Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.043

RESEARCH ARTICLE

The Therapeutic Effect and Mechanism of *Physalin* on LPS-Induced Acute Lung Injury in Rats

Qiu Zhong[1], Yaogui Sun[1], Yinlan Xu[1], Ajab Khan[1], Jianhua Guo[2], Zhirui Wang[3], Na Sun[1] and Hongquan Li[1*]

[1]Shanxi Agricultural University, No. 1, Mingxian South Road, Taigu County, Jinzhong City, Shanxi Province, P.R. China; [2]USA Texas A&M university, College Station, TX, USA; [3]University of Colorado Denver Anschutz Medical Campus RC2-6013, Mail Stop 8621 12700 E 19th Ave. Aurora, CO 80045
*Corresponding author: livets@163.com

ARTICLE HISTORY (20-536)

Received: October 24, 2020
Revised: December 31, 2020
Accepted: January 01, 2021
Published online: May 11, 2021

Key words:
LPS
lung injury
MAPK
NF-κB
Physalin

ABSTRACT

Physalin is the main component of *Physalis alkekengi L.var.franchetii.* Based on research on Chinese herbal medicine, it is mainly used to treat respiratory diseases. LPS-induced acute lung injury (ALI), characterized by pulmonary edema and respiratory distress, was treated with *Physalin*. Firstly, Rats were treated with LPS followed by the gavages of *Physalin*. In vitro, *Physalin* was applied to RLE-6TN cell line pre-treated with LPS. LPS caused inflammation, hemorrhages, and lung dysfunction and apoptosis in rats. *Physalin* significantly attenuated these adverse effects, decreased the expression of IL-1β and TNF-α in lung tissues. Increased the expression of inhibitor of NF-κB (IκB), decreased the translocation of NF-κB and its downstream protein (COX-2 and IL-1β), and MAPK phosphorylation. In vitro, *Physalin* increased alveolar epithelial cells viability, inhibited activation of MAPK and release of inflammatory factors. *Physalin* can achieve the effect of treating inflammation caused by LPS in rats through reducing the phosphorylation level of IκB, NF-κB, ERK1/2, JNK and p38 MAPK, thereby reducing the expression of inflammatory factors TNF-α, COX2 and IL-β, and reducing the apoptosis of alveolar epithelial cells both in vivo and in vitro.

To Cite This Article: Zhong Q, Sun Y, Xu Y, Khan A, Guo J, Wang Z, Sun N and Li H, 2021. The therapeutic effect and mechanism of *Physalin* on LPS-induced acute lung injury in rats. Pak Vet J, 41(3): 372-378. http://dx.doi.org/10.29261/pakvetj/2021.043

INTRODUCTION

Acute lung injury (ALI) is a diffuse lungs lesions caused by severe infection, shock, trauma, severe hypoxemia and respiratory distress. Sepsis caused by lipopolysaccharide (LPS) is the most common cause of ALI (Dong *et al*., 2008). ALI is characterized by inflammatory cell invasion, alveolar epithelium destruction and leakage of proteins into the alveolar cavity. Results in alveolar and interstitial effusion, pulmonary edema and ultimately respiratory failure (Abraham *et al*., 2000; Coimbra *et al*., 2006). Acute respiratory distress syndrome (ARDS) is the most serious state of ALI (He *et al*., 2012). Therefore, the development of new drugs to treat LPS-induced ALI has become very important.

Currently, protective mechanical ventilation, optimal fluid management and nutritional supports are some of the measure adopted for the prevention of ALI/ARDS (Raghavendran and Napolitano, 2011; Nieman *et al*., 2015). Low-tide pulmonary ventilation strategies (6-8mg /kg) have been shown to significantly improve survival among non-drug therapies (Samransamruajkit *et al*., 2005). Treatment with exogenous surfactant (Infasurf) extracted from bovine lungs has been proved to significantly improve survival in patients with ALI/ARDS caused by pediatric direct lung injury. Therefore, the development of highly effective treatment drugs for LPS-induced ALI has become urgent.

The inflammatory response produced by LPS mediated by the NF-κB signaling pathway can damage lungs (Gris *et al*., 2008; Fan *et al*., 2015). Increase in the expression of inflammatory cytokines and activation of MAPK pathway causes inflammatory response, and lung injury induced by the NF-κB signaling pathway (Mukhopadhyay *et al*., 2008; Wu *et al*., 2015). Due to accumulation of inflammatory factors, a large amount of fluid exudes from the alveoli accompanied by hemorrhage and results in the formation of micro thrombi. Anti-inflammatory substances in this regard can increase resistance to LPS induced lungs tissue injury.

Recently, use of natural compounds against pathological conditions has gained considerable popularity. *Physalin* is the main component of *Physalis alkekengi L. var. franchetii.* According to the TCM treatment concept, it is mainly used to treat respiratory diseases. The 50% EtOH extract of *Physalis alkekengi L.var.franchetii.* showed good anti-inflammatory and antioxidant activity in THP-1 cells induced by lipopolysaccharide (LPS) (Zunpeng *et al*., 2016). The methanol extract of *Physalis alkekengi L.var.franchetii.* showed a good therapeutic effect in ovalbumin-induced mouse asthma model, effectively reducing the number of inflammatory cells and the production of IL-4, IL-5 and IL-13 in the alveolar lavage fluid (Li *et al*., 2017). Januário *et al*. (2010) and Pinto *et al*. (2010) have also confirmed that *Physalin* has good anti-inflammatory activity (Januário *et al*., 2010; Pinto *et al*., 2010). However, the underlined hidden molecular mechanism of *Physalin* treatment involved in LPS-induced lung injury is still unclear.

In this study, we investigated the effect of *Physalin* on the production of inflammatory factors, lung tissue injury, and cell apoptosis, as well as the role of the activation of the NF-κB signaling pathway.

MATERIALS AND METHODS

Physalin: *Physalin* isolated from the stems of *Physalis alkekengi L.var.franchetii*. The plant was identified by Dr. Qiongming Xu from Collage of Pharmaceutical Science, Soochow University. *Physalin* was purified and the purity of *Physalin* is 72.39% by chromatography. The chromatographic results are shown in Appendices Fig. 1 A.

Culture of alveolar epithelial cells: RLE-6TN cells (ATCC® CRL-2300™) were seeded in 96-well plate and incubated for 24h. The various concentrations of *Physalin* (100, 75, 50and 25μg/mL) were added and incubated again for 24h. The maximum non-toxic concentration (MNTC) of *Physalin* was determined using CCK8 kit. To calculate cell proliferation, different concentrations of LPS (10, 8, 4, 2, 1, 0.5 and 0.25μg/mL) were added to a 24h grown RLE-6TN cells in 96-well plate and incubated for the next 24h. To develop a cellular inflammation model, LPS (1μg/ml) was added to each well except the blank group and incubated for the next 24h. Different concentrations of *Physalin* (50, 25 and 12.5μg/mL) were added as treatment and incubated again for12h.

Animals and treatments: Sixty male SD rats (220±20g, 6~8weeks) were purchased from Experimental Animal Centre of Shanxi Medical University and were randomly divided into blank, Model and three *Physalin* treatment groups (high, medium and low dose). The rats were housed under basic environmental conditions (20±2°C, 50±5% relative humidity with 12h-dark/light-cycle) with standard maintenance diet. LPS (2mg/kg) was administered via trachea to each group except the blank one. After 24h of LPS administration, rats were treated for 7 consecutive days with *Physalin* by gavages (100, 50 and 25 mg/kg). The equivalent volume of saline was given to the blank and model groups by gavage. At day 1, 3, 5 and 7of treatment, blood and lungs from 3 rats within each group were collected. All animal experiments were conducted in accordance with the guidance of the Animal Experiment Ethics Committee of Shanxi Agricultural University.

Complete blood count: The whole blood was collected from the tail vein of rats and complete blood count was obtained immediately using an Auto Hematology Analyzer HF-3800Vet (Healife, China).

Lung inflammatory factor levels: Lungs from euthanized rats were collected and homogenized. Then, levels of TNF-α and IL-1β were measured with ELISA kit (MSKBIO, China).

Calculation of wet/dry lung weight ratio: Half of the lungs from euthanized rats were collected and weighed after removing adipose tissues. After 48 h of drying in oven at 80°C, the lungs were weighted again and wet/dry weight ratio was calculated.

Lung histopathology: The Bouin's Fluid-fixed 7th day lung tissues were embedded in paraffin. The paraffin-embedded tissues were cut into 5-μm-thick sections and stained with hematoxylin and eosin. The histopathology was observed using a light microscope (Olympus BX51).

TUNEL staining method to detect apoptosis: Apoptosis on the 7th day lung tissue was observed by TUNEL staining (Promega, Beijing, China). The apoptosis of lung tissue was observed in each sample using fluorescence microscope (Olympus BX51).

Western blot: RLE-6TN cells and Lung tissues were homogenized with RIPA lysate (Solarbio, China). 40μg equivalent protein samples were separated by SDS-PAGE gel electrophoresis. GAPDH protein (Sanying, China) was used as loading control. The antibodies for ERK1/2, p-ERK1/2, JNK, p-JNK, p38, p-p38, IκB, p- IκB, NF-κB p65, p- NF-κB p65, COX2, IL-1β were purchased by Abcam (UK). All secondary antibodies were from Sanying. Protein expression was analyzed by scanning densitometry using Image J software.

Statistical analysis: The data are represented in the figures as the mean ± SEM. The differences between groups were tested with a t test with statistical processing by SPSS 19.0 software. A P-value of $P<0.01$ was taken to indicate statistically significant differences.

RESULTS

The effect of *Physalin* on RLE-6TN pretreated with LPS: The working concentration for *Physalin* in RLE-6TN was determined. The result was shown in Fig.1A, compared with the blank group, RLE-6TN cells treated with 75μg/mL *Physalin* for 24h, resulted in a significant reduction in cell viability, while 50μg/mL *Physalin* had negligible effect on cell viability. Therefore, we choose 50μg/mL *Physalin* as the working concentration.

Then we determined the concentration of LPS used in RLE-6TN. From Fig.1B we can see that RLE-6TN cells treated with LPS 1μg/mL for 24h resulted in a significant decrease in cell viability.

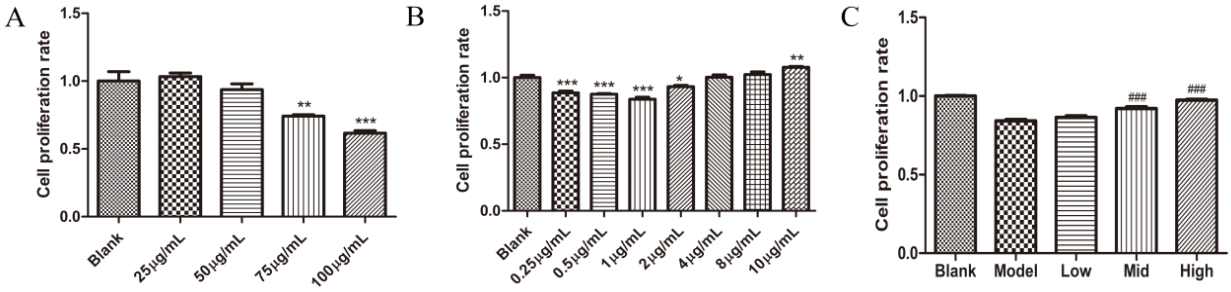

Fig. 1: Cell viability (CCK8) assay results of RLE-6TN cells A: *Physalin*-induced cell proliferation rate; B: LPS-induced cell proliferation rate; C: *Physalin* treatment of the LPS challenged cells. Low: 12.5 μg/mL *Physalin* treatment group; Mid: 25 μg/mL *Physalin* treatment group; High: 50 μg/mL *Physalin* treatment group, the same as below. Compared with the control group, **p*< 0.05, ***p*< 0.01, *** *p*< 0.001. Compared with the LPS + 0% physalin group, #*p*<0.05, ## *p*<0.01, ###*p*<0.001.

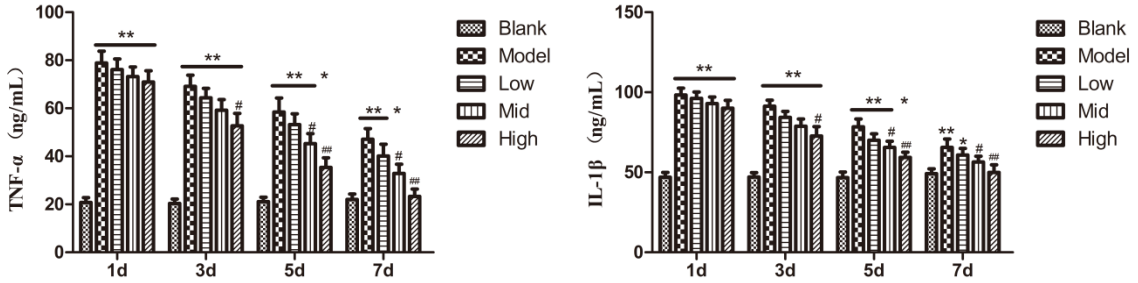

Fig. 2: Effect of *Physalin* on TNF-α and IL-1β expression in rat lung tissue. Compared with the control group, **p*< 0.05, ***p*< 0.01. Compared with the LPS + 0% *Physalin* group, #*p*< 0.05, ## *p*< 0.01.

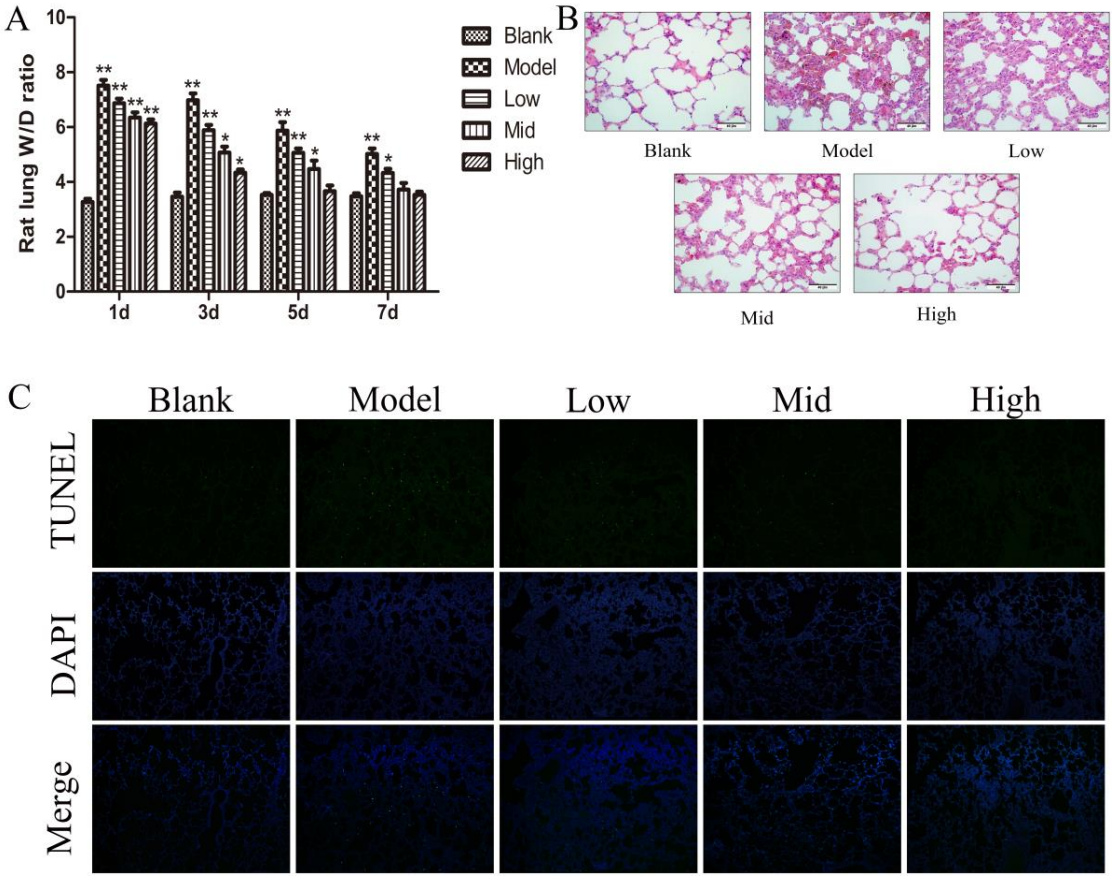

Fig. 3: Efficacy of *Physalin* on LPS induced Lung (rats) histopathological and apoptosis. A: W/D ratio in lung tissue; Representative digital micrographs of histological sections of formalin-fixed paraffin-embedded lung tissue with H&E staining (B) and TUNEL staining (C).

Finally, we first used 1μg/mL of LPS to treat RLE-6TN and then added *Physalin* to the cells to examine the cell viability. As shown in Fig.1C, *Physalin* had significantly reversed the cell viability reduction caused by LPS in a dose dependant manner, and 50μg/mL *Physalin* shows the strongest effect.

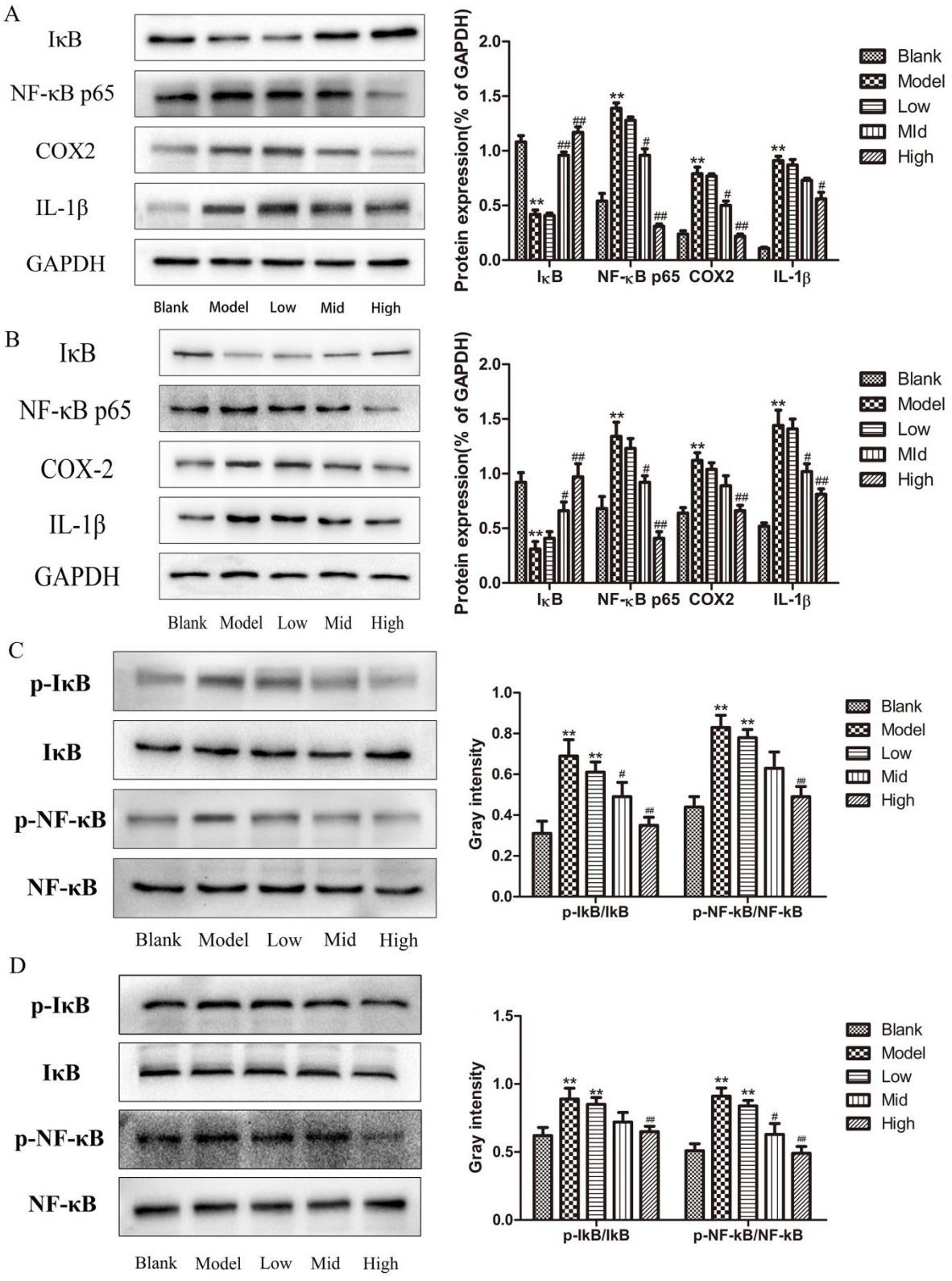

Fig. 4: Effect of *Physalin* on NF-κB pathway proteins expression level. A: IκB, NF-κB, COX2, IL-1β and GAPDH protein expression in RLE-6TN cells and the quantified band intensity data. B: IκB, NF-κB, COX2, IL-1β protein expression in lung tissue of rats and the quantified band intensity data. C: p-IκB, IκB, p-NF-κB and NF-κB protein expression in RLE-6TN cells and the quantified band intensity data. D: p-IκB, IκB, p-NF-κB and NF-κB protein expression in lung tissue of rats and the quantified band intensity data. Compared with the control group, **p*<0.05, ***p*<0.01. Compared with the LPS + 0% *Physalin* group, #*p*<0.05, ## *p*<0.01.

***Physalin* affected the complete blood cell count of rats LPS treated with LPS:** Red blood cells (RBCs) counts, hemoglobin concentration (HGB) and blood platelet (PLT) are specific Indicators for anemia and white blood cells for inflammation and infection. As shown in Table1, in the LPS-treated group, RBC and HGB showed significant decrease while PLT and WBC were increased as compared with blank group. *Physalin* treatment significantly reversed all LPS-induced effects in a dose dependent manner.

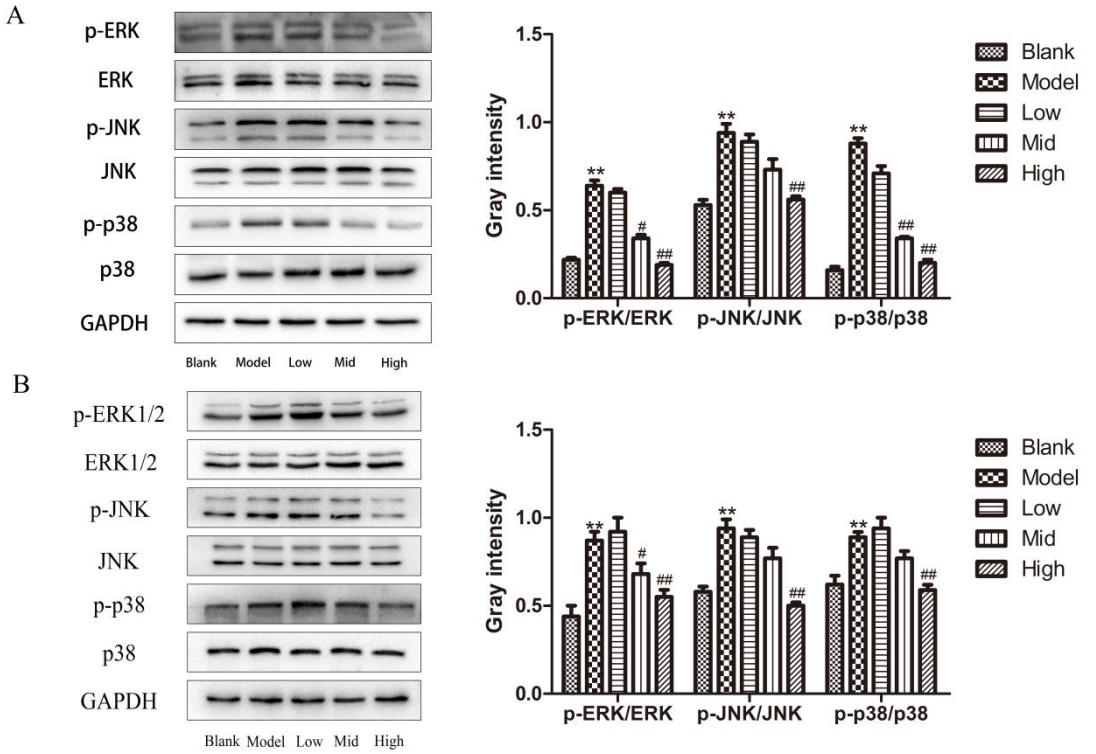

Fig. 5: Western blot analysis of MAPK pathway protein. A:ERK1/2, p-ERK1/2, JNK, p-JNK, p38, p-p38 and GAPDH protein expression in RLE-6TN cells and the quantified band intensity data. B:ERK1/2, p-ERK1/2, JNK, p-JNK, p38, p-p38 and GAPDH protein expression in lung tissue of rats and the quantified band intensity data. Compared with the control group, * P<0.05, ** P<0.01. Compared with the LPS + 0% *Physalin* group, # P<0.05, ## P<0.01.

Table 1: Complete blood count of rats

Complete blood count of rats in each group on day 1 of *Physalin* treatment

Index	Reference	Blank	Model	Low	Mid	High
RBC (×10^9/L)	5.0~9.0	7.02±0.33	5.31±0.21*	5.57±0.24*	5.85±0.48*	6.01±0.29*
HGB (g/L)	100~170	155.36±3.43	100.56±3.98*	108.34±4.42*	112.17±6.21*	116.33±5.37*
PLT (×10^9/L)	700~1500	688. 4±23.57	989.3±32.19*	954. 5±33.24*	928. 1±34.18*	900. 6±29.86*
WBC (×10^9/L)	3.0~15.0	9.00±0.67	34.81±2.07*	31.27±1.99*	30.29±1.85*	29.28±1.34*

Complete blood count of rats in each group on day 3 of *Physalin* treatment

Index	Reference	Blank	Model	Low	Mid	High
RBC (×10^9/L)	5.0~9.0	7.57±0.49	5.52±0.21*	5.88±0.31*	6.02±0.51*	6.22±0.44*
HGB (g/L)	100~170	148.28±3.68	108.88±4.41*	113.35±3.85*	115.45±3.97*	123.38±4.08*
PLT (×10^9/L)	700~1500	703. 7±25.38	952.25±34.41*	917.7±28.86*	894.4±30.31*	855.2±33.35*
WBC (×10^9/L)	3.0~15.0	9.17±1.03	29.22±2.88*	26.24±1.89*	22.25±2.21*	17.91±2.51*

Complete blood count of rats in each group on day 5 of *Physalin* treatment

Index	Reference	Blank	Model	Low	Mid	High
RBC (×10^9/L)	5.0~9.0	7.31±0.29	6.02±0.36*	6.28±0.44*	6.42±0.29*	6.82±0.31
HGB (g/L)	100~170	157.73±4.48	116.68±6.62*	122.34±5.51*	135.31±3.98*	142.41±5.02
PLT (×10^9/L)	700~1500	665.2±18.89	914.4±40.12*	866.3±33.31*	810.2±39.21*	755.9±31.35*
WBC (×10^9/L)	3.0~15.0	8.85±0.97	25.42±2.43*	21.23±2.02*	17.89±1.88*	12.21±1.43*

Complete blood count of rats in each group on day 7 of *Physalin* treatment

Index	Reference	Blank	Model	Low	Mid	High
RBC (×10^9/L)	5.0~9.0	7.48±0.42	6.65±0.54	6.86±0.31	7.02±0.49	7.17±0.33
HGB (g/L)	100~170	152.88±4.79	128.21±5.17*	137.19±4.52	148.29±3.18	155.53±4.12
PLT (×10^9/L)	700~1500	692.3±22.47	803.3±11.83*	752.4±15.24	707.1±13.44	699.1±21.25.
WBC (×10^9/L)	3.0~15.0	8.98±0.67	20.24±3.31*	15.35±2.18*	11.27±0.59	7.71±1.26

*P<0.05 vs. Blank group, **P<0.01 vs. Blank group.

***Physalin* reduces the expression of inflammatory factors in the lung tissue of rats treated with LPS:** The content of IL-1β and TNF-α are important indicators for detecting inflammation in rats. In LPS-treated group, the content of IL-1β and TNF-α in rat lung tissue was significantly increased as shown in Fig. 2. After *Physalin* treatment, the level of inflammatory factors in the lungs of rats was significantly reduced.

Therapeutic effect of *Physalin* on LPS induced rat ALI: The changes of wet–dry (W/D) ratio in lung tissue reflects permeability of alveolar blood vessels and degree of interstitial changes in lungs. In LPS-treated group, W/D of lung tissue was significantly increased as shown in Fig. 3A. In contrast, *Physalin* treatment showed significant decrease in W/D ratio of LPS treated lung tissue in a dose dependent manner. Histopathology results showed clear alveolar space, absence of edema and inflammatory cells infiltration in blank group. In LPS treated group, Alveolar wall edema and hemorrhage, pulmonary interstitial wall thickening, numerous inflammatory cells infiltration in alveolar cavity, destruction of alveolar structure and other

obvious lung injuries were observed. In contrast, *Physalin* treatment significantly alleviated these LPS induced damages in lung tissue. The results were shown in Fig. 3B. Surprisingly, high-dose *Physalin* treatment has almost restored the structure of damaged lung tissues as that of the blank group.

***Physalin* mitigates LPS-induced apoptosis in lung:** TUNEL staining method was used to detect the effect of *Physalin* on LPS-induced apoptosis of alveolar epithelial cells. As shown in Fig. 3C, compared with the blank group, LPS caused apoptosis of alveolar epithelial cells, which can be observed from the increase in the number of TUNEL-positive cells. However, after *Physalin* treatment, the apoptosis of alveolar epithelial cells was significantly reduced.

The effect of *Physalin* on NF-κB-dependent signaling pathway in LPS-induced lung injury: NF-κB signaling pathway proteins were detected by Western-blotting. LPS in vitro treatment significantly reduced the expression of IκB while increased the expression of NF-κB, simultaneously enhanced the activation of IκB and NF-κB. LPS treatment can also increase the expression of COX2, IL-1β and related inflammatory products as shown in Fig.4A and 4C. However, *Physalin* treatment has significantly reversed these effects induced by LPS. The results obtained from rat tissues are consistent with that of the in vitro (Fig. 4B and 4D).

The effect of *Physalin* on MAPK-dependent signaling pathway in LPS-induced lung injury: As ERK1/2 is a key protein involved in NF-κB signaling pathway initiation, therefore the level of ERK1/2 and MAPK signaling pathway proteins were checked. LPS treatment showed no significant change in total amount of ERK1/2, JNK and p38 but significantly increased the level of their phosphorylation as shown in Fig. 5A. On contrary, *Physalin* treatment significantly reversed all these effects induced by LPS. The in vivo results are also in alliance with these results as shown in Fig.5B.

DISCUSSION

Various intrapulmonary factors lead to pulmonary injury and uncontrolled inflammatory response, which is one of the important pathogenesis of ALI/ARDS. Edema and hemorrhage are the prominent features of pulmonary inflammatory injury(Guo *et al*., 2012). The results of this study show that LPS treatment leads to lung injury, edema and bleeding. Erythrocytopenia and leukocytosis suggest inflammation and bleeding. The higher wet - dry weight ratio of lung tissue further indicates the occurrence of edema. Histological observation provided further evidence of lung injury induced by LPS. However, treatment with *Physalin* can reduce pulmonary hemorrhage and edema caused by LPS, confirming its excellent anti-inflammatory activity.

Apoptosis is a kind of programmed cell death, which is very important for maintaining lung homeostasis in rats (Yang *et al*., 2014). CCK8 results showed that 50μg/mL *Physalin* had no significant effect on the growth of RLE-6TN cells, but 1μg/mL LPS could significantly inhibit the proliferation of RLE-6TN cells, and *Physalin* could reverse the inhibitory effect of LPS on cell proliferation. TUNEL assay has confirmed LPS-induced apoptosis of alveolar epithelial cells, which was significantly blocked by *Physalin*. Changes in apoptosis are known to occur through a variety of pathways. Studies have shown that the activation of JNK pathway is related to the effect of promoting apoptosis (Kenyon, 2002; Zhang *et al*., 2014). According to the results of this experiment, the phosphorylation of JNK was significantly increased after LPS treatment, while the phosphorylation of JNK was decreased after *Physalin* treatment. This suggests that *Physalin* may prevent apoptosis of alveolar epithelial cells by reducing the phosphorylation of JNK.

There is growing evidence that the interaction of inflammatory factors is a typical characteristic of many acute pulmonary diseases(Güngör *et al*., 2010; Kaczmarek *et al*., 2010). LPS induces the progression of inflammation by changing the expression of inflammation-related proteins (Li *et al*., 2015; Lv *et al*., 2010). After LPS activates NF-κB, it accelerates the inflammatory response by inducing the release of pro-inflammatory cytokines such as IL-1β and COX2 (Giorgi *et al*., 2012; Wu *et al*., 2015). Our results showed that LPS-induced inflammatory responses activate NF-κB. *Physalin* effectively inhibited the activation of NF-κB, reduced the expression of IL-1β and COX2, and alleviated LPS-induced inflammation. In fact, phosphorylation of MAPK leads to phosphorylation of IκB, resulting in depolymerization of IκB and NF-κB, activates NF-κB and its nuclear translocation, which leads to activation of the NF-κB signaling pathway. Therefore, MAPK is an important target and key factor for the therapy of LPS-induced lung injury.

The MAPK signaling pathway is mainly composed of ERK1/2, JNK and p38 MAPK cascade reactions. After being phosphorylation by growth factors, hydrogen peroxide and other factors, ERK1/2 enters the nucleus and exerts effects on factors such as NF-κB to regulate their activities(Budagian *et al*., 2003; Liu, 2004). p38 MAPK acts as an important regulator of the NF-κB pathway by regulating the phosphorylation of IκB under physiological and pathological conditions. There is increasing evidence that the activated p38 MAPK moves from the cytoplasm to the nucleus, promotes the phosphorylation and degradation of IκB (Wei *et al*., 2008), regulates the expression of specific genes, and participates in the inflammatory response of cells under stress conditions (Lin *et al*., 2009), thereby affecting the balance between inflammatory and anti-inflammatory factors in the organism, determining the process of inflammation that causes tissues damage (Li *et al*., 2014). Our results suggested that LPS can significantly increase the phosphorylation levels of ERK1/2 and p38 MAPK, *Physalin* treatment effectively inhibited the phosphorylation of ERK1/2 and p38 MAPK, reduced the activation of IκB and inhibited NF-κB nuclear translocation. These results showed that *Physalin* inhibited the activation of ERK1/2 /JNK/p38 MAPK signaling pathway and reduced the nuclear heterotopic NF-κB, thus preventing LPS-induced ALI.

Conclusions: In conclusion, *Physalin* can achieve the effect of treating inflammation caused by LPS in rats

through reducing the phosphorylation level of IκB, NF-κB, ERK1/2, JNK and p38 MAPK, thereby reducing the expression of inflammatory factors TNF-α, IL-β and COX2, and reducing the apoptosis of alveolar epithelial cells both in vivo and in vitro. From the perspective of treatment, *Physalin* with a great potential can be used as an anti-inflammatory and anti-apoptotic agent. Therefore, nutritional strategies in prevention and treatment for LPS-induced lung injury may become an effective way to replace antibiotics and reduce side effects in the future.

Funding acknowledgement: Funding: This work was supported by the National Key Research and Development projects [grant number: 2017YFD0501500] and the Key Research and Development Plan of Shanxi Province [grant number: 201803D221023-3].

Authors contribution: QZ and YS conceived and designed the experiments; QZ performed the experiments; QZ and YX analyzed the data; AK, JG, ZW and NS provided manuscript editing. All authors statistically analyzed, discussed, critically revised the contents, and approved the final manuscript.

REFERENCES

Abraham E, Carmody A, Shenkar R, *et al*., 2000. Neutrophils as early immunologic effectors in hemorrhage- or endotoxemia-induced acute lung injury. Am Physiol Lung Cell Mol Physiol 279:1137-45.

Budagian V, Bulanova E, Brovko L, *et al*., 2003. Signaling through P2X7 receptor in human T cells involvesp56lck, MAP kinases and transcription factors AP-1and NF-κB. J Biolog Chem 278:1549-60.

Coimbra R, Melbostad H, Loomis W, *et al*., 2006. LPS-induced acute lung injury is attenuated by phosphodiesterase inhibition: effects on proinflammatory mediators, metalloproteinases, NF-kappaB, and ICAM-1 expression. J Trauma Acute Care Surg 60:115-25.

Dong L, Wang S, Chen M, *et al*., 2008. The activation of macrophage and upregulation of CD40 costimulatory molecule in lipopolysaccharide-induced acute lung injury. J Biomed Biotechnol 2008:852571-1.

Fan X, Zhang Y, Dong H, *et al*., 2015. Trilobatin attenuates the LPS-mediated inflammatory response by suppressing the NF-κB signaling pathway. Food Chem 166:609-15.

Güngör N, Pennings JL, Knaapen AM, *et al*., 2010. Transcriptional profiling of the acute pulmonary inflammatory response induced by LPS: role of neutrophils. Respir Res 11:24.

Giorgi VS, Peracoli MT, Peracoli JC, *et al*., 2012. Silibinin modulates the NF-κb pathway and pro-inflammatory cytokine production by mononuclear cells from preeclamptic women. J Reprod Immunol 95:67-72.

Gris D, Hamilton EF, Weaver LC, 2008. The systemic inflammatory response after spinal cord injury damages lungs and kidneys. Experim Neurol 211:259-70.

Guo Z, Li Q, Yang H, *et al*., 2012. Prevention of LPS-induced acute lung injury in mice by progranulin. Mediat Inflam 2012:540794.

He ZG, Huang J, Zhou SG, *et al*., 2012. Peripheral leukocytapheresis attenuates acute lung injury induced by lipopolysaccharide in vivo. Mediat Inflamm 2012:694635.

Januário AH, Filho ER, Pietro RCLR, *et al*., 2010. Antimycobacterial physalins from Physalis angulata L. (Solanaceae). Phytoth Res 16:445-8.

Kaczmarek P, Sładek K, Skucha W, *et al*., 2010. The influence of simvastatin on selected inflammatory markers in patients with chronic obstructive pulmonary disease. Polskie Archiwum Medycyny Wewnetrznej-polish Arch Inter Med 120:11-7.

Kenyon LC, 2002. Elevated JNK activation contributes to the pathogenesis of human brain tumors. Oncogene 21:5038-46.

Li A-L, Chen B-J, Li G-H, *et al*., 2017. Physalis alkekengi L. var. franchetii (Mast.) Makino: an ethnomedical, phytochemical and pharmacological review. J Ethnopharmacol S0378874117313429.

Li J, Peng L, Du H, *et al*., 2014. The Protective effect of beraprost sodium on diabetic cardiomyopathy through the inhibition of the p38 mapk signaling pathway in high-fat-induced SD rats. Int J Endocrinol 2014:901437.

Li L, Tang J, Sun Y, *et al*., 2015. Upregulation of HO-1 attenuates LPS-stimulated proinflammatory responses through downregulation of p38 signaling pathways in rat ovary. Inflammation 38:1085-92.

Lin CC, Lin WN, Wang WJ, *et al*., 2009. Functional coupling expression of COX-2 and cPLA2 induced by ATP in rat vascular smooth muscle cells: role of ERK1/2, p38 MAPK, and NF-kappaB. Cardiov Res 82:522-31.

Liu Y, 2004. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. Am J Physiol Renal Physiol 287:7-16.

Lv XJ, Li YY, Zhang YJ, *et al*., 2010. Over-expression of caveolin-1 aggravate LPS-induced inflammatory response in AT-1 cells via up-regulation of cPLA2/p38 MAPK. Inflamm Res 59:531-41.

Mukhopadhyay S, Mukherjee S, Smith M, *et al*., 2008. Activation of MAPK/AP-1 signaling pathway in lung injury induced by 2-chloroethyl ethyl sulfide, a mustard gas analog. Toxicol Lett 181:112-7.

Nieman GF, Gatto LA, Habashi NM, 2015. Impact of mechanical ventilation on the pathophysiology of progressive acute lung injury. J Appl Physiol 119:1245-61.

Pinto NB, Morais TC, Carvalho KMB, *et al*., 2010. Topical anti-inflammatory potential of Physalin E from Physalis angulata on experimental dermatitis in mice. Phytomedicine 17:740-3.

Raghavendran K and Napolitano LM, 2011. ALI and ARDS: Challenges and Advances. Crit Care Clin 27:xiii-xiv.

Samransamruajkit R, Prapphal N, Deelodegenavong J, *et al*., 2005. Plasma soluble intercellular adhesion molecule-1 (sICAM-1) in pediatric ARDS during high frequency oscillatory ventilation: a predictor of mortality. Asian Pacific J Allerg Immunol 23:181.

Wei L, Kwang J, Wang J, *et al*., 2008. Porcine circovirus type 2 induces the activation of nuclear factor kappa B by IkappaBalpha degradation. Virology 378:177-84.

Wu WY, Wu YY, Huang H, *et al*., 2015. Biochanin A attenuates LPS-induced pro-inflammatory responses and inhibits the activation of the MAPK pathway in BV2 microglial cells. Int J Mol Med 35:391-8.

Wu XL, Liou CJ, Li ZY, *et al*., 2015. Sesamol suppresses the inflammatory response by inhibiting NF-κB/MAPK activation and upregulating AMP kinase signaling in RAW 264.7 macrophages. Inflamm Res 64:577-88.

Yang J, Moon H, Chettimada S, *et al*., 2014. Cross-talk between apoptosis and autophagy in lung epithelial cell death. J Biochem Pharmacol Res 2:99.

Zhang Y, Hong-Yan LI, Zhang ZH, *et al*., 2014. Garlic-derived compound S-allylmercaptocysteine inhibits cell growth and induces apoptosis via the JNK and p38 pathways in human colorectal carcinoma cells. Oncol Lett 8:2591-6.

Zunpeng S, Na X, Qiuhong W, *et al*., 2016. Antibacterial and Anti-Inflammatory Activities of *Physalis alkekengi L.var.francheti.i* and Its Main Constituents. Evidence-Based Complem Alter Med 2016:1-10.