Original Article

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# Shufeng Jiedu Capsule protect against acute lung injury by suppressing the MAPK/NF-κB pathway

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**Summary** **This study sought to investigate the protective effect of an alternative medicine, Shufeng Jiedu Capsule, in acute lung injury and inflammation signaling pathways related to that action. Hematoxylin and eosin (HE) staining was used to observe pathological changes in rat lung tissue, arterial blood was subjected to blood gas analysis and lactic acid levels were determined, immunofluorescent staining for interleukin-1β (IL-1β) was performed, enzyme linked immunosorbent assay (ELISA) was used to detect biomarkers of the nuclear factor-κB (NF-κB) inflammation pathway including IL-1β and tumor necrosis factor α (TNF-α), biomarkers of the mitogen-activated protein kinase (MAPK) signal pathway including P-selectin, transforming growth factor β (TGF-β), keratinocyte-derived chemokine (KC), and C-Jun/AP-1 were measured, and real-time PCR was used to detect NF-κB mRNA. Results in rats with lipopolysaccharide-induced acute lung injury suggested that Shufeng Jiedu Capsule may increase the partial pressure of oxygen in lung tissue, decrease lactic acid levels, inhibit inflammatory factors such as IL-1β and TNF-α, and suppress the levels of P-selectin, TGF-β, KC, C-Jun/AP-1, and NF-κB mRNA. Thus, Shufeng Jiedu Capsule is a traditional medicine that may alleviate acute lung injury by suppressing the MAPK/NF-κB signaling pathway.**

***Keywords:*** Acute lung injury, Shufeng Jiedu Capsule, dexamethasone, rat model, MAPK/NF-κB signaling pathway

## 1. Introduction

H7N9 avian influenza, a disease caused by the H7N9 avian influenza virus, can lead to acute lung injury. In March 2013, H7N9 avian influenza was found in Shanghai and Anhui Province in China and recurred in eastern and southern China in early 2014. Human infection with the virus has resulted in a high mortality rate of about 20-30% according to recent data (*1*). Human infection with H7N9 avian influenza has received a great deal of attention from China's Ministry of Health and the World Health Organization. In the early stages of H7N9 influenza, patients have flu-like symptoms such as a fever, coughing, and headaches. Some patients have more severe symptoms, such as a high fever that remains above 39°C, expiratory dyspnea, hemoptysis, and acute lung injury; patients usually die of acute respiratory distress syndrome caused by respiratory failure (*2*).

Acute lung injury (ALI) is characterized by acute hypoxemic respiratory failure with several potential causes, including trauma, shock, viruses, and bacterial endotoxins (*3*). ALI can lead to injury of alveolar epithelial cells and capillary endothelial cells and diffuse pulmonary interstitial and alveolar edema. When lung tissue is affected by a virus or bacterial endotoxin, G protein-coupled receptors (membrane proteins) in lung cells are activated. Cells are increasingly exposed to intracellular oxidative stress and the downstream

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inflammatory pathway is triggered, leading to ALI (*4,5*).

Dexamethasone (DXMS) is a type of synthetic glucocorticoid and is a routine medication to control the inflammatory response (*6*). DXMS alleviates hypernomic inflammation and injury by inhibiting NF-κB activity. However, there is disagreement as to the clinical value of DXMS since it cannot improve prognosis in the early stages (*7*). Thus, only a small dose of DXMS is recommended to treat advanced cases of fibrosis. DXMS causes several adverse reactions such as decreased immune function, blood pressure and blood glucose fluctuations, hemorrhaging of the digestive tract, and femoral neck necrosis (*8,9*). Thus, there is an urgent need for an alternative in clinical settings.

Shufeng Jiedu Capsule (SFJDC) is a traditional Chinese medicine that is mainly used to treat upper respiratory tract infections such as the flu, swelling and pain in the throat, mumps, and strep throat (*10*). A previous study has suggested that SFJDC can inhibit viruses such as Adv-7, RSV, HSV-1, and the flu virus and bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* (*11*). In China, SFJDC has been listed as drug to combat avian influenza. Studies have reported that active constituents of SFJDC such as resveratrol and quercetin may alleviate inflammation by suppressing the mitogen-activated protein kinase (MAPK)/nuclear factor-κB (NF-κB) signaling pathway (*12-14*). However, few studies have examined the effect of SFJDC on cellular signaling pathways. Thus, the current study sought to explore the effect of treatment with DMXS and SFJDC in rats with lipopolysaccharide (LPS)-induced ALI.

## 2. Materials and Methods

### 2.1. *Materials*

Endotoxin was purchased from Sigma Aldrich (St. Louis, MO, USA). SFJDC were purchased from Jiren Pharmaceutical (Anhui, China). A real-time PCR kit and enzyme linked immunosorbent assay (ELISA) kit for interleukin-1β (IL-1β) were purchased from Univ-Bio (Shanghai, China). ELISA kits for P-selectin, transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), C-Jun/AP-1, and keratinocyte-derived chemokine (KC) were purchased from Shanghai United Cell Biotechnology Corporation (Shanghai, China).

### 2.2. *Animals*

Eighty specific-pathogen-free male Sprague-Dawley rats weighing 250 ± 20 g were purchased from Fudan University's Animal Research Center (Shanghai, China). ALI model rats were induced by intraperitoneal injection of 10 mg/kg LPS. Rats were randomly divided into four groups: a saline control group, rats with LPS-induced ALI, rats with LPS-induced ALI that were treated with dexamethasone (DXMS), and rats with LPS-induced ALI that were treated with SFJDC. There were 20 rats in each group.

The saline control group and rats with untreated ALI were administered 2 mL/kg saline. Rats treated with DXMS were intraperitoneally injected with dexamethasone 5 mg/kg, and rats treated with SFJDC were intragastrically administered SFJDC 100 mg/kg. The above treatments were given once daily and continued for 7 days. On day 1, day 3, day 5, and day 7, rats were anesthetized with ether and sacrificed. Tissue specimens were taken from 5 random rats in each of the 4 groups. Arterial blood was collected and the lungs were removed. Tissue of the right lower lung was preserved in liquid nitrogen. The left lung was fixed in 40% paraformaldehyde and then sliced into paraffin sections. These paraffin sections were used in hematoxylin and eosin (HE) staining and confocal laser scanning microscopy (Leica TCS SP8, Solms, Germany) of IL-1β fluorescence.

### 2.3. *ELISA assay for IL-1β, P-selectin, TGF-β, TNF-α, KC, and C-Jun/AP-1*

The right lower lung was removed from liquid nitrogen, washed with cold saline, and dried with filter paper. One hundred mg of lung tissue was made into a homogenate with cold saline. ELISA was used to detect levels of IL-1β, P-selectin, TGF-β, TNF-α, KC, and C-Jun/AP-1 expression in this homogenate.

### 2.4. *Real-time PCR detection of NF-κB mRNA*

Total RNA was collected using Trizol (Gibco-BRL, Gaithersburg, USA). cDNA was prepared using a 1-step PCR kit (Promega, USA) and PTC-200 PCR device (MJ Research, USA). The primer sequence was as follows: rGAPD (5'-AGTGCCAGCCTCGTCTCATAG-3' and 5'-CGTTGAACTTGCCGTGGGTAG-3'). The primer had a melting temperature (Tm) of 59°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal reference. PCR products were analyzed using Quantity One software (Bio-Rad, Hercules, USA).

### 2.5. *Confocal laser scanning imaging*

Tissue slices were washed 3 times with PBS, fixed in methanol for 30 min, washed 3 times with PBS, denatured with HCl for 40 min, washed 3 times with PBS, blocked with sheep serum for 30 min, washed 3 times with PBS, and incubated with IL-1β monoclonal body overnight. Slices were washed 3 times with PBS and incubated with the secondary antibody. Confocal laser scanning microscopy (Leica TCS SP8, Solms, Germany) was used to observe fluorescence.

### 2.6. *Statistical analysis*

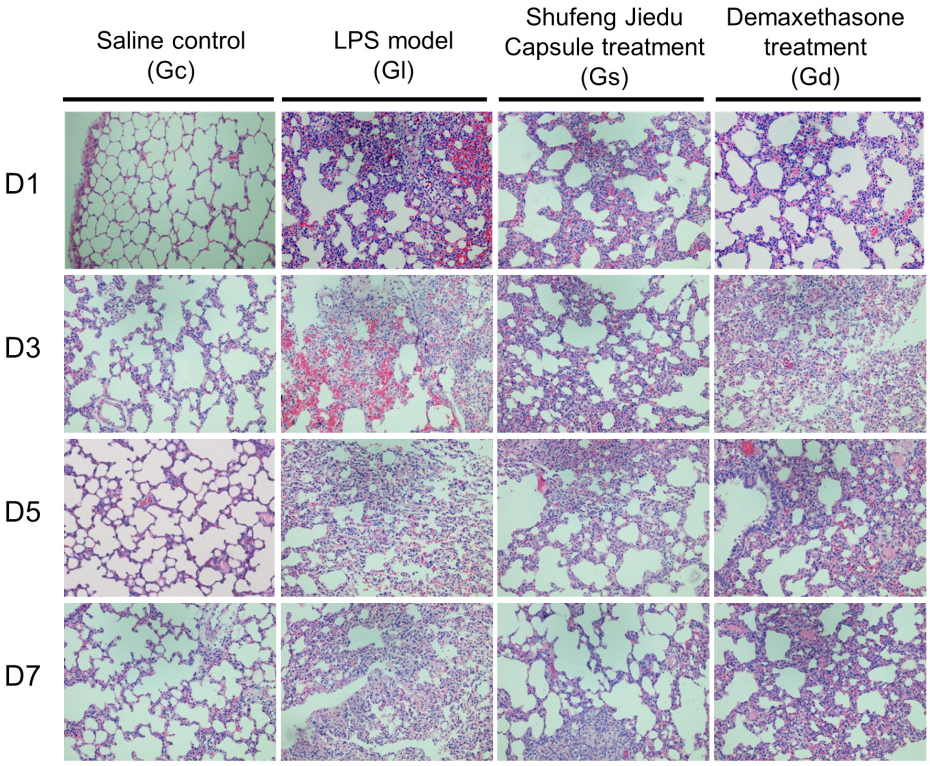


**Figure 1. HE staining of rat lung tissue.** D1: day 1; D3: day 3; D5: day 5; D7: day 7.

Data were analyzed using SPSS 18.0 software and are presented as the mean ± standard deviation (S.D.). A homogeneity of variance and a normal distribution were verified. Once data were found to have a homogeneity of variance and a normal distribution, one-way ANOVA was performed. If results were statistically significant, a pair-wise comparison was done using the least significant difference test. A $p < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. *Morphological changes in lung tissue*

As shown in Figure 1, lung tissue from rats with untreated ALI had severe alveolar exudation, hemorrhaging, edema, small airway collapse, and necrosis of airway epithelial cells on day 1. Exudation and hemorrhaging were not noted in tissue from rats treated with DXMS and rats treated with SFJDC. Lung tissue retained its basic structure, and the inflammatory response was milder in rats treated with DXMS or SFJDC than in rats with untreated ALI. On day 3, more severe inflammation was noted in lung tissue from rats with untreated ALI with obvious exudation and destruction of alveolar structures. Rats treated with DXMS and rats treated with SFJDC also had inflammation but alveolar structures remained and inflammation was mild. Rats treated with SFJDC had milder inflammation than rats treated with DXMS. On day 5 and day 7, inflammation had subsided in all of the rats. The inflammatory response was much milder in rats treated with SFJDC.

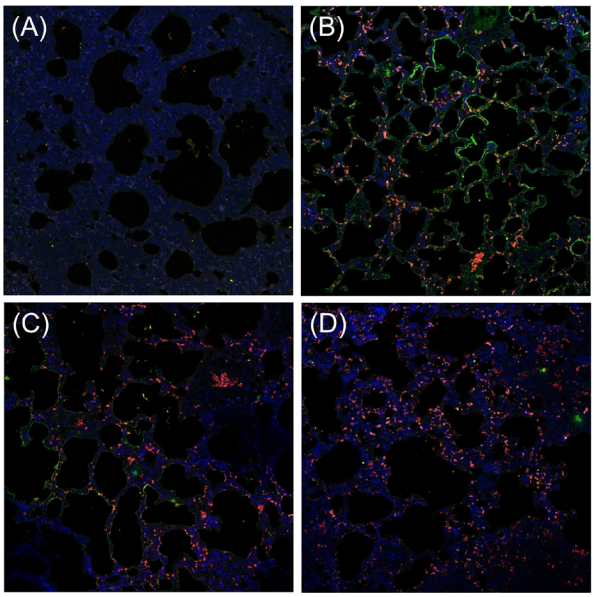


**Figure 2. Confocal laser scanning microscopy image of rat lung tissue.** Rat lung tissue sections were labeled with IL-1β (Green) and were then observed using confocal laser scanning microscopy. **(A)**: saline control group; **(B)**: rats with untreated ALI; **(C)**: rats treated with Shufeng Jiedu Capsule; **(D)**: rats treated with dexamethasone.

### 3.2. *Immunofluorescent staining for IL-1β*

As shown in Figure 2, the saline control group had no obvious green fluorescence for IL-1β, indicating

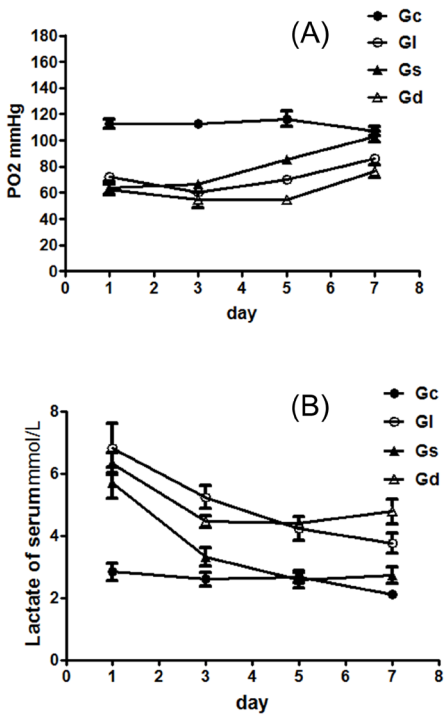


**Figure 3. Partial pressure of oxygen and lactic acid levels.** The partial pressure of oxygen and lactic acid levels in arterial blood from rats were measured. **(A)**: measurement of the partial pressure of oxygen; **(B)**: determination of lactic acid levels. Gc: saline control group; Gl: rats with untreated ALI; Gs: rats treated with Shufeng Jiedu Capsule; Gd: rats treated with dexamethasone.

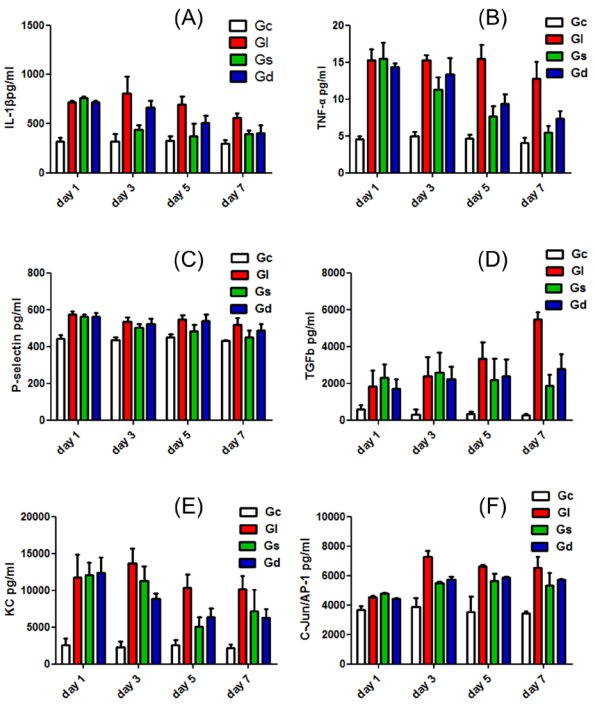


**Figure 4. ELISA detection of IL-1β, TNF-α, P-selectin, TGF-β, KC, and C-Jun/AP-1 in rat lung tissue.** Levels of IL-1β **(A)**, TNF-α **(B)**, P-selectin **(C)**, TGF-β **(D)**, KC **(E)**, and C-Jun/AP-1 **(F)** expression in rat lung tissue were detected using ELISA. Gc: saline control group; Gl: rats with untreated ALI; Gs: rats treated with Shufeng Jiedu Capsule; Gd: rats treated with dexamethasone.

a very low level of IL-1β. Rats with untreated ALI had significant green fluorescence. Compared to the saline control group, rats treated with SFJDC and rats treated with DXMS had green fluorescence, indicating an increased level of IL-1β. Green fluorescence was markedly less intense than that noted in rats with untreated ALI, indicating that SFJDC and DXMS had decreased the level of IL-1β.

### 3.3. *Blood gas analysis of arterial blood and determination of lactic acid levels*

As shown in Figure 3, the partial pressure of oxygen remained between 107-113 mmHg in the saline control group. On day 1, day 3, day 5, and day 7, the partial pressure of oxygen increased gradually from 60 mmHg to 86 mmHg in rats with untreated ALI. The partial pressure of oxygen generally increased from 64 mmHg to 103 mmHg in rats treated with SFJDC and it increased slightly from 54 mmHg to 76 mmHg in rats treated with DXMS. These results indicate that rats treated with SFJDC had a higher partial pressure of oxygen than did rats with untreated ALI on day 5 and day 7. However, rats treated with DXMS had a lower partial pressure of oxygen than rats did with untreated ALI at these two times. On day 1, day 3, day 5, and day 7, the lactic acid level in the saline control group remained between 2.6-2.8 mmol/L. The lactic acid level ranged from 6.8 mmol/L to 3.8 mmol/L in rats with untreated ALI, from 5.7 mmol/L to 2.7 mmol/L in rats treated with SFJDC, and from 6.3 mmol/L to 4.4 mmol/L in rats treated with DXMS. SFJDC markedly reduced the level of lactic acid on day 3, day 5, and day 7 ($p < 0.05$) in comparison to that in rats with untreated ALI. However, there was no evidence of a decrease in the level of lactic acid in rats treated with DXMS.

### 3.4. *Results of ELISA detection of IL-1β, TNF-α, P-selectin, TGF-β, KC, and C-Jun/AP-1*

As shown in Figure 4, ELISA was used to determine levels of IL-1β, TNF-α, P-selectin, TGF-β, KC, and C-Jun/AP-1 in each group at different times. The level of IL-1β remained between 300-325 pg/mL in the saline control group. The level of IL-1β ranged from 809 to 557 pg/mL in rats with untreated ALI, from 761 to 376 pg/mL in rats treated with SFJDC, and from 715 to 399 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of IL-1β on day 3, day 5, and day 7 ($p < 0.05$). There were no significant differences in the level of IL-1β for rats treated with SFJDC and rats treated with DXMS. The level of TNF-α remained between 4.0-4.9 pg/mL in the saline control group. The level of TNF-α ranged from 15.5 to 12.8 pg/mL in rats with untreated ALI, from

15.5 to 5.5 pg/mL in rats treated with SFJDC, and from 14.3 to 7.4 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of TNF-α on day 3, day 5, and day 7 ($p < 0.05$). There were no significant differences in the level of TNF-α for rats treated with SFJDC and rats treated with DXMS. The level of P-selectin remained between 442-451 pg/mL in the saline control group. The level of P-selectin ranged from 574 to 517 pg/mL in rats with untreated ALI, from 562 to 452 pg/mL in rats treated with SFJDC, and from 561 to 487 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of P-selectin on day 5 and day 7 ($p < 0.05$). The level of TGF-β remained between 253-585 pg/mL in the saline control group. The level of TGF-β ranged from 1,828 to 5,466 pg/mL in rats with untreated ALI, from 2,595 to 1,874 pg/mL in rats treated with SFJDC, and from 1,712 to 2,801 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of TGF-β on day 5 and day 7 ($p < 0.05$). There were no significant differences in the level of TGF-β for rats treated with SFJDC and rats treated with DXMS. The level of KC remained between 2,215-2,606 pg/mL in the saline control group. The level of KC ranged from 13,666 to 10,148 pg/mL in rats with untreated ALI, from 12,099 to 5,034 pg/mL in rats treated with SFJDC, and from 12,400 to 6,309 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of KC on day 3, day 5, and day 7 ($p < 0.05$). There were no significant differences in the level of KC for rats treated with SFJDC and rats treated with DXMS. The level of C-Jun/AP-1 remained between 3,426-3,676 pg/mL in the saline control group. The level of C-Jun/AP-1 ranged from 4,551 to 6,644 pg/mL in rats with untreated ALI, from 4,780 to 5,341 pg/mL in rats treated with SFJDC, and from 4,426 to 5,727 pg/mL in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of C-Jun/AP-1 on day 3, day 5, and day 7 ($p < 0.05$). There were no significant differences in the level of C-Jun/AP-1 for rats treated with SFJDC and rats treated with DXMS.

### 3.5. *Level of NF-κB mRNA expression*

As shown in Table 1, the level of NF-κB mRNA expression in rat lung tissue was determined using real-time PCR and was analyzed using the 2-ΔΔCt method. The mean value of nucleic acid fluorescent intensity was first determined. The mean value for the control group was subtracted from the mean value for an experimental group, and the difference was squared. The resulting values reflected a relative change in mRNA expression. The level of expression remained at around 2.0 in the saline control group, it increased from 1.80 (day 1) to 5.99 (day 3) and then decreased to 3.14 (day 7) in rats with untreated ALI, it increased from 1.5 (day 1) to 2.19 (day 3) and then decreased to 1.37 (day 7) in rats treated with SFJDC, and it increased from 2.57 (day 1) to 2.95 (day 3) and then decreased to 1.83 (day 7) in rats treated with DXMS. Compared to rats with untreated ALI, rats treated with SFJDC had an obvious decrease in the level of NF-κB mRNA ($p < 0.05$).

## 4. Discussion

According to previous studies, DXMS may alleviate the inflammatory response by suppressing the MAPK/NF-κB signaling pathway. Numerous studies have indicated that the active constituents of SFJDC, such as resveratrol, quercetin, and other flavonoids, inhibit

**Table 1. Level of NF-κB mRNA expression in rat lung tissue**

| Day | Saline control | Rats with untreated ALI | Rats treated with SFJDC | Rats treated with DXMS |
|---|---|---|---|---|
| 1 | 2.18 ± 0.95 | 1.80 ± 0.58* | 1.50 ± 0.36# | 2.57 ± 0.65 |
| 3 | 1.80 ± 0.28 | 5.99 ± 1.37* | 2.19 ± 0.62# | 2.87 ± 1.06 |
| 5 | 2.14 ± 0.69 | 4.22 ± 1.08* | 1.84 ± 0.29# | 2.95 ± 0.92 |
| 7 | 2.15 ± 0.70 | 3.14 ± 0.70* | 1.37 ± 0.13# | 1.83 ± 1.09 |

*$p < 0.05$; rats with untreated ALI *vs*. saline control; #$p < 0.05$ rats treated with SFJDC *vs*. rats with untreated ALI.

**Table 2. Active constituents of Shufeng Jiedu Capsule and responding signaling pathways**

| Herb | Constituent type | Constituent | Anti-inflammation pathway | *Ref.* |
|---|---|---|---|---|
| *Polygonum cuspidatum* | stilbene | resveratrol | NF-κB pathway and PI3K pathway | (*12*) |
| | anthraquinone | emodin | MAPK and NF-κB pathway | (*15*) |
| | | rhein | MAPK and NF-κB pathway | (*19*) |
| | flavonoid | quercetin | MAPK and NF-κB pathway | (*13*) |
| *Forsythia suspensa* | flavonoid | rutin | NF-κB pathway | (*20*) |
| *Bupleurum chinense DC.* | flavonoid | kaempferol and quercetin | MAPK and NF-κB pathway | (*16,13*) |
| *Glycyrrhiza uralensis* | flavonoid | liquiritigenin and liquiritin | NF-κB pathway | (*17*) |

the MAPK/NF-κB signaling pathway in several cell lines (Table 2) (*15-21*). The current study sought to investigate whether SFJDC could provide protection from ALI by inhibiting inflammatory signaling pathways.

NF-κB is a key factor in the inflammatory response and is involved in the development of ALI (*22,23*). When patients are affected by trauma, shock, a virus, or a bacterial endotoxin, the NF-κB signaling pathway is activated. Inflammatory cells such as neutrophil granulocytes and macrophages are subsequently triggered, inflammatory mediators such as IL-1β and TNF-α are released, and oxidative stress is promoted (*24,25*). IL-1β and TNF-α may serve as biomarkers of the NF-κB inflammatory pathway. A previous study using DXMS as a positive control determined levels of IL-1β and TNF-α to assess the protective effect of SFJDC in a rat model of LPS-induced ALI (*26*). Confocal laser scanning imaging revealed an obvious decrease in the levels of IL-1β in both rats treated with SFJDC and rats treated with DXMS compared to rats with untreated ALI. This finding suggests that SFJDC may alleviate inflammation by inhibiting IL-1β in the NF-κB signaling pathway. Real-time PCR indicated that expression of NF-κB mRNA was effectively suppressed in rats treated with SFJDC in comparison to that in rats with untreated ALI ($p < 0.01$). Thus, SFJDC may alleviate ALI by inhibiting the NF-κB-dependent inflammatory response.

MAPKs (also known as MAP kinases) are serine/threonine/tyrosine-specific protein kinases belonging to the CMGC (CDK/MAPK/GSK3/CLK) kinase group, and the P38δ MAPK receptor is expressed at high levels in lung tissue (*27*). MAPK phosphorylation is associated with activation with NF-κB in the inflammatory response (*28*). MAPKs function in stress, inflammation, tumor, cell growth, differentiation, apoptosis, and fibrosis. P-selectin, TGF-β, KC, and C-Jun/AP-1 are involved in the MAPK signaling pathway, and they may serve as biomarkers of the MAPK pathway (*29-32*). An ELISA assay revealed that rats treated with SFJDC had a more obvious decrease in the level of P-selectin, TGF-β, KC, and C-Jun/AP-1 in lung tissue in comparison to rats with untreated ALI ($p < 0.01$). This indicates that SFJDC may alleviate LPS-induced stress injury, decrease lung cell apoptosis, and protect lung tissue by inhibiting the MAPK signaling pathway.

The pathology of rat lung tissue was observed using HE staining. Like rats treated with DXMS, rats treated with SFJDC had a much milder inflammatory response than rats with untreated ALI. Blood gas analysis of arterial blood and determination of lactic acid levels indicated that rats treated with SFJDC had an obvious increase in the partial pressure of oxygen and a lower lactic acid level than rats with untreated ALI. Thus, SFJDC effectively decreased the hypoxia-induced inflammatory response and protected lung tissue. In conclusion, this study revealed that SFJDC alleviates LPS-induced inflammation in a rat model by suppressing the MAPK/NF-κB signaling pathway. Thus, SFJDC might serve as an alternative medication to inhibit inflammation in different organs and SFJDC has great potential for wide clinical use.

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