema. We found the lung W/D ratio was elevated by LPS stimulation, while 6-Gingerol administration decreased the ratio. However, ML385 elevated the lung W/D ratio again (Fig. 1C). H&E staining was performed to analyze the histopathologic changes of lung tissues in different groups. The results indicated that the ALI scores in the LPS group were significantly higher than the control group and the pathological changes were characterized by increased accumulation of inflammatory cells, alveolar hemorrhage, and pulmonary interstitial edema. However, the degree of pathological injury of lung tissues and the ALI scores in LPS + 6-Gingerol groups were significantly improved and decreased. However, in the LPS + 6-Gingerol + ML385 group, we found the lung injury was aggravated again (Fig. 2A, B). Thus, we found that 6-Gingerol treatment alleviates LPS-induced lung injury in rats.

### *6-Gingerol represses oxidative stress and inflammatory reactions in LPS-induced ALI*

Oxidative stress and inflammatory reactions are the two main contributing factors for ALI [38], and therefore we determined their indices. The contents of oxidative stress markers (MDA, GSH, and SOD) in the lung tissues of rats were detected. We found


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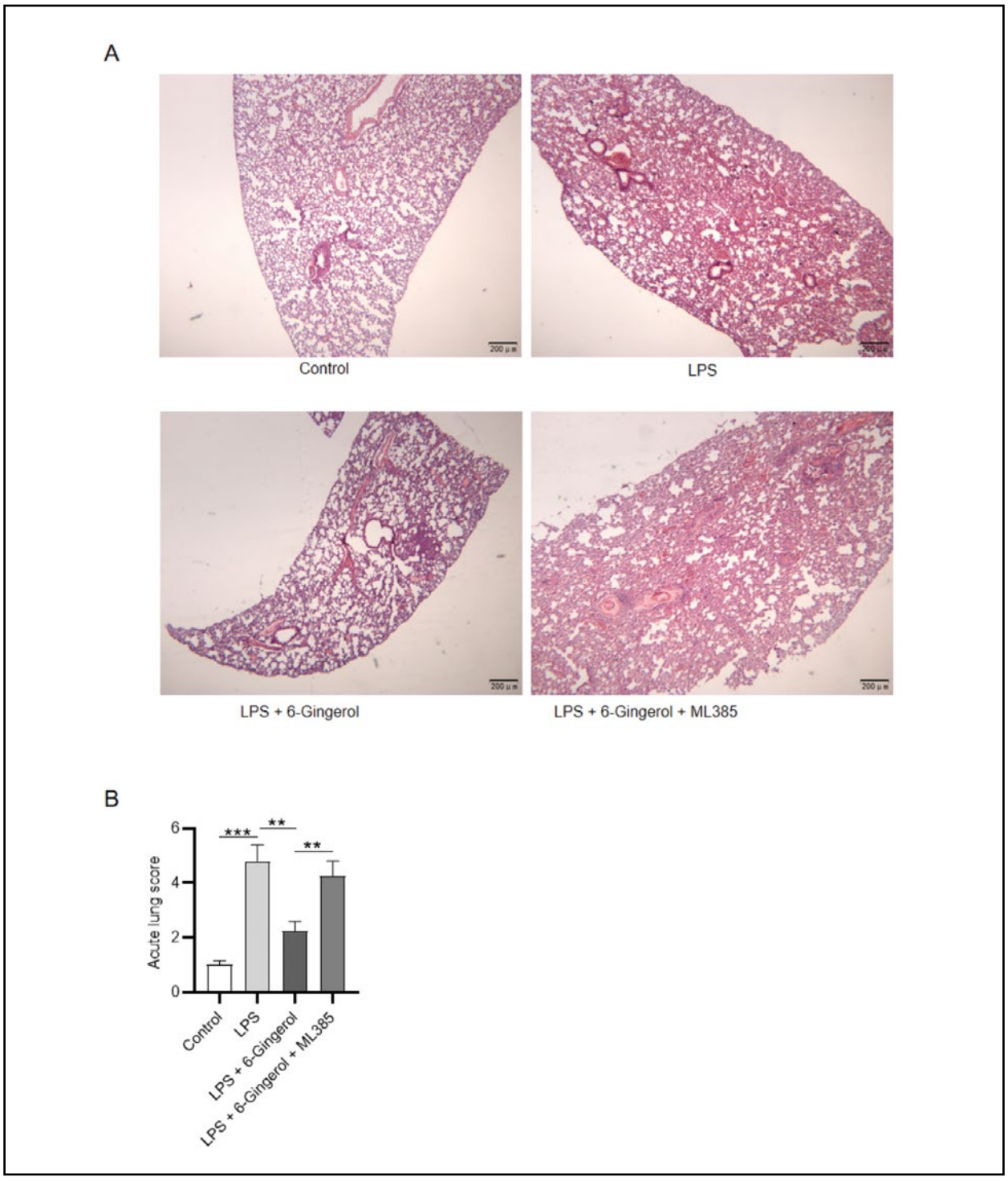


**Figure 2.** Pathological changes in the lung tissues. **A.** H&E staining was utilized to assess the histopathological changes of lungs in different groups. **B.** The detection of lung injury score in different groups. $^{**}P < 0.01$, $^{***}P < 0.001$.

that MDA content was elevated in the lung tissues of LPS-treated rats. However, 6-Gingerol treatment decreased its content, which was then increased by ML385 (Fig. 3A). On the contrary, the contents of GSH and SOD in lung tissues were reduced in LPS-induced rats and increased by 6-Gingerol treatment. However, pretreatment with ML385 counteracted the effect of 6-Gingerol (Fig. 3B, C). Then, ELISA was utilized to evaluate the contents of inflammatory factors (TNF-α, IL-6, and IL-1β) in lung tissues and BALF of rats in different groups. The results showed that, compared with the control group rats, the contents of TNF-α, IL-


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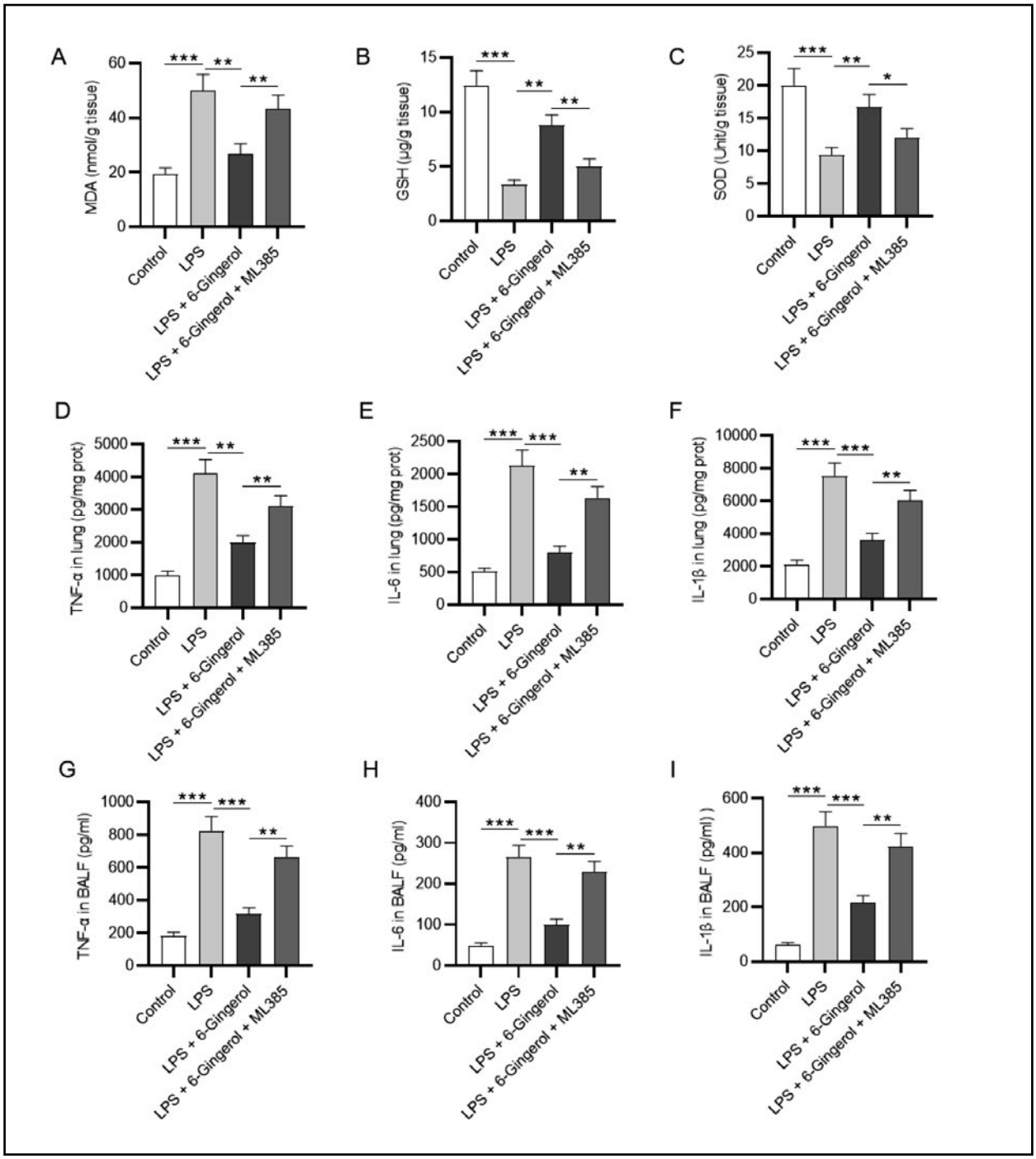


**Figure 3.** 6-Gingerol represses oxidative stress and inflammatory reaction in LPS-induced acute lung injury. **A–C.** The contents of MDA, GSH, SOD in lung tissues of rats in different groups were tested by their corresponding kits. D–I. The contents of TNF-α, IL-6, IL-1β in lung tissues and BALF of rats in different groups were detected by ELISA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

6, and IL-1β in lung tissues and BALF of LPS-treated rats were significantly increased, while their contents were decreased by 6-Gingerol treatment. However, ML385 treatment promoted their contents again (Fig. 3D–I). Thus, these data suggested that 6-Gingerol represses oxidative stress and inflammatory reaction in LPS-induced rats.

### *6-Gingerol attenuates inflammatory cell infiltration in LPS-induced ALI*

As shown in Fig. 4A–F, LPS markedly promoted the infiltration of inflammatory cells into the lung tissues as there was an elevation in the differential and total cell counts in BALF compared with the control group. Then, 6-Gingerol administration reduced the counts of


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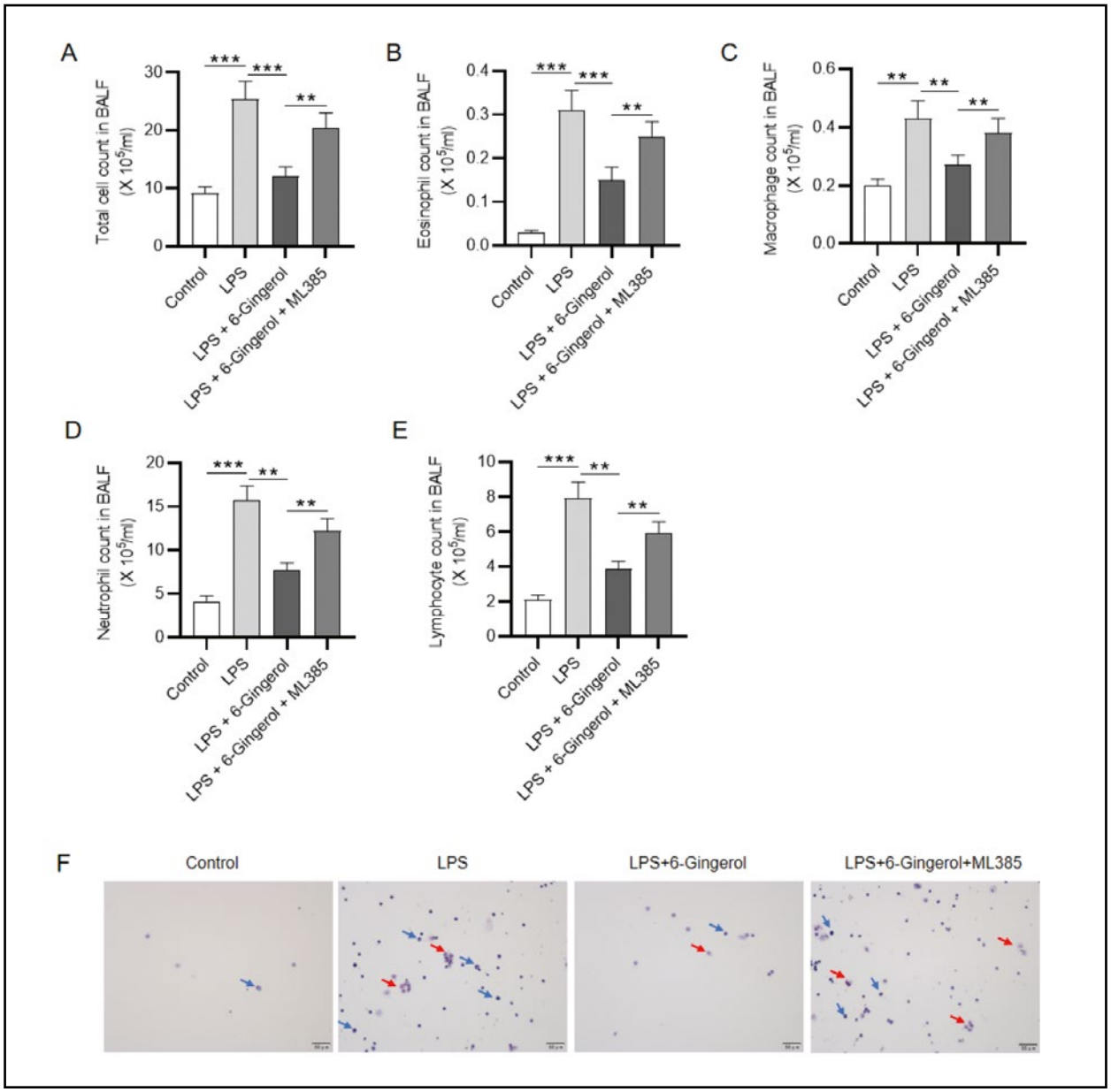


**Figure 4.** 6-Gingerol attenuates infiltration of inflammatory cells in lungs of LPS-treated rats. **A–F.** The counts of total cells, eosinophil, macrophage, neutrophil, and lymphocyte in BALF of rats in different groups were measured using the Wright-Giemsa stained cells. $^{**}P < 0.01$, $^{***}P < 0.001$.

eosinophil, macrophage, neutrophil, and lymphocyte, while ML385 treatment could offset 6-Gingerol effect.

### *6-Gingerol activates Nrf2 in the lungs of LPS-induced ALI*

Since our experiments have proved that ML385 (Nrf2 inhibitor) counteracted the protective effect of 6-Gingerol against oxidative stress and inflammatory response in the lungs of LPS-induced rats, we decided to find out whether Nrf2 and HO-1 could be activated in lungs of ALI rats. Results of RT-qPCR illustrated that the mRNA and protein levels of Nrf2 and HO-1 were reduced in the lung tissues of LPS-treated rats. After the treatment with 6-gingerol, both Nrf2 and HO-1 levels recovered to the level found in the control group. However, ML385 treatment decreased their mRNA and protein levels again (Fig. 5A–C). Thus, we showed that 6-Gingerol activates Nrf2/HO-1 axis in the lungs of LPS-induced rats.

### *6-Gingerol represses NLRP3 inflammasome in lungs of LPS induced-rats by activating Nrf2*

Finally, we detected the effect of 6-Gingerol on NLRP3 inflammasome expression in the lungs of

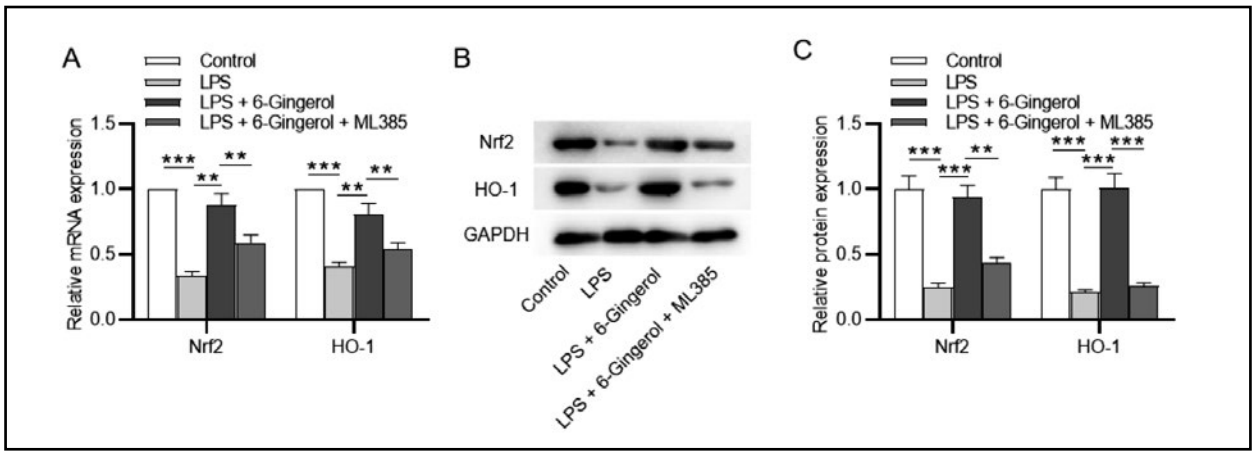


**Figure 5.** 6-Gingerol activates Nrf2 in LPS-induced acute lung injury. **A–C.** RT-qPCR and western blot were utilized to the mRNA and protein levels of Nrf2 and HO-1 in lung tissues of rats of the control group, the LPS group, the LPS + 6-Gingerol group, and the LPS + 6-gingerol + ML385 group. $^{**}P < 0.01$, $^{***}P < 0.001$.

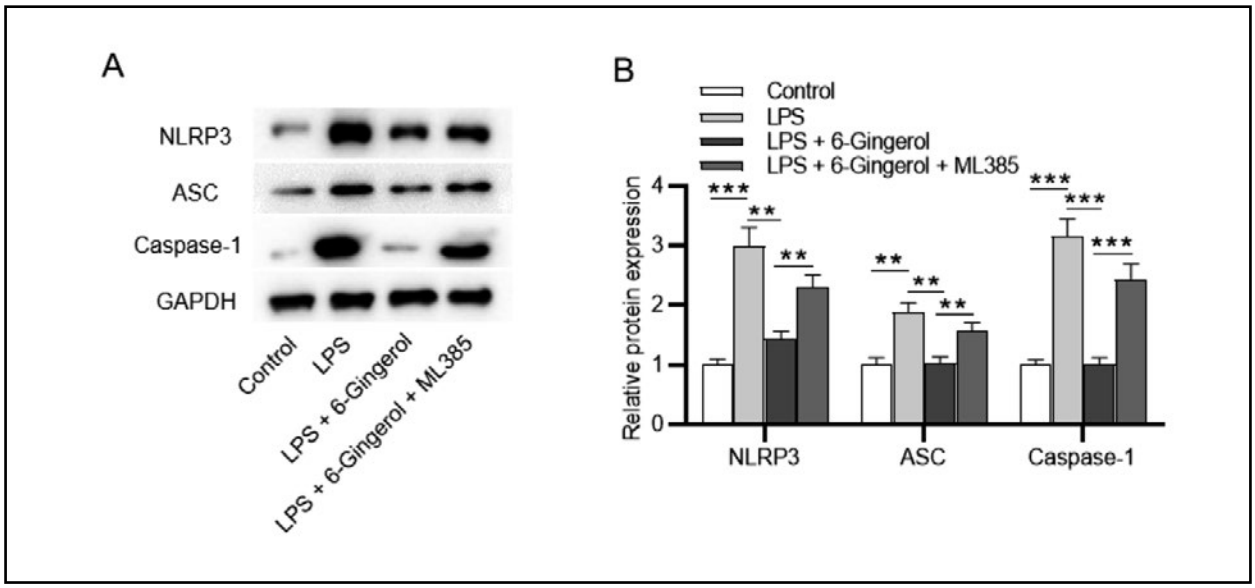


**Figure 6.** 6-Gingerol represses NLRP3 inflammasome by activating Nrf2. **A–B.** Western blot was applied for detecting NLRP3, ASC, and caspase-1 levels in lung tissues of rats of the control group, the LPS group, the LPS + 6-Gingerol group, and the LPS + 6-gingerol + ML385 group. $^{**}P < 0.01$, $^{***}P < 0.001$.

LPS-treated ALI rats. Western blot was utilized for measuring the alterations of main components for NLRP3 inflammasome. The results showed that NLRP3, Apoptosis-associated Speck-like protein containing a Caspase-recruitment domain (ASC), and caspase-1 levels induced by LPS administration in lung tissues of rats were reduced by 6-gingerol treatment, while they were increased in the LPS + 6-Gingerol + ML385 group (Fig. 6A, B). Immunohistochemical staining further indicated that NLRP3 expression was increased in lung tissues of the LPS group and decreased in the LPS + 6-Gingerol group. In the LPS + 6-Gingerol + ML385 group, NLRP3 expression was recovered to the level of the control group (Fig. 7A, B). Thus, these results suggested that 6-Gingerol represses NLRP3 inflammasome expression in LPS-treated rats by activating Nrf2.

## Discussion

Sepsis has become a major etiology of ALI [39]. It is reported that the patients with sepsis-induced ALI had higher illness severity and mortality rates than the patients with non-sepsis-induced ALI [40]. Thus, it is urgent to find an effective treatment for sepsis--induced ALI. 6-Gingerol is one of the main bioactive compounds of ginger, and it has been confirmed as a potential therapeutic agent in different diseases due


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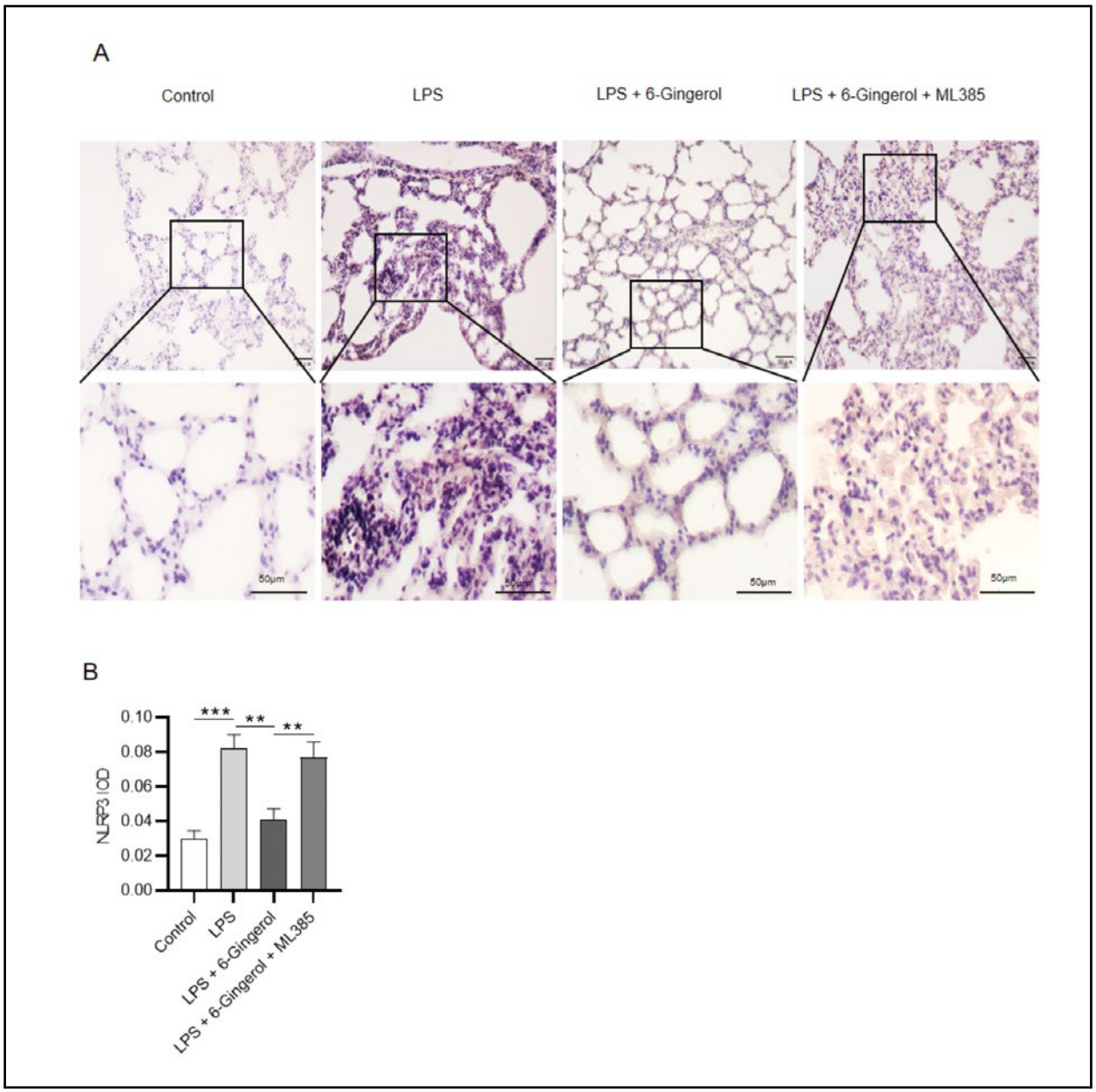


**Figure 7.** Immunohistochemical staining of NLRP3 in the lung. **A.** Immunohistochemical staining was utilized to estimate NLRP3 expression in lung tissues of different groups of rats. **B.** Quantification of NLRP3 expression. $^{**}P < 0.01$, $^{***}P < 0.001$.

to its effects against inflammation and oxidative stress [41–43]. Thus, we examined the specific function of 6-Gingerol in ALI. In this study, we utilized LPS to establish the ALI rat model. Through histopathological analysis, we found that the lungs of rats treated with LPS showed infiltration of inflammatory cells into the alveolar space, peribronchial wall thickening, and vascular congestion. After 6-Gingerol treatment, these pathological alterations were significantly alleviated. Previously, 6-Gingerol was reported to attenuate ventilator-induced lung injury by modulating the PPARγ/NF-κB signaling pathway in rats [44]. Similarly, our study also confirmed the protective effect of 6-Gingerol against LPS-induced lung injury in rats.

Inflammatory mechanism exerts vital function to eliminate pathogens from human bodies, while the excessive release of inflammatory cytokines may cause tissue injury [45]. The migration and activation of neutrophils is the earliest response to ALI, resulting in capillary permeability and edema [7]. Neutrophils promote inflammatory and immune reactions by activating the production of proinflammatory cytokines, chemokines, and metalloproteinases [46]. It has been confirmed that the severity of ALI is associated with


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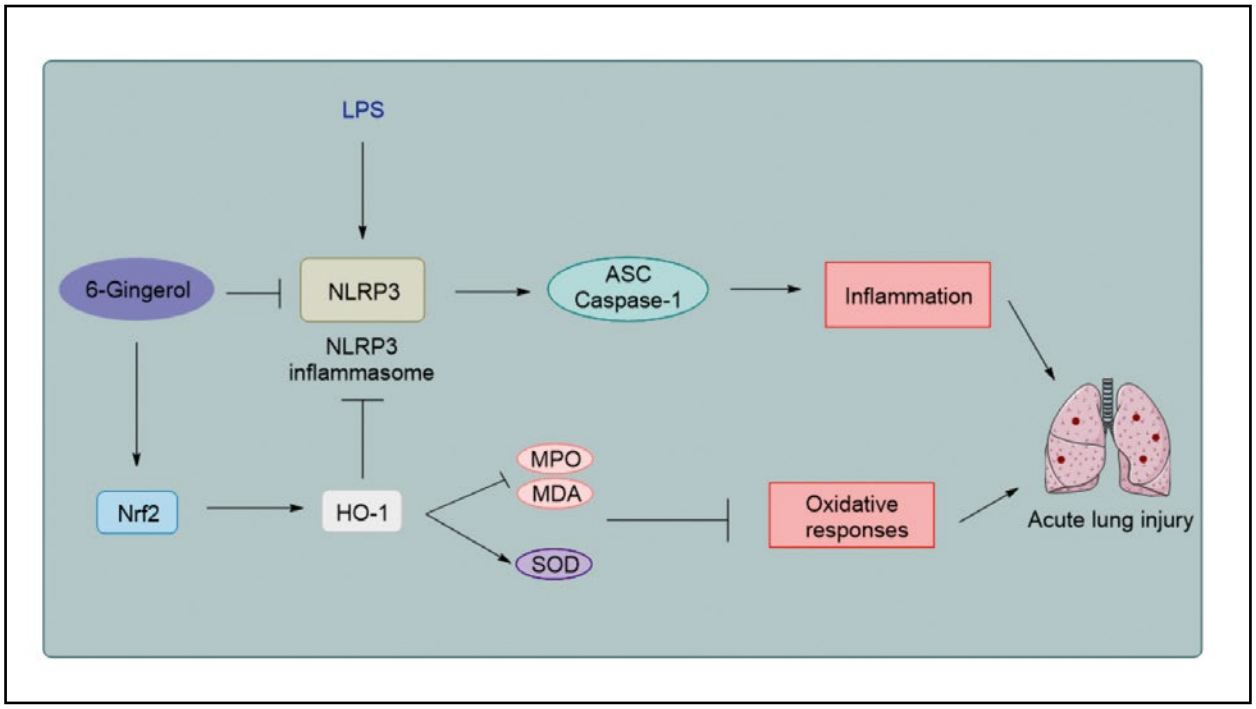


**Figure 8.** Schematic overview of 6-Gingerol regulating LPS-induced acute lung injury (ALI). 6-Gingerol administration repressed LPS-induced activation of NLRP3 inflammasome, thereby repressing inflammation in ALI. Meanwhile, 6-Gingerol activates the Nrf2/HO-1 signal axis to repress NLRP3 inflammasome, the activities of MPO and MAD, and promote SOD activity,which results in the repression of oxidative responses in ALI.

the number of inflammatory cells in BALF [47, 48]. In this study, we found the contents of proinflammatory factors (TNF-α, IL-6, and IL-1β) in lung tissues and BALF of LPS-treated rats were significantly increased, while their contents were decreased by 6-Gingerol addition. Furthermore, 6-Gingerol treatment could reduce the counts of inflammatory cells (eosinophil, macrophage, neutrophil, and lymphocyte) in BALF. These findings suggested that 6-Gingerol repressed inflammatory reactions in LPS-induced ALI. Additionally, the NLRP3 inflammasome also plays a crucial role in the process of inflammatory reactions [49, 50]. The NLRP3 inflammasome is the core of inflammatory response, and it can modulate caspase-1 activation and promote the secretion of cytokine precursors pro-IL-1β, thereby causing an inflammatory response [51]. In this study, we observed that NLRP3, ASC, and caspase-1 levels induced by LPS were reduced by 6-Gingerol administration. It has been reported that 6-Gingerol represses the release of TNF-α and IL-6 in LPS-induced astroglioma cells [52]. Also, 6-Gingerol suppresses cerebral ischemia/reperfusion injury by repressing NLRP3 inflammasome [53]. 6-Gingerol relieves renal damage in streptozotocin-induced diabetic rats by regulating oxidative stress and inflammation [54]. These studies further support our findings that 6-Gingerol alleviated ALI by inhibiting inflammatory reaction *via* NLRP3 inflammasome.

Oxidative stress also plays a key role in ALI development [38, 55]. ROS attack different organs, leading to lipid peroxidation, mutations in the DNA matations, and protein inactivation [56]. ROS overproduction exacerbates the development of pulmonary edema and infiltration of inflammatory cells [57]. In this study, we found that the content of the lipid peroxidative marker MDA was elevated in lung tissues of LPS-treated rats, while 6-Gingerol treatment decreased its content. The contents of antioxidants GSH and SOD reduced by LPS stimulation were increased by 6-Gingerol treatment. These results proved the antioxidant activity of 6-Gingerol against LPS-induced ALI. Previously, 6-Gingerol is reported to repress liver injury by inhibiting oxidative stress [58]. 6-Gingerol relieves colonic injury via repressing oxidative stress in mice [59].

The Nrf2 is responsible for the regulation of the level of antioxidant proteins, such as HO-1 [60, 61]. It has been confirmed to be involved in regulating the progression of lung injury [37]. Accumulating evidence has confirmed that the Nrf2/HO-1 axis participates in the progression of the different diseases [62–64]. For example, suppression of the Nrf2/HO-1 axis causes the increased activation of NLRP3 inflammasome in osteoarthritis [65]. Furthermore, etomidate relieves


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hyperoxia-induced ALI in mice by modulating the Nrf2/HO-1 axis [66]. In this study, we observed that Nrf2 and HO-1 levels that were reduced in lung tissues of LPS-induced rats were recovered by 6-Gingerol administration. Moreover, we also found that ML385 (Nrf2 inhibitor) could counteract the protective effect of 6-Gingerol against oxidative stress and inflammatory response in the lungs of LPS-induced rats. Previously, 6-Gingerol was demonstrated to repress sepsis-induced liver injury by activating Nrf2 pathway [31]. In this study, we confirmed that 6-Gingerol activated Nrf2/HO-1 axis in LPS-induced ALI rats for the first time.

Overall, this study demonstrates that 6-Gingerol attenuates sepsis-induced ALI by suppressing oxidative stress and inflammatory reaction by inhibiting NLRP3 inflammasome *via* the Nrf2/HO-1 axis (Fig. 6). These findings may provide a new therapeutic strategy for ALI. However, the limitation of this paper is that there is no in-depth study on the molecular mechanism of how 6-Gingerol regulates the Nrf2/HO-1 axis, which will become the focus of our further research.

## Conflict of interests

No conflicts of financial interest are enclosed in this study.

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