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**ORIGINAL ARTICLE**

# Protective Effect of Gui-Hong Herb Pair on Lipopolysaccharide-Induced Acute Lung Injury via Regulation of the MAPK and NF-Kb Pathways

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**Abstract:**
The compatibility between Danggui (*Angelica sinensis* (Oliv.) Diels, DG) and Honghua *(Carthamus tinctorius* L., HH) is a known as Gui-Hong herb pair with the functions of "activate blood and dissolve stasis". The effect of Gui-Hong herb pair on acute lung injury (ALI) is not clear. Therefore, we provided a basis for the protective effect of Gui-Hong herb pair on ALI induced by lipopolysaccharides (LPS). The content of hydroxysafflor yellow A in HH was 35.80 mg/g and the content of ferulic acid in DG was 2.85 mg/g. Gui-Hong herb pair inhibited the excessive oxidative stress response and production of NO, and reduced the inflammatory factors IL-6 and IL-1β and TNF-α in RAW264.7 cells. Moreover, they decreased the phosphorylation of p38MAPK and ERK and IκBα-NF-κB trimer degradation as well as nuclear transport of NF-κB p65. In ALI model, Gui-Hong herb pair improved pulmonary edema and lung permeability, enhanced antioxidant stress and anti-inflammatory ability. Our study showed that the Gui-Hong herb pair had the potential for the prevention and treatment of ALI by inhibiting inflammation.
**Keywords: Honghua; Danggui; Acute lung injury; MAPK; NF-κB p65**

## Introduction

Acute lung injury (ALI) has been defined since 1994 by the North American European consensus classification with a clinical research history of more than 50 years. It affects the breathing in the early stage and develops into life-threatening respiratory distress syndrome, which seriously threatens the survival and quality of life of patients [1]. The difficulty of treatment for ALI is high incidence rate, various causes and mortality rate [2]. At present, corona virus disease 2019 (COVID-19) affects the world, leading to severe lung injury and respiratory distress syndrome. Therefore, the development of drugs to prevent and treat lung injury is also helpful for the treatment of COVID -19 [3].

Lung injury can be caused by many reasons, including dust, internal and external lung injury, pneumonia, etc. [4]. The pathogenesis of ALI is related to pulmonary fibrosis, pulmonary edema, increased oxidative stress, inflammatory cascade and excessive apoptosis [3]. The excessive inflammatory response of the lung is a main feature of ALI. At the same time, the apoptosis of lung epithelial cells and the destruction of barrier function are another feature of ALI. Therefore, the pathways of inflammation and apoptosis can become the therapeutic targets of ALI [5, 6]. Inflammation and oxidative stress caused by ALI are associated with the MAPK and NF-κB signaling pathways, which is due to depolymerization of IκBα and NF-κB dimers and lead to the activation of downstream signals by NF-κB nuclear transport [7].

Most traditional Chinese medicine play a role in fighting various inflammation with less toxicity and side effects, so it is widely used in the treatment of respiratory diseases [8]. Gui-Hong

herb pair is an ancient and classic formula comprised of Danggui (DG, *Angelica sinensis* (Oliv.) Diels) and Honghua (HH, *Carthamus tinctorius* L.) and it has the activating blood circulation and dissipating blood stasis effects [9], which is frequently used for treatment of blood stasis syndrome (BSS) in China [10-12]. The therapeutic characteristics of Gui-Hong herb pair are in line with the definition of between inflammation and thrombosis, so it can be applied for the treatment of ALI [13]. Proper compatibility of traditional Chinese medicine can make single traditional Chinese medicine play the characteristics of multi-component, multi-target and multi-pathway [14]. The combination of the different herbals can increase the exposure of active ingredients that play a positive role in traditional Chinese medicine and reduce the exposure of toxic ingredients, resulting in higher utilization of active ingredients, on the contrary, the bioavailability of toxic ingredients is lower [15]. In the present study, we explored the effect and mechanism of the Gui-Hong herb pair on LPS induced ALI *in vitro* and *in vivo*. The MAPK and NF-κB signaling pathways were investigated to estimate the mechanism of Gui-Hong herb pair in the treatment of ALI. Our study proved that Gui-Hong herb pair can be used as a safe and feasible program for the treatment of patients with ALI.

## 2 Materials and methods

### 2.1 Reagents

HH (batch number: 201012006) and DG (batch number: 20120184) were purchased from Beijing Tongrentang Chain Pharmacy Co., Ltd (Tianjin, China). Lipopolysaccharides (LPS), berberine hydrochloride and dexamethasone were purchased from Solebo Technology Co. Ltd., (Beijing, China). Hydroxysafflor yellow A and ferulic acid were obtained from Shanghai Acmec Biochemical Co., Ltd with purity > 98%. All other materials were commercially available unless otherwise stated.

Gui-Hong herb pair was prepared as following method: HH (10.0 g) and DG (10.0 g) were exhaustively extracted with ethanol. Ethanol was re-moved under reduce pressure resulting in dried crude ethanolic extract.

### 2.2 Determination of the content of Gui-Hong herb pair

HH and DG extracts were dissolved in methanol and passed through a 0.22 μm membrane filter before injected. Agilent 1260 HPLC was used for the chromatographic analysis. Samples were separated on an Venusil MP C18 column (4.6 × 250 mm, 5 μm). The flow rate was 1.0 mL/min, and the volume of sample injection was 10 μL. The detection wave length was 360 nm for HH extract and the mobile phase consisted of acetonitrile (A) and 0.1% formic acid solution (B) with the gradient elution (0-8 min, 10% A; 8-40 min, 10-36% A; 40-50 min, 36-40% A; 50-63 min, 40-62% A). DG extract was detected at 320 nm with the same mobile phase as above with following gradient elution: 0-10 min, 10% A; 10-15 min, 10-12% A; 15-30 min, 12-45% A; 30-40 min, 45-54% A; 40-60 min, 54-62% A; 60-75 min, 62-70% A. Figure 1 showed the HPLC chromatograms of HH and DG extracts as well as the reference compounds hydroxysafflor yellow A and ferulic acid. The content of hydroxysafflor yellow A in HH extract was 35.80 mg/g and the content of ferulic acid was 2.85 mg/g.

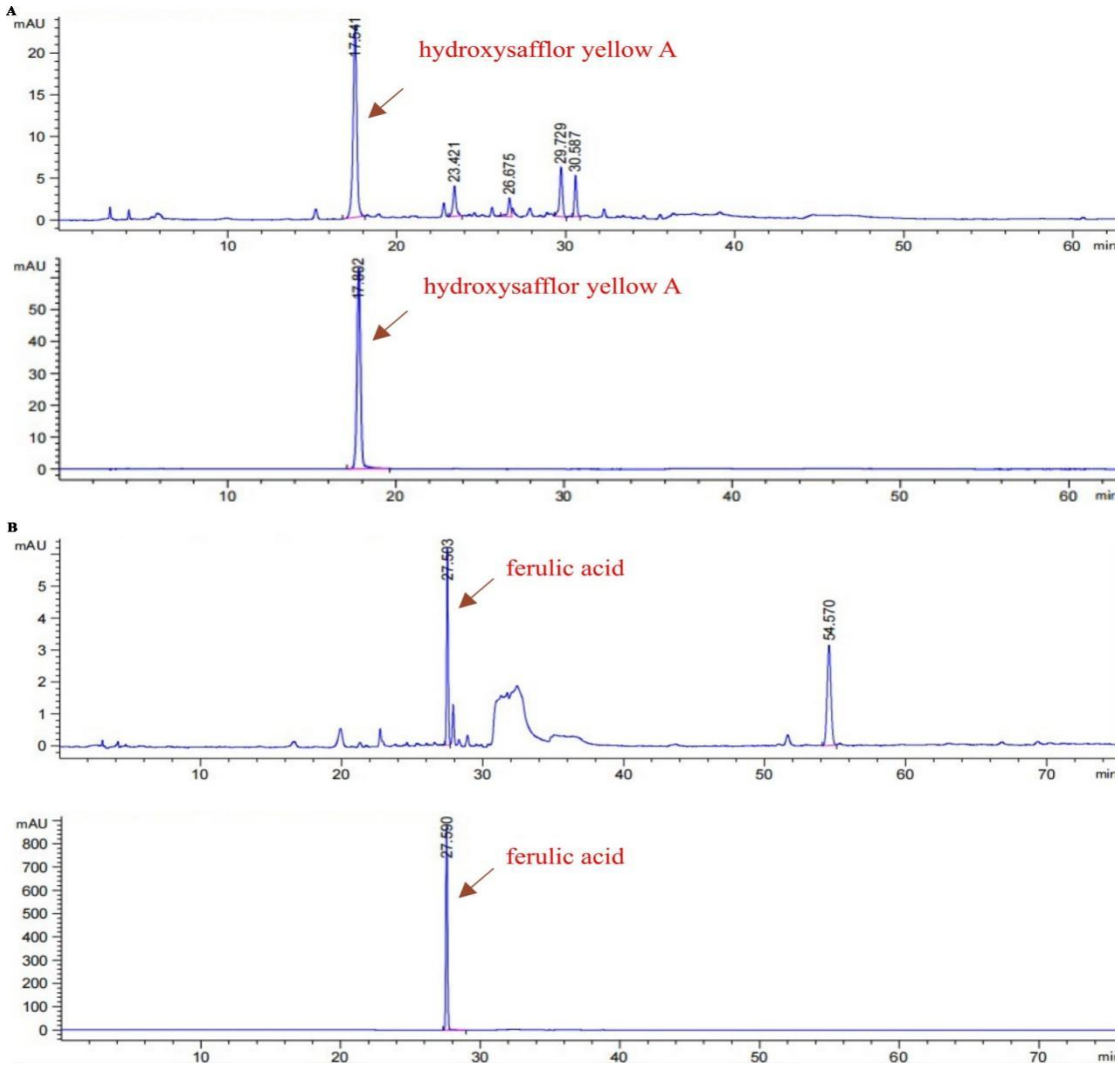


**Figure 1 HPLC chromatograms of HH extract and hydroxysafflor yellow A (A) as well as DG extract and ferulic acid (B).**

The total flavonoid and polyphenols contents of HH and DG were detected by the NaNO2-Al(NO3) 3-NaOH method and Folin-Ciocalteu assay, respectively. The total polysaccharide of HH and DG were evaluated by phenol-sulfuric acid method. The total flavonoid, polyphenols, and polysaccharide of HH were 14.00%, 3.60%, and 22.00%, respectively, and those in DG were 11.10%, 2.40%, and 24.40%, respectively.

### 2.3 Cell culture and cell viability assay

Mouse monocyte macrophage leukemia cells RAW264.7 was purchased from Guangzhou Huatuo Biological Technology Co., LTD. The cells were cultured in GIBCO DMEM high glucose medium and were supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/mL penicillin and 100 IU/mL streptomycin at 37 ℃ in a humidified atmosphere of 5% $CO_2$.

MTT assays were used to determine cell viability. RAW264.7 cells were seeded into 96-well plates with 1 × $10^5$ cells/mL and exposed to HH (0-100 μg/mL) or DG (0-200 μg/mL) extracts with or without LPS (1 μg/mL) for 24 h. Cells were incubated with MTT solution (5 mg/mL) for 4 h. Subsequently, 100 μL DMSO was added to the plate, and the absorbance was measured at 492 nm/630 nm.

### 2.4 Determine the NO production and ROS releasing

NO production was detected by NO kit (Nanjing Jiancheng Bioengineering Institute, China). RAW264.7 cells were treated with HH (5, 10, 25, 50, and 100 μg/mL), DG (5, 10, 25, 50, and 100 μg/mL) or berberine (positive drug, 10 μg/mL) for 1 h before adding LPS (1 μg/mL) for 24 h. After drug treatment, the cell culture supernatants were collected and measured the concentration of NO

according the NO kit. The inhibition rate was calculated by the following formula.

NO inhibition rate (%) = ($NO_{LPS}$ - $NO_{drug}$) / $NO_{LPS}$ × 100

According to the NO inhibition rate, the $IC_{50}$ values of HH and DG were obtained by Graphpadprism7.0 software. Then the effect of Gui-Hong herb pair at the ratio 1:5 was assessed on the NO production and the combined index (CI) value was calculated by CompuSyn software. CI > 1 indicates that the two drugs have antagonistic effect, CI = 1 indicates additive effect, and CI < 1 indicates synergistic effect.

The level of intracellular ROS was detected by ROS detection kit (Solebo Technology Co., Ltd, Beijing.). RAW264.7 cells were collected and incubate in serum-free medium containing DCFH-DA (1 μM) for 10 min without light after treatment with HH (4 μg/mL) and DG (20 μg/mL) alone or Gui-Hong herb pair with LPS for 24 h. The cell fluorescence signal was evaluated by FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA).

### 2.5 Animal and LPS induced ALI model

Male BALB/c mice (18-20 g, 6-8 weeks) were purchased from Liaoning Changsheng Biotechnology Co. Ltd.. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Tianjin University of Science and Technology and approved by the Animal Ethics Committee of Tianjin University of Science and Technology. Mice were fed with free access to food and water on a 12 h light/dark cycle. ALI model was induced by intraperitoneal injection of LPS as the same previous reported method [16]. Mice were randomly divided into six groups with 10 mice in each group. The mice in control, model, HH, DG, and Gui-Hong herb pair groups were administered intragastrically by 5% CMC-Na (10 mL/kg), 5% CMC-Na (10 mL/kg), HH extract (2 g/kg), DG extract (8 g/kg), and Gui-Hong herb pair, respectively. Dexamethasone group (DEX) mice were injected with 5 mg/mL dexamethasone. After treatment for nine days, the mice in model, HH, DG, and Gui-Hong herb pair groups were intraperitoneally injected 5 mg/kg LPS at ten days. All the mice were killed by dislocation after 24 h of LPS injection. Blood was taken from femoral artery and centrifuged at 4000 rpm/min at 4℃ for 10 min to obtain plasma, which were stored in the refrigerator at -80℃. The organs of lung and spleen were weighted and observed the morphology. The indexes of lung and spleen were calculated by the formula.

Lung index = lung weight (mg)/body weight (g) ×100%

Spleen index = spleen weight (mg)/body weight (g) ×100%

### 2.6 Biochemistry analysis and pathological study

We measured wet-to-dry ratio of the lungs and lung permeability. After 24 h of LPS stimulation, part of the left lung was removed, and the wet weight was measured. Then, the lungs were placed in a 70℃ for 48 h to remove all water to measure the dry weight and the ratio of wet-to-dry weight calculated.

According to the reported, we evaluated lung permeability by the Evans blue dye extravasation method [17]. Briefly, Evans blue dye (45 mg/kg) was injected into the caudal vein before 30 min of killing the mice. Evans blue dye was extracted from the lung using formamide for 18 h at 60℃. The absorbance of the supernatant was determined at 620 nm using a microplate reader (Tecan, Austria).

The levels of inflammatory factor protein in cells or serum were determined by IL-6 and TNF-α and IL-1β kit (Nanjing Jiancheng Bioengineering Institute, China), and analyzed with corresponding ELISA kits according to the instructions. Superoxide dismutase (SOD) and malondialdehyde (MDA) were performed by the relevant kits for the determination of lung tissue homogenates (Nanjing Jiancheng Bioengineering Institute, China). The absorbance was measured at 450 nm and 532 nm, respectively.

To evaluate lung injury, the right lung of mice was excised after 24 h of LPS stimulation, fixed in 4% (v/v) paraformaldehyde, embedded in paraffin, stained with H&E solution and observed under microscope.

### 2.7 Western blotting

The cell samples were quantified using Coomassie Brilliant blue. The antibodies including total p38

MAPK, phosphorylated p38 MAPK (p-p38 MAPK), total ERK1/2, and phosphorylated ERK1/2 (p-ERK1/2) were obtained from Cell Signaling Technology. The total IκBα, phosphorylation IκBα (p-IκBα), total NF-κB p65, phosphorylated NF-κB p65 (p-NF-κB p65), and Lamin B1 were purchased from Beijing Biosynthesis Biotechnology. Lamin B1 was a nucleus standard and α- Tubulin used as a standard was purchased from Sigma Aldrich. The gray analysis of protein bands was quantified with ImageJ software (Wayne DGband National Institutes of Health, USA).

### 2.8 Statistical analysis

All data were presented as mean ± standard deviation (SD) of the results of three independent experiments. The statistical differences were analyzed by one-way analysis of variance (ANOVA) of Tukey test. $P < 0.05$ were considered as significant.

## 3 Results

### 3.1 Gui-Hong herb pair inhibited the NO production in RAW264.7 cells

The concentration of the safe effect of HH and DG on RAW264.7 cells was determined using MTT experiments. The concentrations of HH and DG below 100 μg/mL caused no toxic damage to RAW264.7 cells (Figure 2).

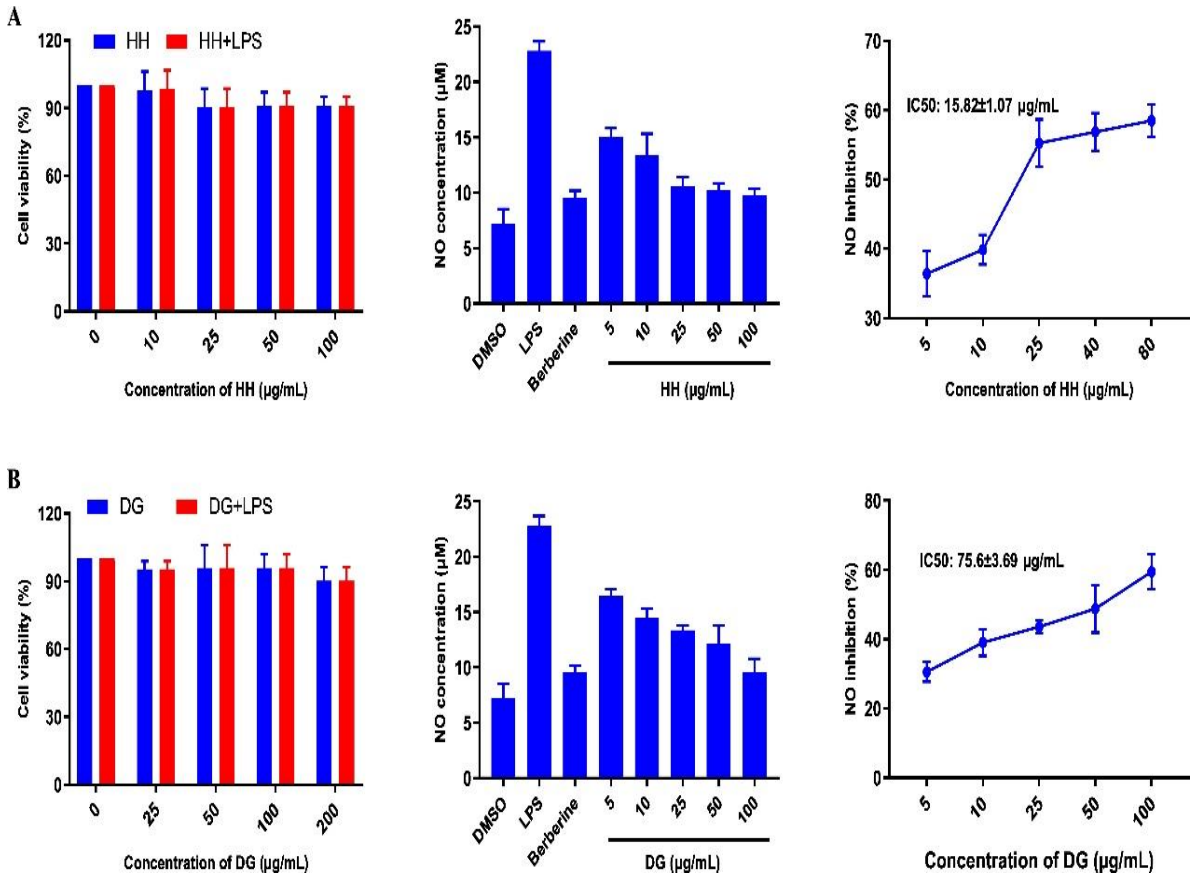


**Figure 2 The toxic effects and NO production of HH (A) and DG (B) on RAW264.7 cells were measured. Cells were treated with different concentrations of HH and DG for 24 h. The cytotoxicity was detected by MTT assay. Cells are acted upon with different concentrations of HH and DG (5, 10, 25, 50, 100 μg/mL) for 24 h. The production of NO was reduced with the increase of HH and DG concentration. The IC50 was calculated by GraphPad software.**

Within 100 μg/mL, HH and DG inhibited the NO production and the $IC_{50}$ values were 15.82 ± 1.07 and 75.6 ± 3.69 μg/mL, respectively. Berberine was selected as the positive drug in this study due to the application for the treatment of inflammatory diseases such as ulcerative colitis [18], mastitis [19] and rheumatoid arthritis [20]. Berberine could significantly inhibit the NO releasing.

According to the IC50, we set the ratio as 1:5 of

HH and DG as Gui-Hong herb pair and used different concentration to detect the appropriate dose. As shown in Fig. 3, HH and DG alone induced weak inhibition of NO, whereas Gui-Hong herb pair group exhibited strong NO inhibition at low doses of the two herbals (Figure 3A-C), but not in high doses (Figure 3D and E). The calculated CI value was less than 1, indicating that the HH and DG had synergistic effect on inhibiting NO production (Figure 3F). The highest inhibition rate could reach 82.11% at 4 μg/mL of HH and 20 μg/mL of DG (Figure 3C).

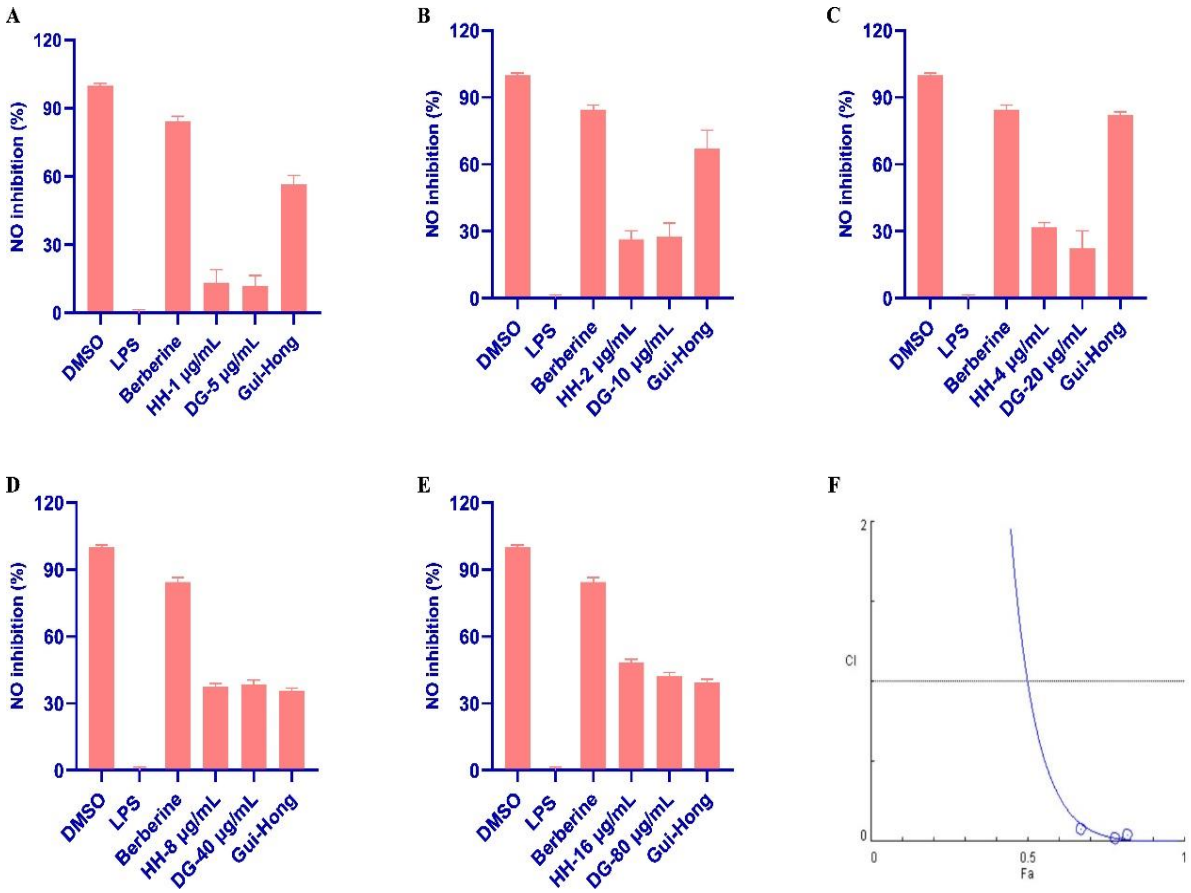


**Figure 3 The effects of Gui-Hong herb pair on NO production in RAW264.7 cells. The different concentrations of HH (1, 2, 4, 8, 16 μg/mL) and DG (5, 10, 20, 40, 80 μg/mL) alone or Gui-Hong herb pair were used to treat RAW264.7 cells for 24 h and NO inhibition was measured (A-E). The dose of 1-4 μg/mL of HH combined with 5-20 μg/mL of DG significantly increased the inhibition of NO (A-C). However, the inhibitory effect of Gui-Hong herb pair at 8-16 μg/mL of HH and 40-80 μg/mL of DG had no change compared with alone group (D and E). The CI value was calculated by CompuSyn software and (F).**

### 3.2 Gui-Hong herb pair synergistically inhibited the inflammatory related factors on RAW264.7 cells

LPS caused a large amount of intracellular ROS production, resulting in cell damage. When HH and DG alone or Gui-Hong herb pair treated the cells, the ROS production were significantly inhibited (Figure 4A and B). The Gui-Hong herb pair showed the remarkable inhibitory effect on ROS releasing, which confirmed the synergistic effect between HH and DG. Similarly, the production of NO was obviously reduced by the Gui-Hong herb pair compared with alone groups (Figure 4C). Meanwhile, we detected the expressions of IL-1β, IL-6, and TNF-α after RAW264.7 cells exposure to Gui-Hong herb pair. HH and DG alone could significantly reduce the levels of IL-1β, IL-6, and TNF-α. Most notably, Gui-Hong herb pair strongly increased the production of the three inflammatory factors (Figure 4D-F), which further indicated that HH and DG played a synergistic anti-inflammatory effect on RAW264.7 cells.

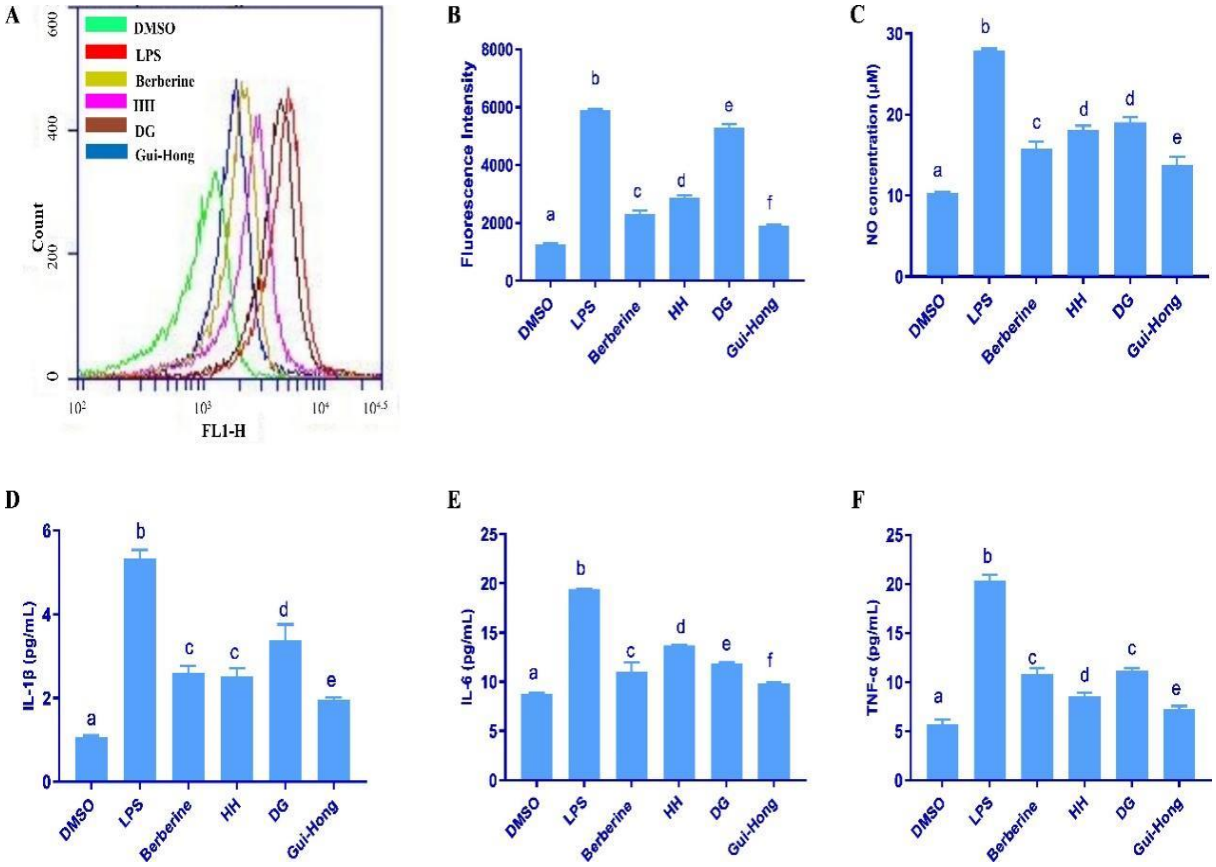


**Figure 4 Effects of Gui-Hong herb pair on LPS-induced inflammatory related factors of RAW264.7 cells. Cells were treated with HH (4 μg/mL) and DG (20 μg/mL) alone or Gui-Hong herb pair for 1 h and LPS was added for 24 h. The cells were stained with DCFH-DA fluorescence and detected by flow cytometry (A and B). The intracellular ROS was increased in LPS group, but was inhibited by the treated groups. The concentration of NO was determined by kit (C). ELISA kit testing was performed to analysis the levels of IL-1β, IL-6 and TNF-α (D-F). Different letters indicated significant differences, $P < 0.05$.**

### 3.3 Gui-Hong herb pair regulated the MAPK and NF-κB signaling pathways

In order to further explore the synergistic mechanism between HH and DG, the expression of the proteins associated to MAPK and NF-κB pathways were assessed by western blotting. Both of ERK and p38 MAPK were significantly activated by LPS, which were obviously inhibited by HH and DG alone and reversed by the Gui-Hong herb pair (Figure 5A). When RAW264.7 cells were stimulated by LPS, the trimer of IκBα and NF-κB in the cell depolymerized, which lead to IκBα phosphorylated and NF-κB released. As shown in Figure 5B-D, LPS upregulated the level of p-IκBα and decreased the expression of p-NF-κB p65 in cytoplasm, but increased the p-NF-κB p65 in nucleus. DG and the Gui-Hong herb pair improved the nucleation response of dimers, further controlled the activation of the NF-κB pathway, which were proved by that they significantly downregulated the level of p-IκBα and upregulated the expression of p-NF-κB p65 in cytoplasm as well as reduced the p-NF-κB p65 in nucleus. The results showed that Gui-Hong herb pair played a synergistic role in anti-inflammatory effect by inactivation of MAPK and NF-κB signaling pathways.

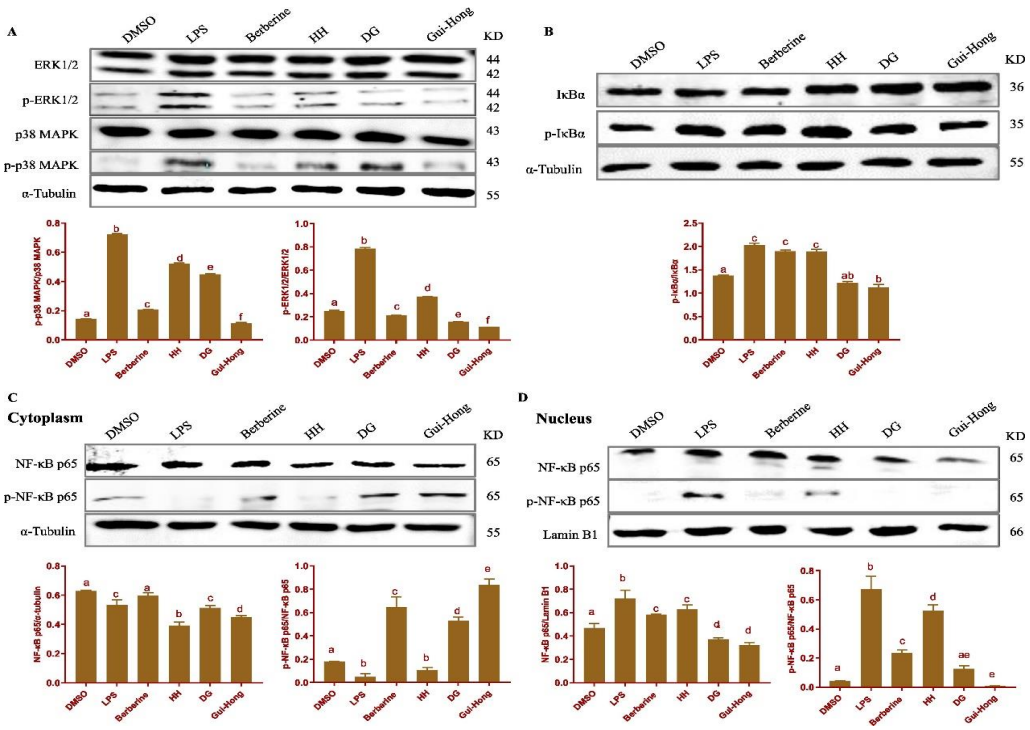


**Figure 5 The regulation of Gui-Hong herb pair on MAPK and NF-κB pathways in LPS-induced injury of RAW264.7 cells. Western blot analysis the expressions of ERK and p-38 MAPK (A), IκBα (B), and NF-κB p65 in cytoplasm (C) and nucleus (D) in LPS-treated RAW264.7 cells after different drug treatment. Different letters indicated significant differences, $P < 0.05$.**

### 3.4 The protective effect of Gui-Hong herb pair on ALI model

We established the ALI mouse model and selected dexamethasone as the positive drug. LPS caused a significant decrease of body weight in ALI mice, while HH and DG alone or Gui-Hong herb pair could prevent weight loss (Figure 6A). Meanwhile, the two herbals alone or Gui-Hong herb pair improved the elevation of spleen index (Figure 6B) and lung index (Figure 6C) caused by LPS. According to literature [21], the lung injury score was obtained and the treated drugs ameliorated the lung injury induced by LPS (Figure 6D). HE staining was used to observe the pathological changes of lung in mice. The control group mice had normal lung morphology, and the LPS stimulated group showed thickening of alveolar wall, rupture or disappearance of septum, and certain inflammatory cell infiltration. HH improved the thickening of alveolar wall, while DG still had certain inflammatory infiltration and lung consolidation. However, Gui-Hong herb pair made the lung tissue of mice basically return to normal. The results showed that Gui-Hong herb pair played a synergistic role in the prevention of ALI.

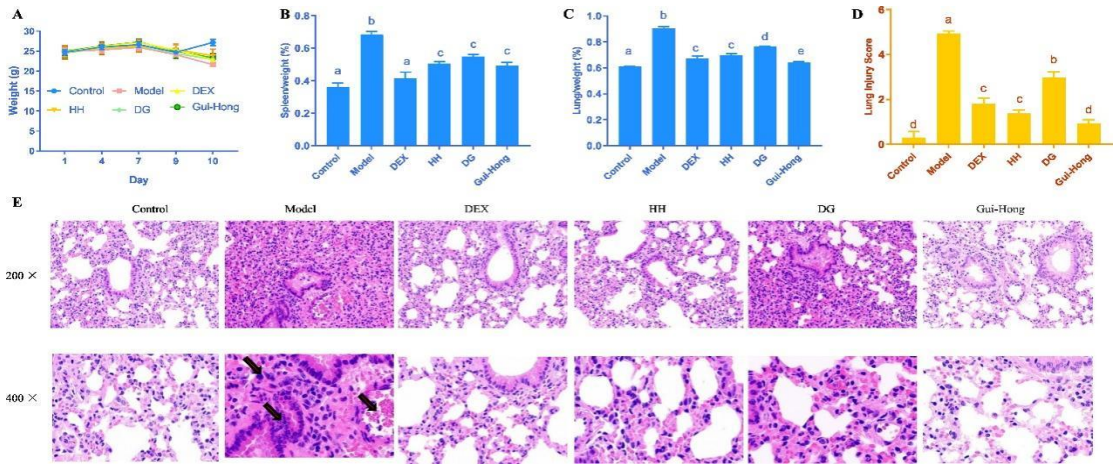

**Figure 6 The Gui-Hong herb pair treatment protected against LPS-induced ALI. The drugs could change the body weight loss of mice (A), ameliorate the spleen index (B) and lung index (C), decrease the lung injury score (D), and improve the pathological changes of lung by HE staining (E, magnification×200, ×400, scale bar; 20 μm). Different letters indicated a significant difference, $P < 0.05$.**

### 3.5 Gui-Hong herb pair reduced LPS-induced pulmonary edema, enlarged capillary permeability, oxidative stress, and inflammatory response

The ratio of wet to dry weight of lung tissue was used to express the degree of pulmonary edema. The results showed that the wet/dry ratio of lung tissue was decreased from 7.9 to 4.0 after the treatment of Gui-Hong herb pair (Figure 7A), which greatly improved pulmonary edema. The content of Evans blue extracted from lung tissue was reduced from 63 μg/100mg to 21 μg/100mg after Gui-Hong herb pair treated (Figure 7B), indicating that the permeability of alveolar capillaries decreased and the integrity of alveoli were enhanced. Therefore, Gui-Hong herb pair could improve the physiological state of mice lungs and maintain normal in mice.

Malondialdehyde (MDA) is a marker of oxidative stress and its large increase will cause body damage. We tested the ability of HH and DG alone or Gui-Hong herb pair on SOD and MDA in ALI mice. LPS decreased the level of SOD and increased the expression of MDA. HH and DG alone or Gui-Hong herb pair increased the content of SOD and reduce the excessive release of MDA caused by ALI (Figure 7C and D). We used the ELISA kit to detect the expression of IL-6 and TNF-α in serum (Figure 7E and F). Prophylactic administration of HH and DG alone or Gui-Hong herb pair reduced the production of IL-6 and TNF-α in ALI mice. In this process, the Gui-Hong herb pair showed stronger effects than single drugs, indicating that the two drugs had a synergistic effect on the improvement of oxidative stress and inflammatory response.

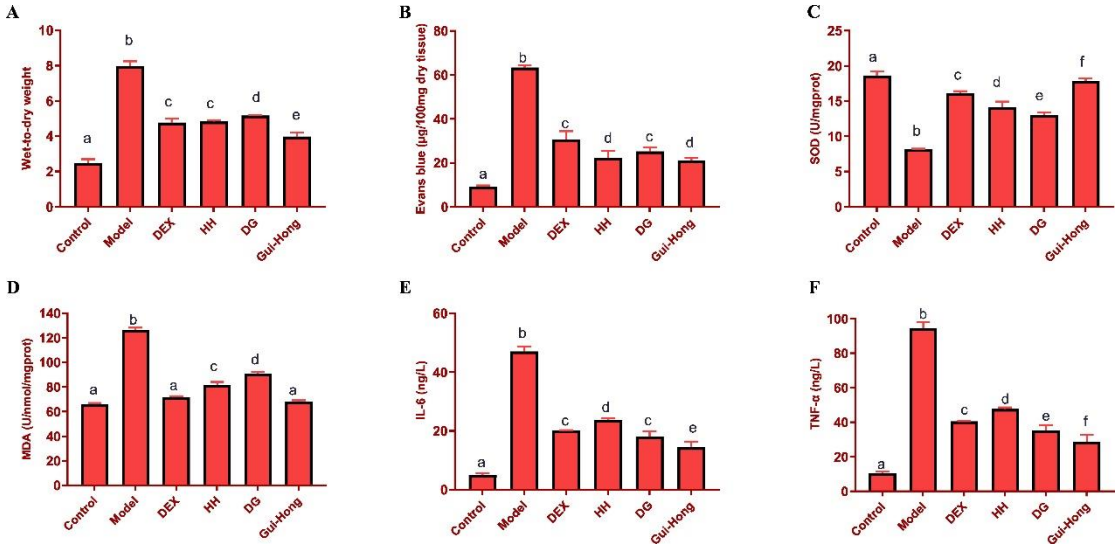


**Figure 7 Gui-Hong herb pair attenuated pulmonary edema, lung permeability, oxidative stress and inflammatory response in the lung of mice induced by LPS. The ratio of wet to dry weight of lung tissue indicated the degree of pulmonary edema (A). The amount of lung-internal Evans blue content represented the strength of lung permeability (B). The levels of MDA (C), SOD (D), IL-6 (E), and TNF-α (F) were detected. Different letters indicate significant differences, $P < 0.05$.**

## Discussion

ALI seriously threatens the quality of life of patients. Traditional therapeutic drugs play an anti-inflammatory role in the treatment ALI. Erlotinib can reduce inflammatory response and capillary permeability by inhibiting EGFR and NF-κB signaling pathway [22]. Curcumin can affect the reaction process of inflammation and improve ALI [23]. Aspirin as a nonsteroidal anti-

inflammatory drug inhibits the synthesis of prostaglandins [24]. Glucocorticoid dexamethasone appropriately regulates and terminates the inflammatory response by inhibiting the activity of inflammatory cells [25]. However, the toxic and side effects of these drugs are inevitable, which reduce the quality of life of patients. Traditional Chinese medicine has shown obvious advantages in preventing or treating lung injury, which are belonged to the herbals of promoting blood circulation and removing blood stasis [26-28]. In this study, we used Gui-Hong herb pair to evaluate the effect on ALI. Quantitative analysis of the chemical components in HH and DG showed that the largest content was total flavonoids and polysaccharides followed by total polyphenols. Among them, hydroxysafflor yellow A in HH extract was 35.80 mg/g and the content of ferulic acid in DG extract was 2.85 mg/g. HH has the effect of dispersing blood stasis and relieving pain. In previous studies, it was reported that HH could improve pulmonary edema in ALI mice and reduce inflammatory response as well as oxidative stress [29, 30]. DG as an umbrella plant can replenish and activate blood. DG could prevent and improve the inflammatory infiltration in ALI mice by regulating the PI3K/AKT and TLR4/NF- κB pathways [31]. The compatibility of traditional Chinese medicine adopts a scientific combination method to make them play a greater role than the single [15, 32, 33]. Our study found that Gui-Hong herb pair could synergistically inhibit the releasing of ROS and the production of NO caused by LPS stimulation, showing that Gui-Hong herb pair improved the ability of antioxidant stress and inhibited inflammatory response. Meanwhile, the Gui-Hong herb pair significantly reduced the levels of inflammatory factors including TNF-α, IL-1β and IL-6. The reduction of inflammatory response improves the degree of lung injury. TNF-α is a gold-standard inflammatory factor for inflammatory responses, which promotes inflammatory cytokines. The interleukins are the main inflammatory factors and are released in large quantities in the early and middle stages of the inflammatory response, resulting in the intensification of the inflammatory response. Blocking the inflammatory factors in the earliest stage of inflammatory response can avoid the problem of causing the subsequent release of other inflammatory factors. Therefore, the inhibition of the releasing of TNF-α, IL-1β and IL-6 induced by Gui-Hong herb pair indicated that they prevented the occurrence of inflammatory response, which in turn reduced the occurrence of lung injury. In addition, we detected the expression of proteins related to inflammatory and oxidative stress pathways, mainly including MAPK and NF-κB pathways, which play an important role in the occurrence and development of inflammatory response [34, 35]. NF-κB p65/p50 dimer is an important component of NF-κB pathway and a main target of inflammatory therapy. p38 MAPK is involved in the synthesis of inflammatory factors, and the imbalance of ERK is related to chronic inflammatory diseases [36-39]. Gui-Hong herb pair obviously inhibited the expressions of ERK1/2, p38 MAPK, IκBα, and the nucleation reaction of NF-κB p65, thereby preventing the release of inflammatory factors and oxidants.

LPS is often used as the establishment agent of ALI mouse model by various ways of administration and different dosage. The inflammatory reaction caused by intraperitoneal injection of LPS is mainly systemic reaction, which is manifested in the serious inflammatory reaction of the whole body [40-42]. We used intraperitoneal injection of LPS (5mg/mL) for 24 h to establish the ALI model. LPS seriously affects the life of mice and even threatens the survival of mice. LPS caused the mice depression, loss of appetite and significant weight loss. The short-term intervention of HH and DG alone or Gui-Hong herb pair could not improve the weight loss during ALI. However, the mice in the Gui-Hong herb pair had good mental state and the explosion of fur was relieved. We considered whether the long-term administration of the Gui-Hong herb pair can offset the transient stimulation of LPS, which needs to be further explored Gui-Hong herb pair reduced the visceral body coefficient of lung and spleen, which might be related to the inhibition of inflammatory response. Meanwhile, Gui-Hong herb pair improved the problem of pulmonary edema during ALI according to the wet to dry of lung. In addition, the changes of the permeability of pulmonary capillaries and the barrier effect of epithelial cells can cause the infiltration of some inflammatory factors. We used the method of tail vein injection

of Evans blue dye to explore the problem of capillary permeability. Gui-Hong herb pair reduced the abnormal increase of capillary permeability during ALI, so as to prevent the intensification of inflammatory reaction and slow down the process of ALI. We further investigated the effect of Gui-Hong herb pair on oxidation and antioxidation in lung tissue and found that they increased the level of antioxidant SOD and reduced the content of oxide MDA. Gui-Hong herb pair reduced the promotion of inflammatory cytokines IL-6 and TNF-α in vitro and in vivo, and decreased the inflammatory infiltration and damage of lung tissue by HE staining. Through the detection of inflammatory and antioxidant stress pathways related proteins, we found that the treatment of ALI of the Gui-Hong herb pair might be related to blocking the activation of MAPK and NF-κB signaling pathways. We speculated that Gui-Hong herb pair protected the LPS induced ALI through regulating inflammatory signaling pathways (Figure 8).

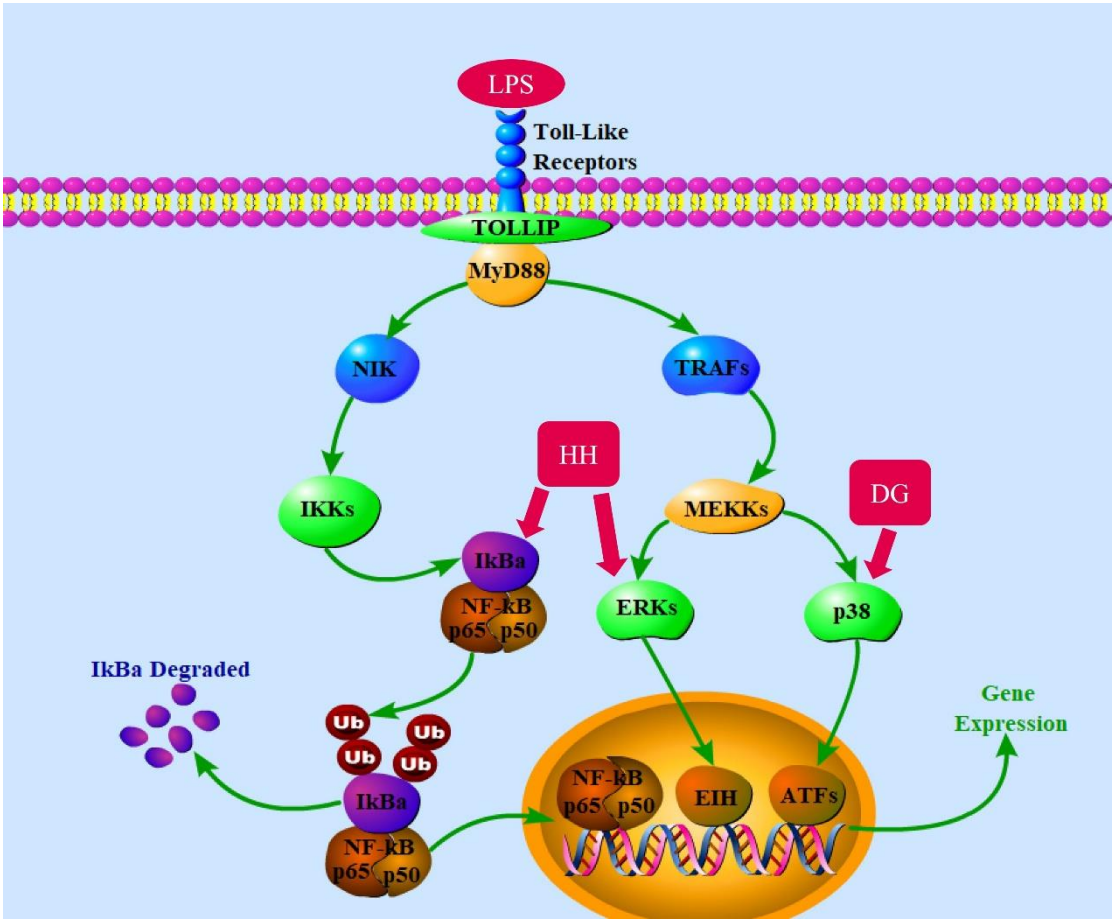


**Figure 8 The proposed mechanism of the synergy of the Gui-Hong herb pair in ALI.**

## Conclusion

Collectively, our study confirmed the potential of the Gui-Hong herb pair in the prevention of ALI through i*n vitro* and *in vivo* experiments. They could protect lung damage by inhibiting the oxidative stress and inflammatory response of lung epithelial cells, which was related to the MAPK and NF-κB pathways. This study provided a scientific basis for further elucidating the effect and clinical application of HH and DG on ALI.

## Author contributions

Conceptualization, Zhen Liu; Investigation, Jinyao Wang, Jiawen Wang, Yang Yu, Yu Wang; Formal analysis, Jinyao Wang and Jiawen Wang; Supervision, Yuou Teng; Writing – original draft, Zhen Liu; Writing – review & editing, Zhen Liu.

## Conflicts of Interest

No potential conflict of interest was reported by the author(s).

## Data availability

All data generated or analyzed during this study are included in this published article.

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Abbreviations: HH, Honghua; DG, Danggui; ALI, acute lung injury; LPS, Lipopolysaccharides; HPLC, High Performance Liquid Chromatography; COVID-19, corona virus disease 2019; CI, combined index; NO, nitric oxide; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor kappa-B; DEX, dexamethasone; SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species.