International Immunopharmacology 66 (2019) 169–176

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Chicoric acid alleviates lipopolysaccharide-induced acute lung injury in mice through anti-inflammatory and anti-oxidant activities

Hui Ding[a], Xinxin Ci[b], Hang Cheng[b], Qinlei Yu[a], Dan Li[a,*]

[a] Department of Respiratory Medicine, The First Hospital of Jilin University, Changchun 130061, China
[b] Department of Translational Medicine, The First Hospital of Jilin University, Changchun 130061, China

ARTICLE INFO

Keywords:
Chicoric acid (CA)
LPS
Inflammation
Oxidative stress
Acute lung injury

ABSTRACT

Acute lung injury (ALI) is a severe clinical disease with high mortality rates. Chicoric acid (CA), an active component extracted from traditional Chinese medicine, was suggested to have anti-inflammatory and anti-oxidant activities. Inflammation and oxidative damage are implicated in the pathogenesis of ALI. In this study, we explored the protection effect of CA on LPS-induced ALI, and further discussed the possible molecular mechanisms. The results showed that CA could significantly improve the histological changes of LPS-induced acute lung injury. In addition, CA not only decreased LPS-stimulated protein leakage and lung wet/dry ratio but also reduced inflammatory cell infiltration, myeloperoxidase (MPO) activity and the generation of pro-inflammatory cytokines in bronchoalveolar lavage fluid (BALF). Meanwhile, CA lessened the reactive oxygen species (ROS) generation, and malondialdehyde (MDA) formation, and decreased glutathione (GSH) and superoxide dismutase (SOD) depletion, which were caused by LPS challenge. Furthermore, CA dramatically inhibited LPS-stimulated MAPK and NLRP3 activation and increased the expression of NAD (P) H: quinone oxidoreductase (NQO1), and dismutase (SOD), glutamate-cysteine ligase catalytic/modifier (GCLC/GCLM) subunit and heme oxygenase-1 (HO-1), as well as its upstream genes nuclear factor-erythroid 2-related factor 2 (Nrf2), which might be central to the protective effects of CA. In conclusion, these data indicated that the protective effects and mechanisms of CA on LPS-induced ALI and provided new insights for its application.

1. Introduction

Acute lung injury (ALI) is a clinically common disease with a high rate of mortality. ALI can cause neutrophil infiltration, pro-inflammatory cytokines generation, lung epithelium and endothelium injuries, which lead to the pulmonary edema and the impairment of gas exchange [1]. Although recent a series of intense studies have been made to understand the mechanisms of ALI, there are still no effective measures or specific drugs to treat the disease [2,3]. Furthermore, oxidative stress and inflammatory plays a crucial role in the pathogenesis of ALI [4]. Lipopolysaccharide (LPS) is a gram-negative bacterial, which can result in a series of inflammatory reactions and promote the production of reactive oxygen species (ROS). Therefore, LPS is the ideal inducer of acute lung injury model [5].

It has been reported that the release of inflammatory mediators, such as interleukin (IL)-1β, tumor interleukin-6 (IL-6) and necrosis factor-α (TNF-α) played an important role in the pathogenesis of LPS-induced ALI, and the inflammatory factors can be modulated by three mitogen-activated protein kinase (MAPK) pathways, including the c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 pathways [6,7]. In addition, ROS induced oxidative stress, as a participant in the pathogenesis of ALI, can stimulate various signaling pathways such as MAPK. Therefore, inhibition of MAPKs signaling pathway can suppress inflammation responses and oxidative stress, and then improve LPS-induced ALI. Moreover, accumulating evidence has showed that nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3) inflammasome plays a key role in the pathogenesis of ALI [8]. Studies indicated that NLRP3 inflammasome pathway consists of caspase-1, NOD like receptor (NLRP3) and the adaptor protein ASC [9]. The activation of NLRP3 inflammasome was responsible for the maturation and secretion of IL-1β, which could promote inflammatory responses [10]. A recent study showed that ROS scavenger could suppress NLRP3 activation, indicating that the presence of oxidative stress contributes to abnormal inflammation [11]. Therefore, the antioxidants alleviate LPS-induced ALI through NLRP3 inhibition. Moreover, previous abundant reports have noted that nuclear factor erythroid-2 related factor 2 (Nrf2), could combat oxidative damage through regulating of various cytoprotective enzymes,

* Corresponding author at: Department of Respiratory Medicine, The First Hospital of Jilin University, Xinmin road 71#, Changchun 130061, China.
E-mail address: lisa080205@163.com (D. Li).

https://doi.org/10.1016/j.intimp.2018.10.042
Received 28 July 2018; Received in revised form 16 October 2018; Accepted 30 October 2018
Available online 19 November 2018
1567-5769/

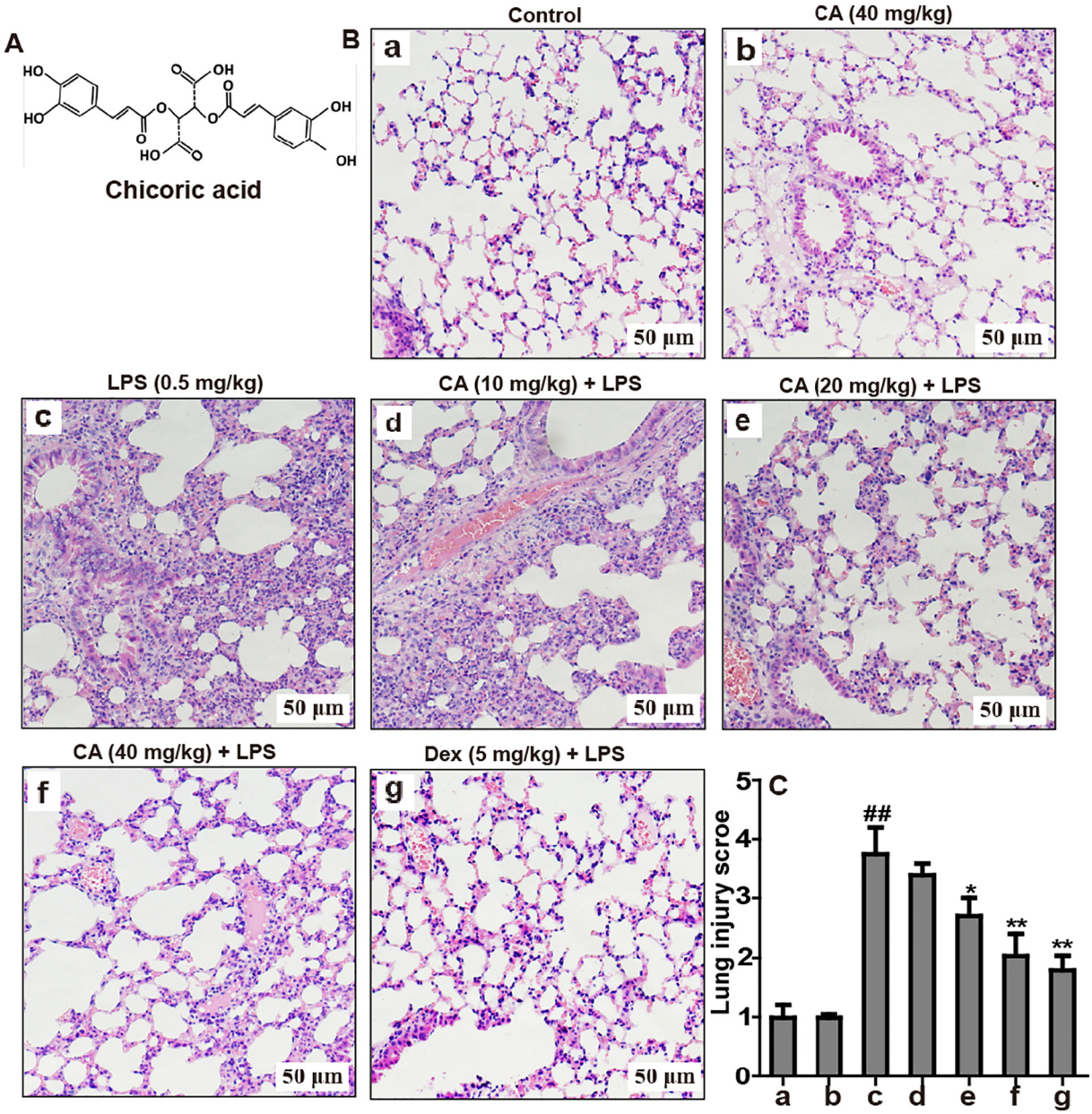

Fig. 1. CA treatment ameliorates the histology of lung in LPS-induced ALI mice. (A) Chemical structure of chicoric acid (CA). (B) Representative histological changes obtained from different groups of mice. a: Control group, b: CA only group (40 mg/kg), c: LPS group (0.5 mg/kg), d: LPS + CA (10 mg/kg) group, e: LPS + CA (20 mg/kg) group, f: LPS + CA (40 mg/kg) group, g: LPS + Dex (5 mg/kg) group (hematoxylin and eosin staining, magnification 200 ×). (C) Lung injury score. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the LPS group.

including NAD (P) H: quinone oxidoreductase (NQO1), dismutase (SOD), glutamate-cysteine ligase catalytic/modifier (GCLC/GCLM) subunit and heme oxygenase-1 (HO-1) [12]. Nrf2 and its downstream antioxidant genes have been recognized to playing an important role in the attenuation of LPS-induced ALI against oxidative stress and inflammatory [13]. In addition, many compounds could suppress LPS-induced ALI by anti-inflammatory and anti-oxidative effects.

Among polyphenols, various caffeyl polyphenol molecules including caffeic acid and chlorogenic acid have antioxidant activity [14,15]. Chicoric acid (CA, Fig. 1A) is one phenolic acid which is obtained from chicory and the echinacea (*purple coneflower*) plant (*Echinacea purpurea*). Additionally, CA is reported to be a daily nutraceutical that enhanced antioxidant activity [16]. Landmann et al. revealed that CA was enabled to reduce acute alcohol-induced hepatic steatosis in mice through suppressing oxidative stress and its anti-inflammatory activity [17]. However, there is still no evidence to explain the protection and mechanism of CA against LPS-induced ALI. In the present study, we evaluated the potential of CA to prevent LPS-induced ALI mice and the possible mechanisms of their effects.

2. Materials and methods

2.1. Reagents and chemical

Chicoric acid (CA), purity > 98%, was supplied by Chengdu Herbpurify CO., LTD (Chengdu, China). Dimethylsulfoxide (DMSO) and LPS (*Escherichia coli* 055:B5) were purchased from Sigma Chemical Co (St. Louis, MO, USA). Mouse TNF-α, IL-6, and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were provided by Biolegend (San Diego, CA, USA). Glutathione (GSH), ROS, myeloperoxidase (MPO) and malondialdehyde (MDA) determination kit were purchased from the Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China).

Antibodies against NLRP3, ASC, caspase-1, IL-1β, Nrf2, HO-1 and β-actin were obtained from Cell Signaling (Boston, MA, USA). The horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG was purchased from protein-tech (Boston, MA, USA). All other chemicals, unless specifically stated elsewhere, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals and treatment

Male BALB/c mice (18–20 g), 6–8 weeks old, were purchased from Liaoning Changsheng Technology Industrial Co., LTD (Certificate SCXK2010-0001; Liaoning, China). All animals were housed in certified standard laboratory cages and experiments were conducted prior to use, feeding food and water ad libitum. All studies were conducted in accordance with the International Guiding Principles for Animal Biomedical Research published by the Council of International Medical Scientific Organizations.

To induce ALI model, all mice were randomly assigned to five groups: control (saline), CA only (40 mg/kg), LPS only (0.5 mg/kg, dissolved in saline), LPS (0.5 mg/kg) + CA (40 mg/kg), LPS (0.5 mg/kg) + CA (20 mg/kg), LPS (0.5 mg/kg) + CA (10 mg/kg), and LPS (0.5 mg/kg) + dexamethasone (Dex, 5 mg/kg dissolved in saline, as positive control). One hour after intraperitoneal injection of CA (10, 20 or 40 mg/kg), the mice received an intranasal injection of LPS. After LPS challenge 12 h, mice were euthanized and bronchoalveolar lavage fluid (BALF) and lung tissue were collected for testing.

2.3. Histological evaluation

After 12 h of LPS challenge, the lung tissues for histological evaluation were not used for the collection of BALF. Lower lobe from left lungs was removed and fixed in 4% formalin, dehydrated with ethanol, paraffin-embedded and observed by HE staining. The lung injury score was graded on a scale of 0 to 4 as follows: 0, l, 2, 3, and 4, representing normal, mild damage, moderate damage, severe damage, and very intense damage, respectively [18].

2.4. Protein concentration assay in BALF

12 h after administration of LPS, 0.5 ml PBS was injected into the trachea and gentle aspiration for 3 times to obtain BALF. A small portion of BALF was used to determine the total protein concentration using BCA (Bicinchoninic acid) method.

2.5. Lung wet/dry (W/D) ratios

The right lung was taken 12 h after LPS administration. The lungs were separated and the wet weight was determined. For dry weight measurements, the lungs were incubated at 80 °C for 48 h. The ratio of wet lung weight to dry lung weight is used to assess the edema of the lungs [19].

2.6. Inflammatory cell counting in BALF

12 h after LPS treatment, BALF was obtained after the mice were sacrificed. The BALF was centrifuged to obtain pelleted cells, and the pelleted cells were re-suspended in PBS and total cell, neutrophils and macrophage counts were performed using a hemocytometer. Wright-Giemsa staining was used for cytosine staining.

2.7. Measure of ROS contents in BALF

The level of ROS in BALF was determined using test kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's kit protocol.

2.8. GSH, SOD, MPO and MDA detection [20]

All of the mice were sacrificed 12 h after LPS treatment, and the mouse lung tissues were homogenized and dissolved in extraction buffer to analyze the GSH, MPO and MDA levels according to the manufacturer's instructions.

2.9. ELISA assay

The levels of TNF-α, IL-1β, and IL-6 in BALF were quantified using a commercially available ELISA kit according to the manufacturer's instructions. The absorbance of each well was read at 450 nm with a microplate reader.

2.10. Western blot analysis

After 12 h of LPS treatment, the mice were sacrificed and lung tissue was taken for western blot. Different groups of lung tissue were lysed using RIPA with protease and phosphatase inhibitors for 30 min. And then centrifuged, the supernatants were collected. The protein concentrations were determined using a BCA protein assay kit (Beyotime, China). Protein extracts were separated by 10% SDS-PAGE and then electrotransferred to PVDF membranes. The membrane was blocked with 5% blocking solution (5% (w/v) nonfat dry milk) for 2 h and then blocked overnight with the primary antibody. The next day, the membrane was washed three times with PBST, followed by incubated for 1 h with HRP-conjugated secondary antibody. Finally, the bands were determined using the ECL western blotting system according to the manufacturer's instruction.

2.11. Statistical analysis

The data have been analyzed using SPSS19.0 (IBM). Additionally, comparisons between experimental groups were conducted using one-way ANOVA, while multiple comparisons were made using the LSD method. When $p < 0.05$ or $p < 0.01$, statistical significance was accepted.

3. Results

3.1. Effects of CA treatment on histological changes in LPS-challenged mice

To determine the effects of CA at different concentrations on histological changes in LPS-challenged mice, lower lobe of left lung was taken at 12 h after LPS challenge. Compared with the control group, LPS caused significant inflammatory cell infiltration and alveolar hemorrhage (Fig. 1B). Pretreatment with CA (20 or 40 mg/kg) and Dex (5 mg/kg, as positive control) significantly attenuated such pathological changes induced by LPS. However, pretreatment with CA (10 mg/kg) did not exhibit this effect. And the changes was also assessed by the lung injury score (Fig. 1C). Thus, CA could significantly improve the pathological changes induced by LPS.

3.2. Effect of CA treatment on permeability and lung water content in LPS-induced mice

The ratio of wet lung weight to dry lung weight is used to assess the edema of the lungs. As shown in Fig. 2A, LPS dramatically increased the wet to dry ratio compared with control group, whereas CA and Dex reduced the change. Total protein concentration change in BALF is a character of capillary permeability increase. LPS exposure markedly increased protein concentration in BALF (Fig. 2B). CA markedly decreased LPS-induced changes.

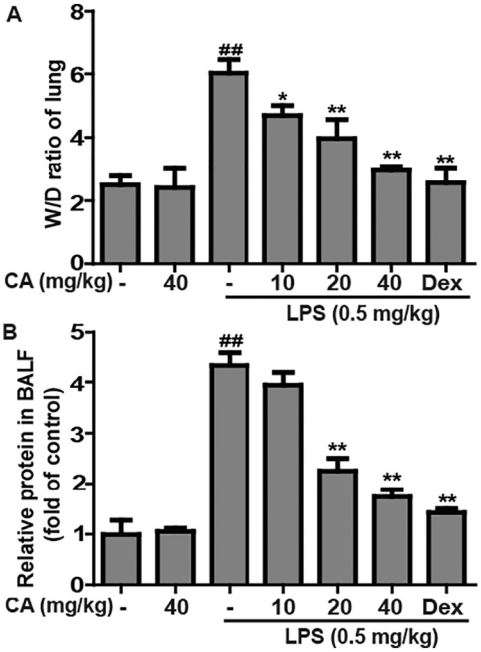

Fig. 2. CA treatment improves LPS-induced protein leakage and lung W/D ratio in BALF. (A) Effects of CA on LPS-induced lung W/D ratio of LPS-induced ALI. (B) Effects of CA on LPS-induced protein leakage in BALF. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{**}p < 0.01$ vs the LPS group.

3.3. Effects of CA treatment on inflammatory cell count in the BALF

MPO is a heme-containing enzyme. MPO and MPO-derived oxidants can surely result in tissue damages. In our results, CA could significantly inhibit the increase of LPS-induced MPO levels (Fig. 3A). To further verify the effect of CA on pulmonary inflammation, the total and different inflammatory cells in BALF were measured. Our results indicated LPS significantly raised the levels of total cells, neutrophils and macrophages in BALF. CA pretreatment markedly reduced the infiltration of total cells, neutrophils and macrophages (Fig. 3B–D).

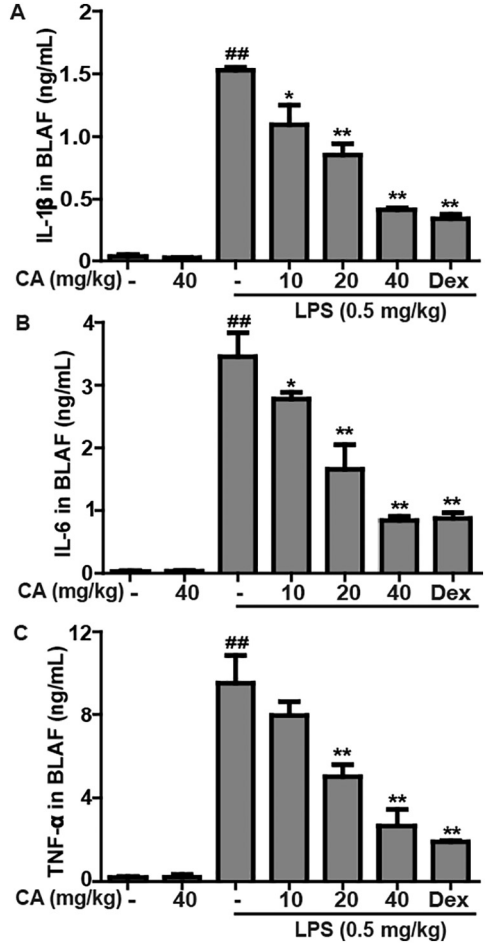

Fig. 4. CA treatment decreased LPS-induced pro-inflammatory cytokines secretion in BALF. BALF supernatant was collected to detect the level of pro-inflammatory cytokines by ELISA. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{**}p < 0.01$ vs the LPS group.

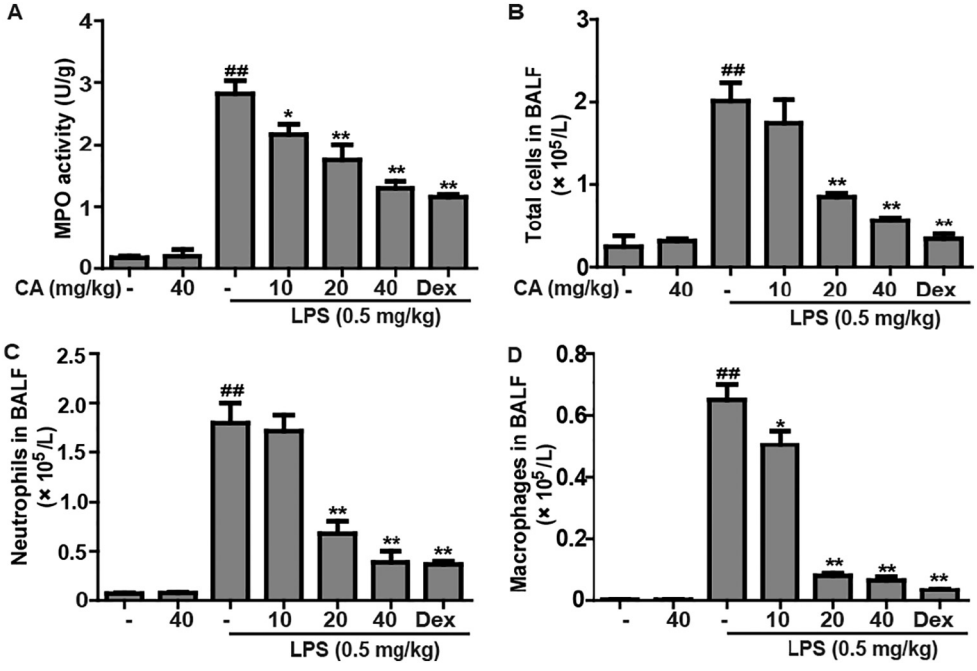

Fig. 3. CA treatment improves LPS-induced inflammatory cell infiltration. (A) MPO activation. (B) The numbers of total cells in BALF. (C) The numbers of neutrophils in BALF. (D) The numbers of macrophages in BALF. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the LPS group.

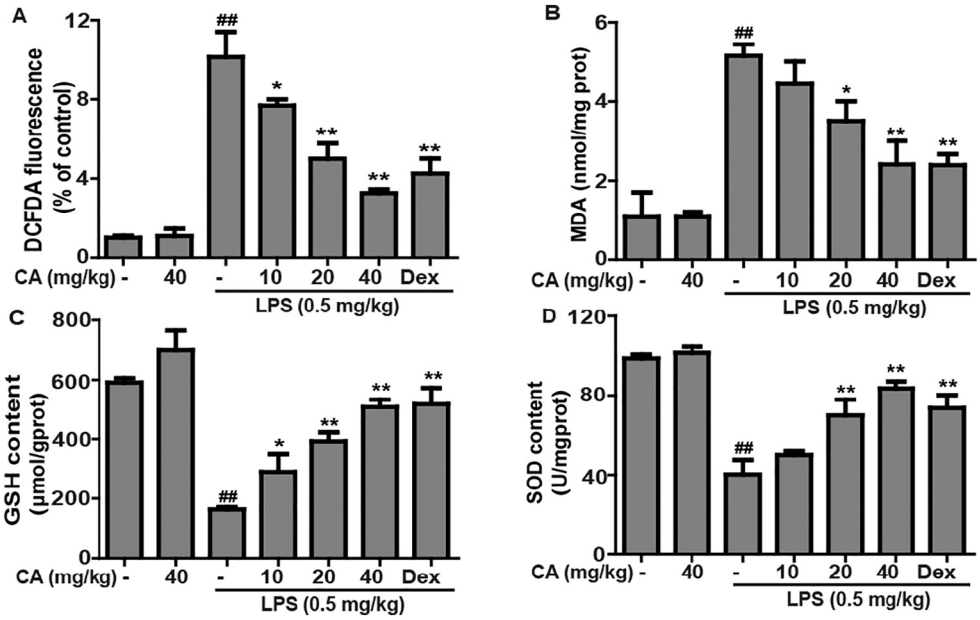

Fig. 5. CA treatment prevents LPS-induced oxidative stress in mice. (A) ROS generation. The ROS of sediment cells in BALF was detected by ROS detection kit. (B) MDA contents. (C) The level of GSH. (D) SOD activation. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the LPS group.

3.4. Effects of CA treatment on LPS-induced cytokine production

Given that inflammatory cytokines such as TNF-α, IL-1β, and IL-6 have shown to be involved in the pathogenesis of LPS-induced ALI, we further tested the levels of cytokine production in BALF by ELISA. In consistent with abundant previous reports, mice subjected to LPS significantly increased TNF-α, IL-1β, and IL-6 production, whereas CA (20, or 40 mg/kg) treatment ameliorated such phenomenon. However, pretreatment with CA (10 mg/kg) did not exhibit this effect (Fig. 4).

3.5. Effects of CA treatment on ROS, GSH, MDA and SOD activity

Next, we tested the potential protective effects of CA against LPS-induced ALI by alleviating oxidant stress. Therefore, the levels of ROS were detected by Fluorescence microplate reader. And the levels of GSH MDA and SOD in the lung tissues were performed following manufacturer's instructions. Our results showed that CA pretreatment decreased LPS-induced ROS generation, and MDA content (Fig. 5A and B) and obviously increased GSH and SOD levels (Fig. 5C and D).

3.6. Effects of CA treatment on LPS-induced MAPK activation

In order to further study the protective mechanism of CA, the effect of CA on the MAPK signaling pathway was determined by Western blot analysis. Our Western blots results indicated that LPS caused JNK, ENK and p38 phosphorylation, but these effects were significantly inhibited by CA pretreatment (Fig. 6).

3.7. Effects of CA treatment on NLRP3, ASC, and caspase-1 expression

Importantly, NLRP3 inflammasome activation is also necessary to regulate the expression of inflammatory mediators, which is related to LPS-induced ALI. Our results implied that LPS challenge led to a significant increase the protein levels of ASC, caspase-1 and NLRP3. On the contrary, CA pretreatment significantly ameliorated such phenomenon (Fig. 7). In addition, it has been reported that NLRP3 inflammasome is essential for the maturation of IL-1β. Western blot showed that mature IL-1β also increased after LPS challenge for 12 h, which was reduced by CA (Fig. 7).

3.8. Effects of CA on Nrf2 and its downstream gens expression

An increasing body of literature has suggested that the protective effects of Nrf2 on ALI [21,22]. As expected, CA treatment also evoked a notable increase in the expression of Nrf2 and its downstream enzymes including HO-1, GCLM, GALC and NQO1 (Fig. 8).

4. Discussion

More and more studies have shown that inflammatory responses can stimulate excessive production of ROS, whereas oxidative stress can also induce inflammatory responses [23]. Inflammation and oxidative stress are considered to be two important factors involved in the pathogenesis of ALI [24]. Moreover, LPS could stimulate the production of excessive cytokines, chemokines and ROS [25]. Therefore, LPS is the ideal inducer of acute lung injury model. Several studies have demonstrated that chicoric acid (CA) suppressed cerebral ischemia-reperfusion injury by its anti-oxidative effect [26], and possessed anti-inflammation capacity [27,28]. However, the role of CA in LPS-induced mine with ALI has not been assessed. In our study, we explore if the anti-Inflammatory and anti-oxidant activities of CA have a role in the treatment of LPS-induced ALI.

We evaluated the protective effect of CA on LPS-induced lung injury in mice by H&E staining for observing histological changes. We found that CA could significantly relieve LPS-induced lung injury and reduce inflammatory cells immersion and alveolar hemorrhage. In addition, the lung wet/dry weight ratios and protein concentration is two important indicators of lung injury, LPS could significantly cause the increase of wet/dry weight ratios and protein concentration in BALF, whereas the CA treatment ameliorated such phenomenon. It has been report that LPS could trigger inflammatory cell infiltration and lead to high expression of cytokines in the exudative phase of LPS-induced ALI [29,30]. In our study, CA significantly inhibited the number of total cells, neutrophils and macrophages, which was associated with inhibition of cytokine production including TNF-α, IL-1β, and IL-6. The results provide evidence of the protection of CA against LPS-induced ALI, which is related to its anti-inflammatory effects.

Accumulating evidence reveals that the inhibition of multiple inflammatory regulatory factors can reduce animal damage [31]. Recently, many studies have focused on LPS-induced inflammation-related signaling pathways [32], including mitogen-activated protein kinase (MAPK) [33–35]. The MAPKs family is composed of c-Jun N-

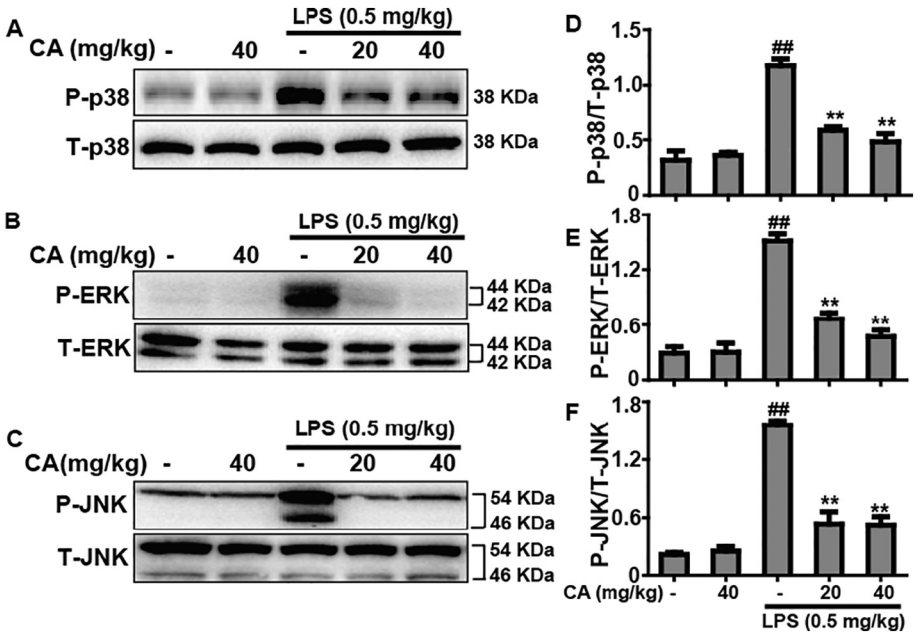

Fig. 6. CA treatment inhibits LPS-induced MAPK pathway activation. The protein levels of lung tissues were analyzed by Western blot. And quantification of the protein levels was normalized to that of β-actin. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{**}p < 0.01$ vs the LPS group.

terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK) and p38, which plays an important role in the inflammatory process [36,37]. In our present study, CA treatment suppressed LPS-activated JNK, ERK and p38 phosphorylation. In addition, NLRP3 inflammasome activation is involved in the development of many inflammatory diseases. Activation of the NLRP3 inflammasome subsequently activates caspase-1, which triggers the maturation of IL-1β by hydrolyzing pro-IL-1β. In the early stages of ALI, IL-1β is a fairly active cytokine that causes the release of proinflammatory mediators such as IL-6 and IL-8 [38]. And then, inflammatory cells are recruited through these inflammatory mediators, excessive inflammation eventually leads to subsequent lung damage [39]. A lot of evidence has showed that LPS can activate NLRP3 inflammasome [40]. In our present study, LPS significantly increased the protein levels of NLRP3, ASC, caspase-1 and IL-1β, whereas CA treatment ameliorated such phenomenon. From the above evidence, the protective effects of CA against LPS-induced ALI may derive from its anti-inflammatory effect through inhibiting MAPK and NLRP3 activation.

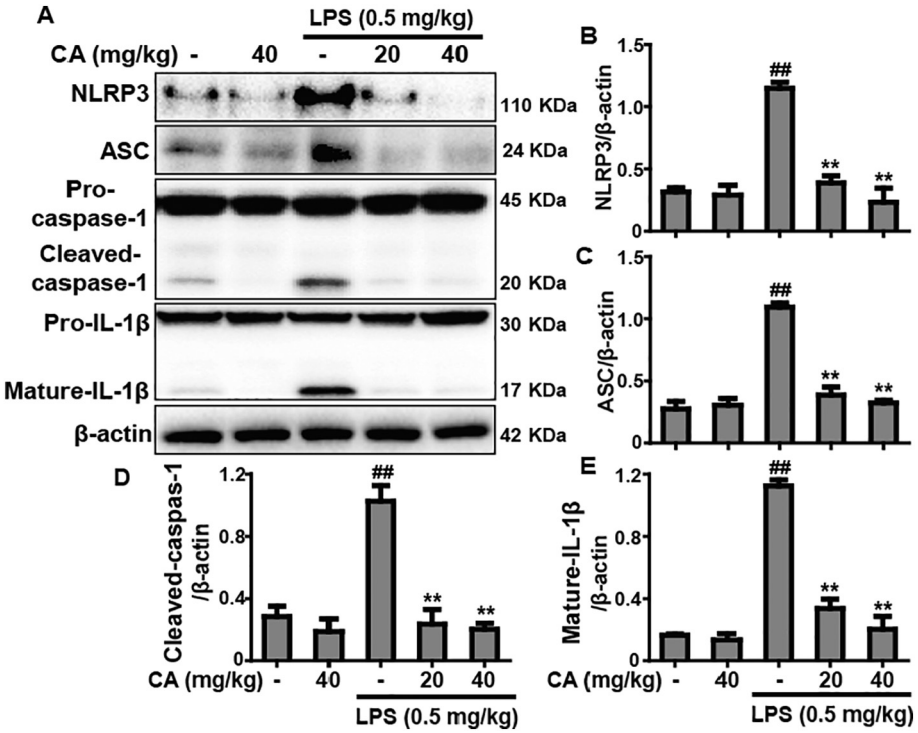

Fig. 7. CA inhibits LPS-induced NLRP3 inflammasome activation. The protein levels of lung tissues were analyzed by Western blot. And quantification of the protein levels was normalized to that of β-actin. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{**}p < 0.01$ vs the LPS group.

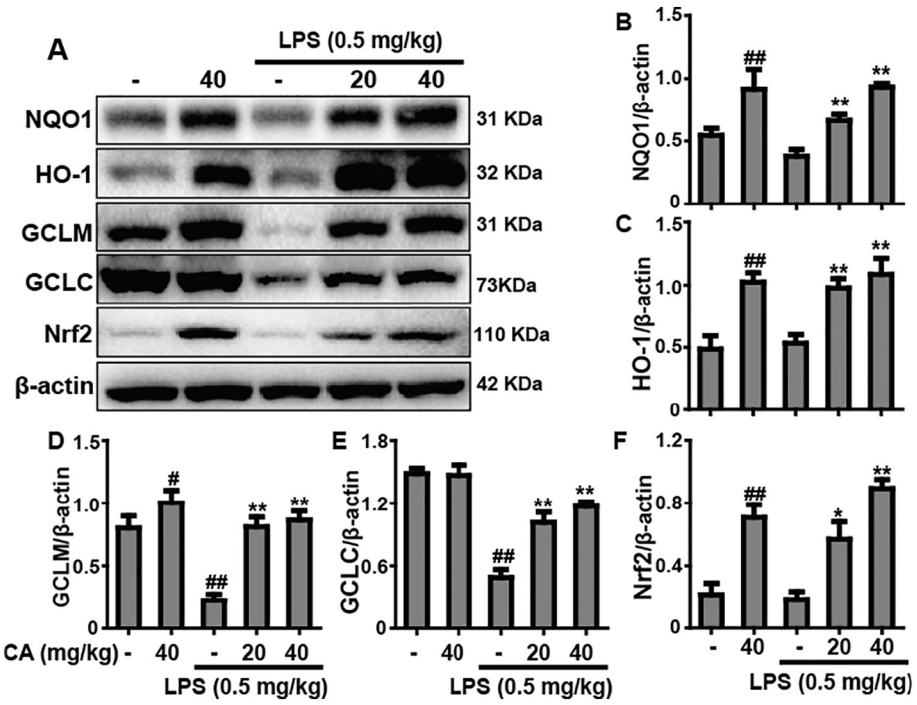

Fig. 8. CA treatment increases the expression of Nrf2 and its downstream gens in LPS-induced ALI. (A) Lung tissues were obtained and homogenized, and then protein expression of NQO1, HO-1, GCLC, GCLM and Nrf2 was determined by western blot. (B–F) Densitometry quantitation of protein levels was normalized to that of β-actin. Data was expressed as means ± SEM (n = 5/group). $^{\#\#}p < 0.01$ vs the control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the LPS group.

LPS exposure not only causes the release of inflammatory cytokines such as TNF-α, IL-1β, but also produces a large amount of ROS, causing parallel tissue damage and aggravating inflammation [41,42]. Oxidative stress is characterized by the overproduction of ROS that is involved in the pathogenesis of ALI [43]. Our further results discovered that LPS significantly increased the production of ROS, whereas was inhibited by CA treatment. Moreover, the activities of antioxidative enzymes including glutathione (GSH) and superoxide dismutase (SOD) could attenuate of oxidative stress [20]. In our experiments, CA could significantly suppress the decrease of GSH and SOD caused by LPS in mice. MDA is a product of lipid peroxidation and is a widely used marker of oxidative stress [44]. In our results, LPS evidently enhanced the formation of MDA reduced by CA treatment. The above results indicated that CA had an antioxidant effect. Based on the critical role of oxidative stress in LPS-induced ALI, strict control of oxidative stress by antioxidants and detoxification enzymes is a possible strategy for mitigating ALI. Furthermore, Nrf2 can combat oxidative damage through regulating of various cytoprotective enzymes, such as HO-1 [12]. Our results showed that CA could significantly up-regulate the expression of HO-1, NQOI, GCLC, and GCLM, as well as its upstream genes Nrf2. Based on these evidence, CA alleviated LPS-induced oxidative damage in ALI by increasing the expression of antioxidative genes.

In conclusion, our study suggested that CA played beneficial role in LPS-induced ALI by inhibiting inflammation and oxidant stress. And the protective effects resulted from the inhibition of MAPK and NLRP3 inflammasome and the activation of the Nrf2 and its downstream genes. Thus, the present results may provide experimental evidence for CA as a potential medication against LPS-induced ALI.

Conflict of interest

The authors declare to have no conflict of interest.

Acknowledgment

This work was supported by the National Science Foundation of China (grant no. 81500051).

References

[1] W.Y. Kim, S.B. Hong, Sepsis and acute respiratory distress syndrome: recent update, Tuberc. Respir. Dis. (Seoul) 79 (2016) 53–57.
[2] M. Bhatia, S. Moochhala, Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome, J. Pathol. 202 (2004) 145–156.
[3] W.L. Lee, G.P. Downey, Neutrophil activation and acute lung injury, Curr. Opin. Crit. Care 7 (2001) 1–7.
[4] V.R. Sunil, K.N. Vayas, J.A. Cervelli, R. Malaviya, L. Hall, C.B. Massa, A.J. Gow, J.D. Laskin, D.L. Laskin, Pentoxifylline attenuates nitrogen mustard-induced acute lung injury, oxidative stress and inflammation, Exp. Mol. Pathol. 97 (2014) 89–98.
[5] D.S. Faffe, V.R. Seidl, P.S. Chagas, V.L. Goncalves de Moraes, V.L. Capelozzi, P.R. Rocco, W.A. Zin, Respiratory effects of lipopolysaccharide-induced inflammatory lung injury in mice, Eur. Respir. J. 15 (2000) 85–91.
[6] N.O. Gekara, N. Dietrich, M. Lyszkiewicz, S. Lienenklaus, S. Weiss, Signals triggered by a bacterial pore-forming toxin contribute to toll-like receptor redundancy in gram-positive bacterial recognition, J. Infect. Dis. 199 (2009) 124–133.
[7] P.D. Maroni, S. Koul, R.B. Meacham, H.K. Koul, Mitogen Activated Protein kinase signal transduction pathways in the prostate, Cell Commun. Signal 2 (2004) 5.
[8] J.J. Grailer, B.A. Canning, M. Kalbitz, M.D. Haggadone, R.M. Dhond, A.V. Andjelkovic, F.S. Zetoune, P.A. Ward, Critical role for the NLRP3 inflammasome during acute lung injury, J. Immunol. 192 (2014) 5974–5983.
[9] S.J. Kim, S.M. Lee, NLRP3 inflammasome activation in D-galactosamine and lipopolysaccharide-induced acute liver failure: role of heme oxygenase-1, Free Radic. Biol. Med. 65 (2013) 997–1004.
[10] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp, A role for mitochondria in NLRP3 inflammasome activation, Nature 469 (2011) 221–225.
[11] J.D. Ren, X.B. Wu, R. Jiang, D.P. Hao, Y. Liu, Molecular hydrogen inhibits lipopolysaccharide-triggered NLRP3 inflammasome activation in macrophages by targeting the mitochondrial reactive oxygen species, Biochim. Biophys. Acta 1863 (2016) 50–55.
[12] B. Wang, X. Zhu, Y. Kim, J. Li, S. Huang, S. Saleem, R.C. Li, Y. Xu, S. Dore, W. Cao, Histone deacetylase inhibition activates transcription factor Nrf2 and protects against cerebral ischemic damage, Free Radic. Biol. Med. 52 (2012) 928–936.
[13] C.H. Yeh, J.J. Yang, M.L. Yang, Y.C. Li, Y.H. Kuan, Rutin decreases lipopolysaccharide-induced acute lung injury via inhibition of oxidative stress and the MAPK-NF-kappaB pathway, Free Radic. Biol. Med. 69 (2014) 249–257.
[14] U.J. Jung, M.K. Lee, Y.B. Park, S.M. Jeon, M.S. Choi, Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice, J. Pharmacol. Exp. Ther. 318 (2006) 476–483.
[15] E.S. Lee, K.O. Uhm, Y.M. Lee, M. Han, M. Lee, J.M. Park, P.G. Suh, S.H. Park, H.S. Kim, CAPE (caffeic acid phenethyl ester) stimulates glucose uptake through AMPK (AMP-activated protein kinase) activation in skeletal muscle cells, Biochem. Biophys. Res. Commun. 361 (2007) 854–858.
[16] J. Azay-Milhau, K. Ferrare, J. Leroy, J. Aubaterre, M. Tournier, A.D. Lajoix, D. Tousch, Antihyperglycemic effect of a natural chicoric acid extract of chicory (*Cichorium intybus* L.): a comparative in vitro study with the effects of caffeic and ferulic acids, J. Ethnopharmacol. 150 (2013) 755–760.
[17] M. Landmann, G. Kanuri, A. Spruss, C. Stahl, I. Bergheim, Oral intake of chicoric acid reduces acute alcohol-induced hepatic steatosis in mice, Nutrition 30 (2014)

882–889.
[18] K. Murakami, R. McGuire, R.A. Cox, J.M. Jodoin, L.J. Bjertnaes, J. Katahira, L.D. Traber, F.C. Schmalstieg, H.K. Hawkins, D.N. Herndon, D.L. Traber, Heparin nebulization attenuates acute lung injury in sepsis following smoke inhalation in sheep, Shock 18 (2002) 236–241.
[19] W. Tao, Y.S. Shu, Q.B. Miao, Y.B. Zhu, Attenuation of hyperoxia-induced lung injury in rats by adrenomedullin, Inflammation 35 (2012) 150–157.
[20] H. Lv, Q. Liu, Z. Wen, H. Feng, X. Deng, X. Ci, Xanthohumol ameliorates lipopolysaccharide (LPS)-induced acute lung injury via induction of AMPK/GSK3beta-Nrf2 signal axis, Redox Biol. 12 (2017) 311–324.
[21] R. Joo Choi, M.S. Cheng, Y. Shik Kim, Desoxyrhapontigenin up-regulates Nrf2-mediated heme oxygenase-1 expression in macrophages and inflammatory lung injury, Redox Biol. 2 (2014) 504–512.
[22] K. Mehla, S. Balwani, A. Agrawal, B. Ghosh, Ethyl gallate attenuates acute lung injury through Nrf2 signaling, Biochimie 95 (2013) 2404–2414.
[23] Q. Bian, S. Gao, J. Zhou, J. Qin, A. Taylor, E.J. Johnson, G. Tang, J.R. Sparrow, D. Gierhart, F. Shang, Lutein and zeaxanthin supplementation reduces photo-oxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells, Free Radic. Biol. Med. 53 (2012) 1298–1307.
[24] S.J. Nicholls, The complex intersection of inflammation and oxidation: implications for atheroprotection, J. Am. Coll. Cardiol. 52 (2008) 1379–1380.
[25] M. Rojas, C.R. Woods, A.L. Mora, J. Xu, K.L. Brigham, Endotoxin-induced lung injury in mice: structural, functional, and biochemical responses, Am. J. Phys. Lung Cell. Mol. Phys. 288 (2005) L333–L341.
[26] L. Jia, Y. Chen, Y.H. Tian, G. Zhang, MAPK pathway mediates the anti-oxidative effect of chicoric acid against cerebral ischemia-reperfusion injury in vivo, Exp. Ther. Med. 15 (2018) 1640–1646.
[27] K.L. Tsai, C.L. Kao, C.H. Hung, Y.H. Cheng, H.C. Lin, P.M. Chu, Chicoric acid is a potent anti-atherosclerotic ingredient by anti-oxidant action and anti-inflammation capacity, Oncotarget 8 (2017) 29600–29612.
[28] Q. Liu, Y. Chen, C. Shen, Y. Xiao, Y. Wang, Z. Liu, X. Liu, Chicoric acid supplementation prevents systemic inflammation-induced memory impairment and amyloidogenesis via inhibition of NF-kappaB, FASEB J. 31 (2017) 1494–1507.
[29] G. Matute-Bello, C.W. Frevert, T.R. Martin, Animal models of acute lung injury, Am. J. Phys. Lung Cell. Mol. Phys. 295 (2008) L379–L399.
[30] J. Grommes, O. Soehnlein, Contribution of neutrophils to acute lung injury, Mol. Med. 17 (2011) 293–307.
[31] G. Di Bonaventura, A. Pompilio, R. Zappacosta, F. Petrucci, E. Fiscarelli, C. Rossi, R. Piccolomini, Role of excessive inflammatory response to Stenotrophomonas maltophilia lung infection in DBA/2 mice and implications for cystic fibrosis, Infect. Immun. 78 (2010) 2466–2476.
[32] G.J. Huang, S.S. Huang, J.S. Deng, Anti-inflammatory activities of inotilone from *Phellinus linteus* through the inhibition of MMP-9, NF-kappaB, and MAPK activation in vitro and in vivo, PLoS One 7 (2012) e35922.
[33] C.Y. Lee, S.P. Chen, C.H. Su, Y.C. Ho, M.L. Yang, S.S. Lee, R. Huang-Liu, C.P. Yang, C.J. Chen, Y.H. Kuan, Zerumbone from *Zingiber zerumbet* ameliorates lipopolysaccharide-induced ICAM-1 and cytokines expression via p38 MAPK/JNK-IkappaB/NF-kappaB pathway in mouse model of acute lung injury, Chin. J. Phys. 61 (2018) 171–180.
[34] L.J. Chen, Y.B. Ding, P.L. Ma, S.H. Jiang, K.Z. Li, A.Z. Li, M.C. Li, C.X. Shi, J. Du, H.D. Zhou, The protective effect of lidocaine on lipopolysaccharide-induced acute lung injury in rats through NF-kappaB and p38 MAPK signaling pathway and excessive inflammatory responses, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 2099–2108.
[35] Y. Hu, L. Tao, H. Tan, M. Zhang, K. Shimizu, F. Zhang, C. Zhang, An active drimane-type lactone from *Polygonum jucundum* attenuates lipopolysaccharide-induced acute lung injury in mice through TLR4-MAPKs signaling pathway, Inflammation 40 (2017) 1204–1213.
[36] C.Y. Lee, J.J. Yang, S.S. Lee, C.J. Chen, Y.C. Huang, K.H. Huang, Y.H. Kuan, Protective effect of *Ginkgo biloba* leaves extract, EGb761, on endotoxin-induced acute lung injury via a JNK- and Akt-dependent NFkappaB pathway, J. Agric. Food Chem. 62 (2014) 6337–6344.
[37] J.S. Chang, H.J. Lin, J.S. Deng, W.T. Wu, S.S. Huang, G.J. Huang, Preventive effects of velvet antler (*Cervus elaphus*) against lipopolysaccharide-induced acute lung injury in mice by inhibiting MAPK/NF-kappaB activation and inducing AMPK/Nrf2 pathways, Evid. Based Complement. Alternat. Med. 2018 (2018) 2870503.
[38] L.J. Cross, M.A. Matthay, Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury, Crit. Care Clin. 27 (2011) 355–377.
[39] R.B. Goodman, J. Pugin, J.S. Lee, M.A. Matthay, Cytokine-mediated inflammation in acute lung injury, Cytokine Growth Factor Rev. 14 (2003) 523–535.
[40] Q. Liu, X. Ci, Z. Wen, L. Peng, Diosmetin alleviates lipopolysaccharide-induced acute lung injury through activating the Nrf2 pathway and inhibiting the NLRP3 inflammasome, Biomol. Ther. (Seoul) 26 (2018) 157–166.
[41] L. Fialkow, Y. Wang, G.P. Downey, Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function, Free Radic. Biol. Med. 42 (2007) 153–164.
[42] Y. Wu, J. Lu, S. Antony, A. Juhasz, H. Liu, G. Jiang, J.L. Meitzler, M. Hollingshead, D.C. Haines, D. Butcher, K. Roy, J.H. Doroshow, Activation of TLR4 is required for the synergistic induction of dual oxidase 2 and dual oxidase A2 by IFN-gamma and lipopolysaccharide in human pancreatic cancer cell lines, J. Immunol. 190 (2013) 1859–1872.
[43] G.J. Quinlan, N.J. Lamb, R. Tilley, T.W. Evans, J.M. Gutteridge, Plasma hypoxanthine levels in ARDS: implications for oxidative stress, morbidity, and mortality, Am. J. Respir. Crit. Care Med. 155 (1997) 479–484.
[44] S.W. Ryter, H.P. Kim, A. Hoetzel, J.W. Park, K. Nakahira, X. Wang, A.M. Choi, Mechanisms of cell death in oxidative stress, Antioxid. Redox Signal. 9 (2007) 49–89.