

JAX2, an ethanol extract of *Hyssopus cuspidatus* Boriss, can prevent bronchial asthma by inhibiting MAPK/NF- κ B inflammatory signaling

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ABSTRACT

Background: *Hyssopus cuspidatus* Boriss has been used to treat bronchial asthma for many years in Uighur medicine. JAX2, an ethanol extract from this plant, has effectiveness against bronchial asthma. However, the molecular basis for the anti-inflammatory effects of JAX2 remains unclear.

Purpose: This study aimed to evaluate the mechanism of JAX2 against bronchial asthma.

Methods: We established an asthma model in rats using ovalbumin (OVA), and an inflammatory model in RAW264.7 cells using lipopolysaccharide (LPS) stimulation. To evaluate the anti-inflammatory effects of JAX2, the concentrations of tumor necrosis factor (TNF)- α , interleukin (IL)-4, IL-6, IL-17, eotaxin and immunoglobulin (Ig)E were measured by enzyme-linked immunosorbent assay (ELISA). Cell viability was investigated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium, inner salt (MTS) assay. Further, nitric oxide (NO) and reactive oxygen species (ROS) were determined using Griess reagent and 2,7-dichlorofluorescein diacetate. The phosphorylation of p-extracellular signal-regulated kinase (ERK), p-Jun-NH2-terminal kinase (JNK), p38 kinases (p38) and p-inhibitor of nuclear factor kappa-B kinase (IKK), and nuclear translocation of p-p65 kinases (p-p65) were determined by immunofluorescence to uncover the effects of JAX2 on the Mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF- κ B) signaling pathways.

Results: After JAX2 administration to rats, Interferon (IFN)- γ concentrations in BALF increased significantly. Further, the concentrations of TNF- α , IL-4, IL-6, IL-17 and eotaxin in BALF, and IgE in serum decreased. JAX2 decreased TNF- α , IL-6 and NO in cell supernatant, and reduced ROS intracellularly. Concurrently, IFN- γ concentrations increased in cell supernatant significantly. In LPS-induced RAW264.7 cells, JAX2 inhibited phosphorylation of p-ERK, p-JNK and p-38 MAPK. The subsequent phosphorylation of p-IKK and nuclear translocation of the p-p65 subunit of NF- κ B were also suppressed.

Conclusion: Based on these findings, we believe that JAX2 has both preventive and treatment effects in bronchial asthma. Furthermore, in the RAW264.7 cell inflammatory model, JAX2 also inhibited NF- κ B and MAPK signaling pathways.

Introduction

Bronchial asthma is a chronic inflammatory respiratory disease characterized by inflammatory cell infiltration, reversible bronchoconstriction, and airways inflammation, obstruction and hyper-

responsiveness (Yuan et al., 2013; Huang et al., 2104; Kang et al., 2013). Asthma-related morbidity results from an immune imbalance and the release of inflammatory mediators (Wang et al., 2016; Yan et al., 2013; Shieh et al., 2015), which include interleukin (IL)-4, IL-6 (Wang et al., 2016), IL-17 (Ji et al., 2014), tumor necrosis factor (TNF-

Abbreviations: BALF, Bronchoalveolar lavage fluid; BSA, Bovine albumin; DCFH-DA, Dichlorofluorescein diacetate; DMSO, Dimethyl sulfoxide; ERK, Extracellular signal-regulated kinase; HPLC, High performance liquid chromatography; IFN- γ , Interferon- γ ; IKK, Inhibitor of nuclear factor kappa-B kinase; IL, Interleukin; JNK, Jun-N-terminal kinase; LPS, Lipopolysaccharide; MAPK, Mitogen-activated protein kinase; NaNO₂, Sodium nitrite; NF- κ B, Nuclear factor-kappa B; NO, Nitric Oxide; OVA, Ovalbumin; PBS, Phosphate buffer saline; p38K, p38 kinases; ROS, Reactive Oxygen species; TNF- α , Tumor necrosis factor- α

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α) (Rana et al., 2016) and interferon (IFN)- γ in the asthmatic rat model, and IL-6, TNF- α and ROS (Samarghandian et al., 2016; Lee et al., 2016) in inflammatory cells. NF- κ B and MAPK are involved in regulating the inflammatory and immune response in humans via effects on gene expression. Activated NF- κ B and MAPK signaling pathways play an important role in inflammatory lung diseases such as chronic obstructive pulmonary disease and asthma (Shieh et al., 2015). Inflammatory factors like TNF- α and IL-6 can also be induced during inflammatory processes (Paplińska et al., 2012; Gao et al., 2006). Therefore, inhibition of inflammatory factors may protect against asthma.

Hyssopus cuspidatus Boriss is a traditional herb used in folk medicine and in Uighur medicine, where it is called “zufaqini”. Like other aromatic plants from the mint (Labiatae) family, the dried ground aerial parts of *H. cuspidatus* Boriss have been used to treat conditions such as cough, asthma, colds, fever and rheumatism, with the chemical constituents including volatile oils, flavonoids, alkaloids, organic acids and lipids (Liu et al., 1999; He et al., 2015). *H. cuspidatus* Boriss has been used to relieve bronchial spasms for several hundred years, with modern pharmacology confirming that it has anti-inflammatory and antibacterial effects. In asthma, its effects are mediated via the hypothalamic pituitary adrenal axis with augmentation of endogenous neuroendocrine hormones to suppress inflammation. It also inhibits the release of inflammatory cytokines (Ma et al., 2011). This has been demonstrated with a water extract of the plant, which inhibited the secretion of IL-4 to correct the imbalance of Th1/Th2 in allergic asthmatic mice (Wang et al., 2008). *H. cuspidatus* Boriss water extract and essential oil also have bacteriostatic effects against *Staphylococcus aureus*, *E. coli*, *Candida albicans* and *Streptococcus timentiaciens* (Yin et al., 2007). Collectively, these findings suggest a beneficial effect of *Hyssopus cuspidatus* Boriss in asthma.

In this study, we obtained and purified a 40% ethanol elution from the dried ground aerial parts of *Hyssopus cuspidatus* Boriss (JAX2), which mainly contained caffeic acid, rosmarinic acid, hyperoside, salvigenin, acacetin, diosmin, luteolin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside, 3,4-dimethoxycinnamic acid and acacetin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (Mao et al., 2017b; Zhao et al., 2013). We evaluated the function and molecular mechanism of JAX2 in bronchial asthma.

Materials and methods

Contents of phenolics and flavonoids of JAX2

JAX2 mainly contains the following phenolics and flavonoids: caffeic acid (1.67%), rosmarinic acid (3.68%), hyperoside (2.52%), salvigenin (2.49%), acacetin (0.97%), luteolin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (1.9%), diosmin (2.44%), acacetin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (1.83%) and 3,4-dimethoxycinnamic acid (2.12%). Our study on the spectrum-effect relationship suggested that rosmarinic acid appeared was most responsible for the anti-inflammatory activity exerted (Mao et al., 2017a). High performance liquid chromatography (HPLC) chromatograms of JAX2 and standards are given in Fig. 1.

Induction of the rat asthma model (Song et al., 2016)

Male Sprague-Dawley rats (~200 g body weight) were purchased from the Experimental Animal Center of Xinjiang Medical University (Urumqi, China). The rats were divided randomly into seven groups ($n = 10$). There was a normal control group and an asthma model group plus five positive drug treatment groups: dexamethasone (Shanghai Xinyi Pharmaceutical Factory Limited Company, China), Feining tablets (the main ingredient is aster tataricus; Zheng He Pharmaceutical Group Limited by Share Ltd, Jilin, China), and JAX2 in three doses (low, medium and high). With the exception of the normal group, all rats were sensitized with intraperitoneal administration of

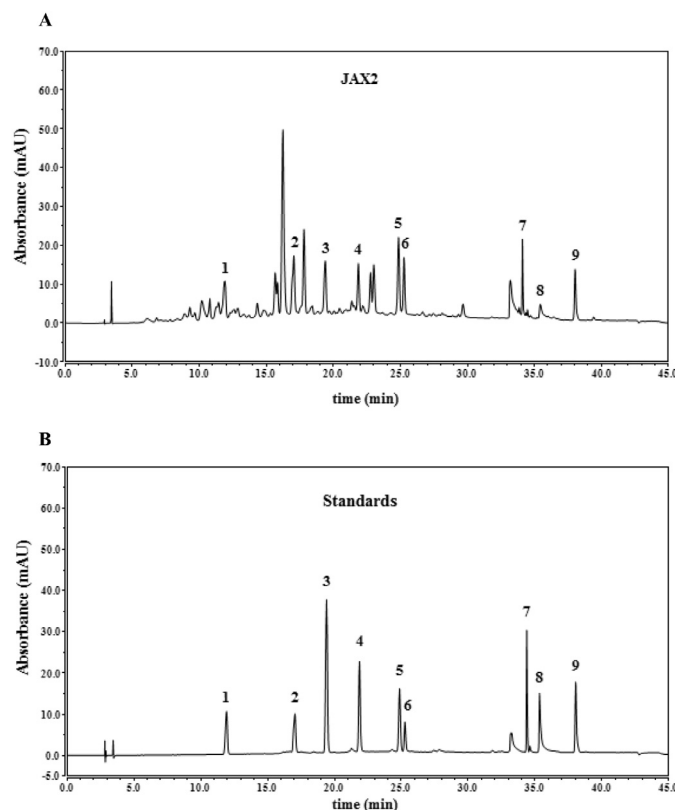


Fig. 1. HPLC chromatograms of JAX2 (A) and standards (B) at 330 nm detection. Peak identification: (1) caffeic acid, (2) hyperoside, (3) diosmin, (4) rosmarinic acid, (5) 3,4-dimethoxycinnamic acid, (6) luteolin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside, (7) acacetin 7-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside, (8) acacetin, (9) salvigenin.

100 mg ovalbumin (OVA; Grade V Sigma) and 10% aluminum hydroxide gel in saline to a total volume of 1 ml. This was administered daily from day 0 to day 15. From days 21 to 32, JAX2 (25, 50 or 100 mg/kg), dexamethasone (1.2 mg/kg) or Feining (500 mg/kg) were given to rats in the relevant treatment groups by intragastric administration approximately 60 min after OVA (100 μ g) in saline (50 μ l) was instilled intranasally. The control rats were treated with phosphate-buffered saline instead of OVA during this airway challenge. Under continuous stimulation for these 12 days, we observed the rats to have symptoms of allergy including restlessness, nose scratching and sneezing, which supported the success of the model. The rats were anesthetized after the last OVA challenge, and bronchoalveolar lavage fluid (BALF), blood and lung tissue were harvested for evaluation. The peripheral blood leukocyte, eosinophil and lymphocyte counts were performed using a Mindray (BC5140, ShenZhen China) hematology analyzer.

Measurement and analysis IgE levels

IgE concentration in rat serum was measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Xitang Shanghai Biotechnology co., Ltd, Shanghai, China). The lowest limit of detection was 3.9 μ g/ml.

Assay of eotaxin, IFN- γ , IL-4, IL-6, IL-17 and TNF- α in BALF

Eotaxin, IFN- γ , IL-4, IL-6, IL-17 and TNF- α concentrations in BALF were measured using ELISA as per the manufacturer's instructions (Boster, Wuhan, China). The lowest detection limit was 15.6 pg/ml for eotaxin, IL-4, IL-17, and TNF- α , 31.2 pg/ml for IFN- γ , and 62.5 pg/ml for IL-6.

Hematoxylin-eosin staining (Ablimit et al., 2013)

The lung samples were fixed in 10% formaldehyde solution, washed with distilled water and then dehydrated and embedded in paraffin. Paraffin-wax sections (4 μ M) were subsequently dewaxed and stained with hematoxylin-eosin (H&E) to enable leukocyte, lymphocyte and eosinophil cells to be counted.

Acute toxic effects

Twenty Kunming mice were divided into two groups: the JAX2 group ($n = 10$, five males and five females) and the control group ($n = 10$, five males and five females). Mice in the JAX2 group were treated with JAX2 (1 g JAX2 equivalent to 62 g raw medicine, dissolved in 1% carboxymethylcellulose sodium water solution) at a dose of 12 g/kg body weight. Mice in the control group were administered the same volume of blank extract vehicle. All tested animals were observed at 1, 7 and 14 days after administration, and changes in body weight, diet and behavior along with occurrence of marked toxicity or death were recorded. On the 15th day, the mice were dissected to observe the effects on mouse organs.

Cell culture and treatment

RAW264.7 cells were purchased from ATCC (USA), and cultured in a humidified incubator at 37 °C under 95% air/5% CO₂ atmospheric conditions until 80% confluency.

JAX2 was dissolved in DMSO to a concentration of 100 mg/ml, and then further diluted to final JAX2 concentrations of 2, 10 and 50 μ g/ml. Different concentrations of JAX2 were added to the cells at the start of LPS stimulation, and incubated with or without LPS for 24 h. The cells were divided randomly into five groups being a control group, an LPS group treated with LPS 1 μ g/ml for 24 h, and three JAX2 groups treated with JAX2 at concentrations of 2, 10 or 50 μ g/ml for 24 h.

Assay of cell viability

MTS assay (Promega, Madison, USA) was used for evaluating cell viability. After JAX2 treatment as described above, the medium was replaced with 100 μ l of MTS solution as directed by the manufacturer. Sample absorbance at 490 nm was read using a SpectraMax Plus microplate reader (Mei Gu molecular Instruments Co., Ltd. USA).

Measurement of NO and intracellular ROS

NO and ROS concentrations in cell supernatant were measured using Griess reagent (Xin et al., 2013) (Promega, USA) and 2,7-dichlorofluorescein diacetate (Li et al., 2015). After treatment, the supernatant from each well was mixed with an equal volume of Griess reagent and incubated at room temperature for 10 min. Optical density was determined at 540 nm with a microplate reader and NO concentration calculated using a standard curve of NaNO₂. The intracellular ROS level was measured using 2,7-dichloro-fluorescein diacetate (DCFH₂-DA, Sigma, St. Louis, USA). The wells were then washed with PBS and incubated with DCFH₂-DA at a final concentration of 10 μ mol/l at 37 °C for 30 min. The cells were washed again with PBS to remove extracellular DCFH₂-DA, and the fluorescence intensity measured with a SpectraMax M2 microplate reader at excitation and emission wavelengths of 485 and 538 nm, respectively. Intracellular ROS concentrations were expressed as fluorescent intensity.

Measurement of TNF- α , IL-6 and IFN- γ in cell-cultured supernatant

Cell supernatant concentrations of TNF- α , IL-6 and IFN- γ were measured using ELISA kits according to the manufacturer's instructions (eBioscience, USA). The lowest detection limits were 8 pg/ml for TNF- α

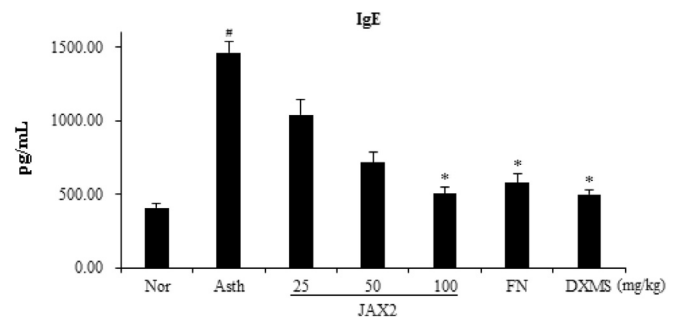


Fig. 2. Effect of JAX2 on serum IgE in rats with bronchial asthma. IgE concentrations in rat serum were measured by ELISA. IgE concentrations in the model group were significantly higher than in the normal group, but were reduced in a dose-dependent manner when treated with JAX2. High dose JAX2 did not differ significantly from the two positive drug groups. The results are shown as means $\pm$ SEMs. [#] $p < 0.05$ compared with the control group; ^{*} $p < 0.05$ compared with the model group.

and IFN- γ , and 4 pg/ml for IL-6.

Immunofluorescence assay

The macrophages, RAW264.7, were incubated with JAX2 for 3 h before being stimulated by LPS (1 μ g/ml) for 30 min. The cells were then fixed with 4% paraformaldehyde, perforated with 0.1% Triton X-100 and closed with 5% BSA. For assay, NF- κ B and MAPK were added to the cells and incubated at 4 °C overnight prior to incubating with the secondary antibody, Alexa 488-conjugate for 2 h. Finally, the cells were incubated with Hoechst33342 for 15 min, and the stained cells monitored with a Laser scanning confocal microscope.

Results

JAX2 significantly decreased IgE in serum

IgE concentrations in the model group were significantly higher than in the normal group, but were reduced in a dose-dependent manner when treated with JAX2 (Fig. 2). High dose JAX2 did not differ significantly from the two positive drug groups.

JAX2 significantly inhibited leukocytes, eosinophils and lymphocytes in peripheral blood

There were significantly more leukocytes, eosinophils and lymphocytes in the peripheral blood of rats in the asthma model group than in the normal group. Both the dexamethasone and Feining tablets groups had significantly fewer of these cell types than the asthmatic rats. JAX2 dose-dependently decreased leukocyte count, and both the eosinophil and lymphocyte counts were also significantly reduced with even a low JAX2 dose. The high dose JAX2 group did not differ significantly from the dexamethasone treatment group in terms of the numbers of leukocytes, eosinophils or lymphocytes. These results showed that JAX2 significantly attenuated the increase in inflammatory cells that occurred in the blood of asthmatic rats (Fig. 3).

Concentrations of IFN- γ , IL-4, IFN- γ /IL-4, TNF- α , IL-6, IL-17 and eotaxin in BALF

Compared with the normal group, the asthma model group had higher IL-4 concentrations and lower IFN- γ and IFN- γ /IL-4 in BALF. The ratio of IFN- γ to IL-4 increased as the JAX2 dose increased. These results indicated that the Th1/Th2 immune imbalance phenomenon was present in the asthma model group. Dexamethasone significantly inhibited expression of the Th2 cytokine, IL-4, compared with the asthma

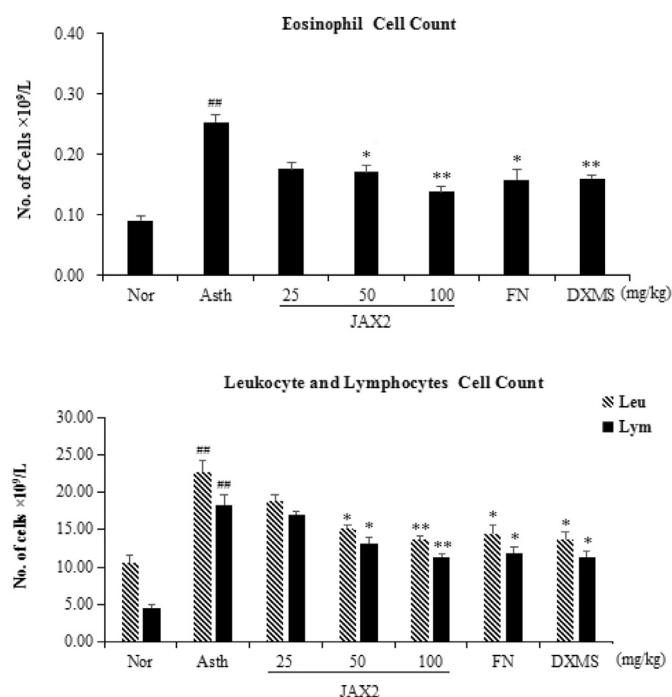


Fig. 3. Effect of JAX2 on leukocytes (Leu), lymphocytes (Lym) and eosinophils (Eos) in peripheral blood of rats with bronchial asthma. The lung samples were fixed in 10% formaldehyde solution, washed with distilled water and then dehydrated and embedded in paraffin. Paraffin-wax sections were subsequently dewaxed and stained with hematoxylin-eosin (H&E) to enable leukocyte, lymphocyte and eosinophil cells to be counted. JAX2 significantly inhibited leukocytes, eosinophils and lymphocytes in peripheral blood of rats with bronchial asthma. The results are shown as means $\pm$ SEMs. $^{##}p < 0.01$ compared with the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the model group.

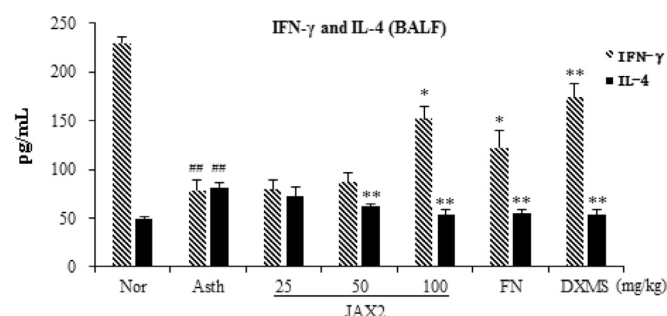


Fig. 4. Effect of JAX2 on IFN- γ and IL-4 in BALF of rats with bronchial asthma. IFN- γ and IL-4 concentrations in rat BALF were measured by ELISA. JAX2 significantly reduced IL-4 concentrations and increased IFN- γ concentrations, compared with the asthma model group. The ratio of IFN- γ to IL-4 increased as the JAX2 dose increased. These findings showed that JAX2 significantly inhibited the immune response of Th2 cytokine (IL-4) and promoted the immune response of Th1 cytokine (IFN- γ) in asthmatic rats. The results are shown as means $\pm$ SEMs. $^{##}p < 0.01$ compared with the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the model group.

model group, while expression of the Th1 cytokine, IFN- γ , was improved. JAX2 also significantly reduced IL-4 concentrations and increased IFN- γ concentrations, compared with the asthma model group. These findings showed that JAX2 significantly inhibited the immune response of Th2 cytokine (IL-4) and promoted the immune response of Th1 (IFN- γ) in asthmatic rats. Therefore, JAX2 can regulate the immune imbalance of Th1/Th2, which may benefit bronchial asthma (Fig. 4).

Concentrations of the proinflammatory cytokines, TNF- α , IL-6, IL-17 and eotaxin increased dramatically in the BALF of rats in the asthma model group, while JAX2 dose-dependently reduced levels of all these

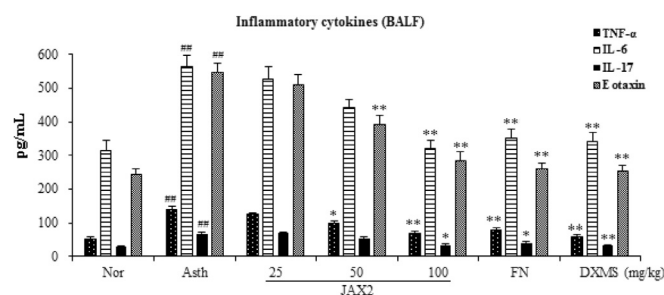


Fig. 5. Effect of JAX2 on inflammatory cytokines in BALF of rats with bronchial asthma. Eotaxin, IL-6, IL-17 and TNF- α concentrations in BALF were determined by immunofluorescence assay. TNF- α , IL-6, IL-17 and eotaxin increased dramatically in the BALF of rats in the asthma model group, while JAX2 dose-dependently reduced levels of all these inflammatory factors. JAX2 suppressed inflammatory factor concentrations in the BALF of asthmatic rats. The results are shown as means $\pm$ SEMs. $^{##}p < 0.01$ compared with the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the model group.

inflammatory factors. There were no significant differences in the JAX2 treated groups compared with either the dexamethasone or Feining tablets group. Therefore, JAX2 suppressed inflammatory factor concentrations in the BALF of asthmatic rats (Fig. 5).

H&E staining assay

The H&E staining method was used to detect pathological changes to lung tissue in rats. Lung tissue was severely damaged in the model group, and the bronchial wall thickened. The low and medium JAX2 dosing groups had some inflammatory cell infiltration, whereas the high dose JAX2, Feining tablets and dexamethasone groups all had significantly less lung tissue damage, and lower inflammatory cell infiltration (Fig. 6).

Acute toxic effect

Over the observation period, no differences in biologic reactions were observed between mice in the JAX2 group and those in the control group. No abnormal behaviors or deaths occurred. There were no alterations in the macroscopic appearance of the mouse organs.

JAX2 cellular toxicity and inhibition of NO generation

The effect of JAX2 on cell viability was examined with the MTS assay. Compared with the normal group, treatment with JAX2 for 24 h did not alter cell viability (Fig. 7A). Indeed, JAX2 did not damage the cells or affect their viability at any experimental concentration.

After stimulation of the cells with LPS (1 μ g/ml) for 24 h, nitrite production increased obviously. Treatment with JAX2 at concentrations of 2, 10 and 50 μ g/ml significantly inhibited nitrite release (Fig. 7B).

JAX2 suppressed production of pro-inflammatory cytokines in RAW264.7 and promoted production of IFN- γ

After stimulation with LPS for 24 h, the cell culture medium showed high TNF- α and IL-6 content, and low IFN- γ content. When LPS stimulation occurred in the presence of JAX2, release of both TNF- α and IL-6 was inhibited while that of IFN- γ was promoted (Fig. 8A–C).

Effect of JAX2 on LPS-induced oxidative stress in RAW264.7

Oxidative stress plays an important role in LPS-induced cytotoxicity. ROS generation was impaired in RAW264.7 cells after pre-incubation with LPS. Thus, we examined the effect of JAX2 on LPS-induced oxidative imbalance in these cells. We observed reduced intracellular ROS

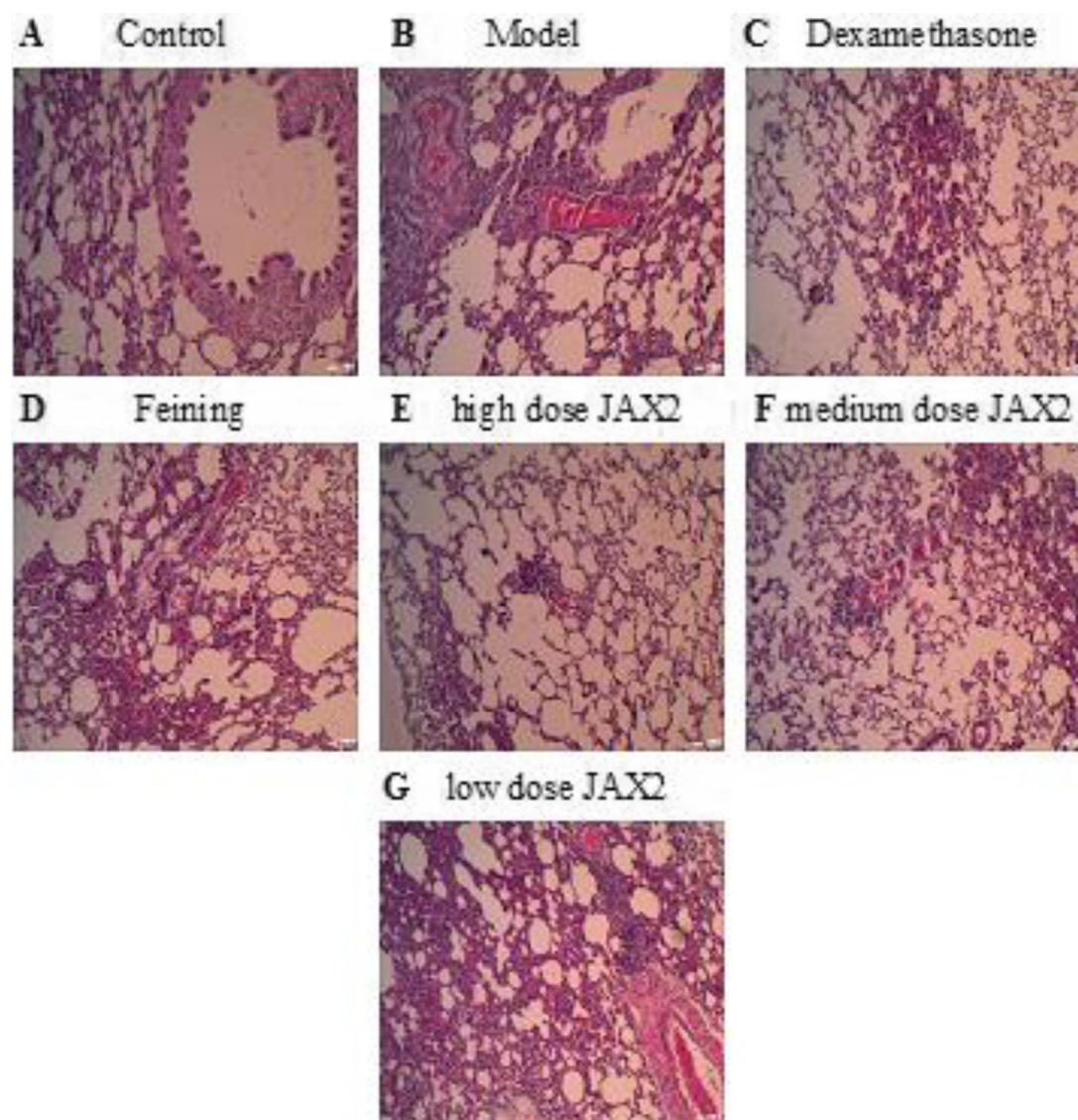


Fig. 6. H&E staining assay. The H&E staining method was used to detect pathological changes in rat lung tissue. Lung tissue was severely damaged in the model group, and the bronchial wall thickened. The low and medium JAX2 dosing groups had some inflammatory cell infiltration, whereas the high dose JAX2, Feining tablets and dexamethasone groups all had significantly less lung tissue damage, and lower inflammatory cell infiltration.

concentrations with all JAX2 concentrations (2, 10 and 50 $\mu\text{g/ml}$) (Fig. 8D).

LPS-induced NF- κ B and MAPK translocation attenuation by JAX2

We used an immunofluorescence assay to evaluate the potential effects on translocation of the NF- κ B and MAPK family from cytoplasm to nucleus. Compared to untreated cells ('control'), intracellular p65, Ikk, ERK, JNK and P38 translocated from cytoplasm to nucleus after LPS treatment. This nuclear migration was inhibited by JAX2 pre-treatment. Fluorescence intensity demonstrated dose-dependency with increasing JAX2 concentration (Figs. 9 and 10).

Discussion

Bronchial asthma is a common and chronic inflammatory disease of the respiratory system (Nixon et al., 2017). While inflammation is an important defense process, an excessive or prolonged inflammatory response can precipitate autoimmune diseases (Kim et al., 2016; Koga et al., 2016). Therefore, a reasonable approach to asthma management is to suppress inflammatory mediators.

NO is a bioactive gas molecule that plays an important regulatory role in the transmission of information between cells. It is also an inflammatory mediator, with the presence of NO correlating with the extent of inflammation. In activated macrophages, inducible nitric oxide synthase (iNOS)-induces NO production, promotes exudation in inflammatory sites, and causes development and progression of inflammatory diseases (Chen et al., 2015). In this study, we demonstrated that JAX2 dose-dependently inhibited NO release from LPS-stimulated RAW264.7 cells.

ROS is a harmful metabolic byproduct that can cause cell damage as well as induce endogenous antioxidant enzymes and iNOS. Previous studies have shown that ROS can directly activate the NF- κ B and MAPK signaling pathways, which can produce cellular damage (Ma et al., 2016). Reduced ROS production can inhibit endothelial cell apoptosis and macrophage formation. We found that JAX2 inhibited the release of ROS from LPS-stimulated RAW264.7 cells thus reducing oxidative stress and inhibition of macrophage differentiation (Lai et al., 2014; Duan et al., 2017).

The NF- κ B and MAPK signaling pathways regulate the expression of many inflammation-related genes, and can affect the inflammatory and immune response. In earlier work, activation of the NF- κ B and MAPK

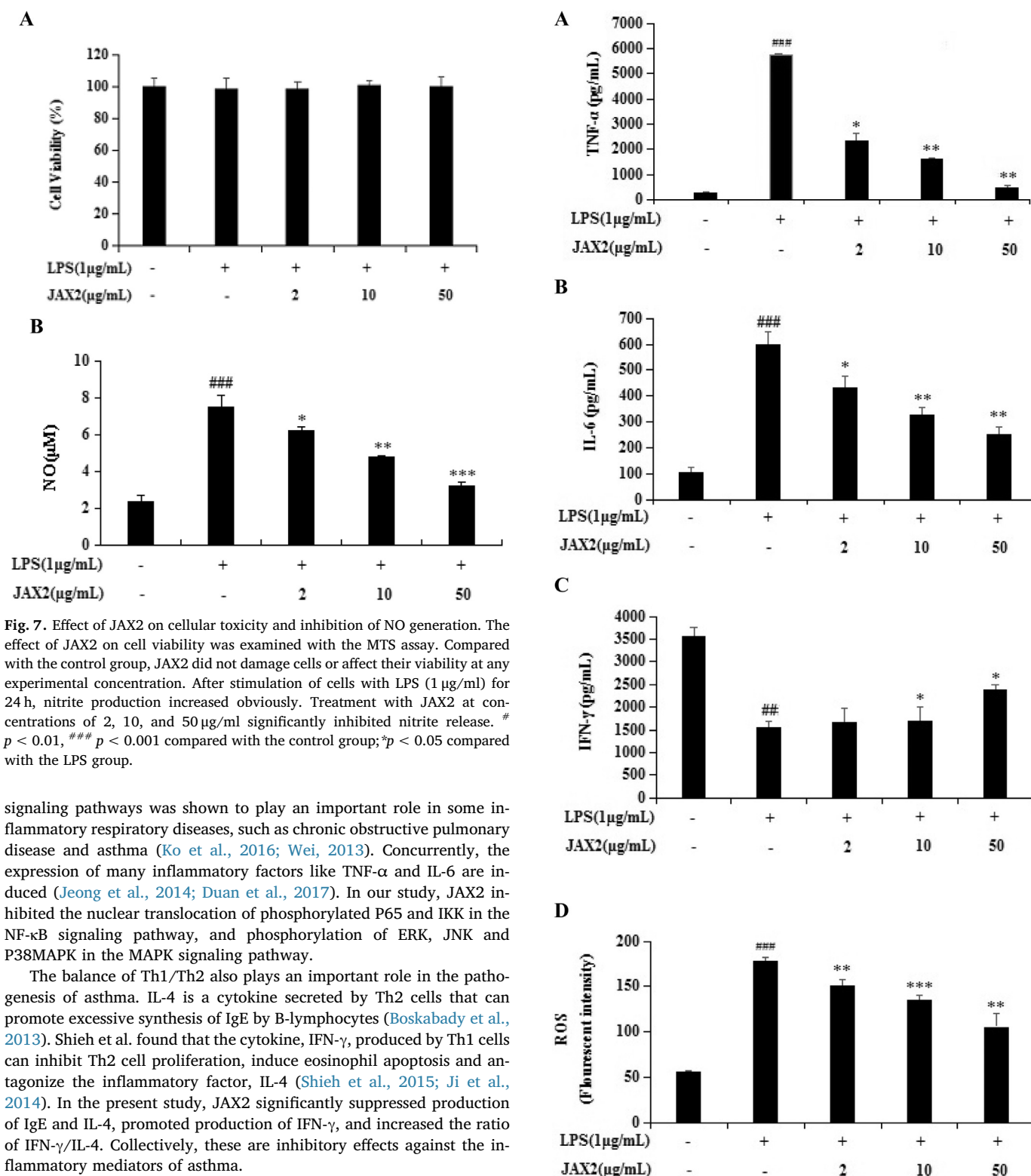


Fig. 8. Effect of JAX2 on production of pro-inflammatory cytokines, IFN-γ and LPS-induced oxidative stress in RAW264.7. Cells were pre-treated with different concentrations of JAX2 (2, 10 and 50 μg/ml) for 3 h, and then stimulated by LPS (1 μg/ml) for 24 h. Release of both TNF-α and IL-6 was inhibited, while that of IFN-γ was promoted. Intracellular ROS concentrations were reduced. ## $p < 0.01$, ### $p < 0.001$ compared with the control group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the LPS group.

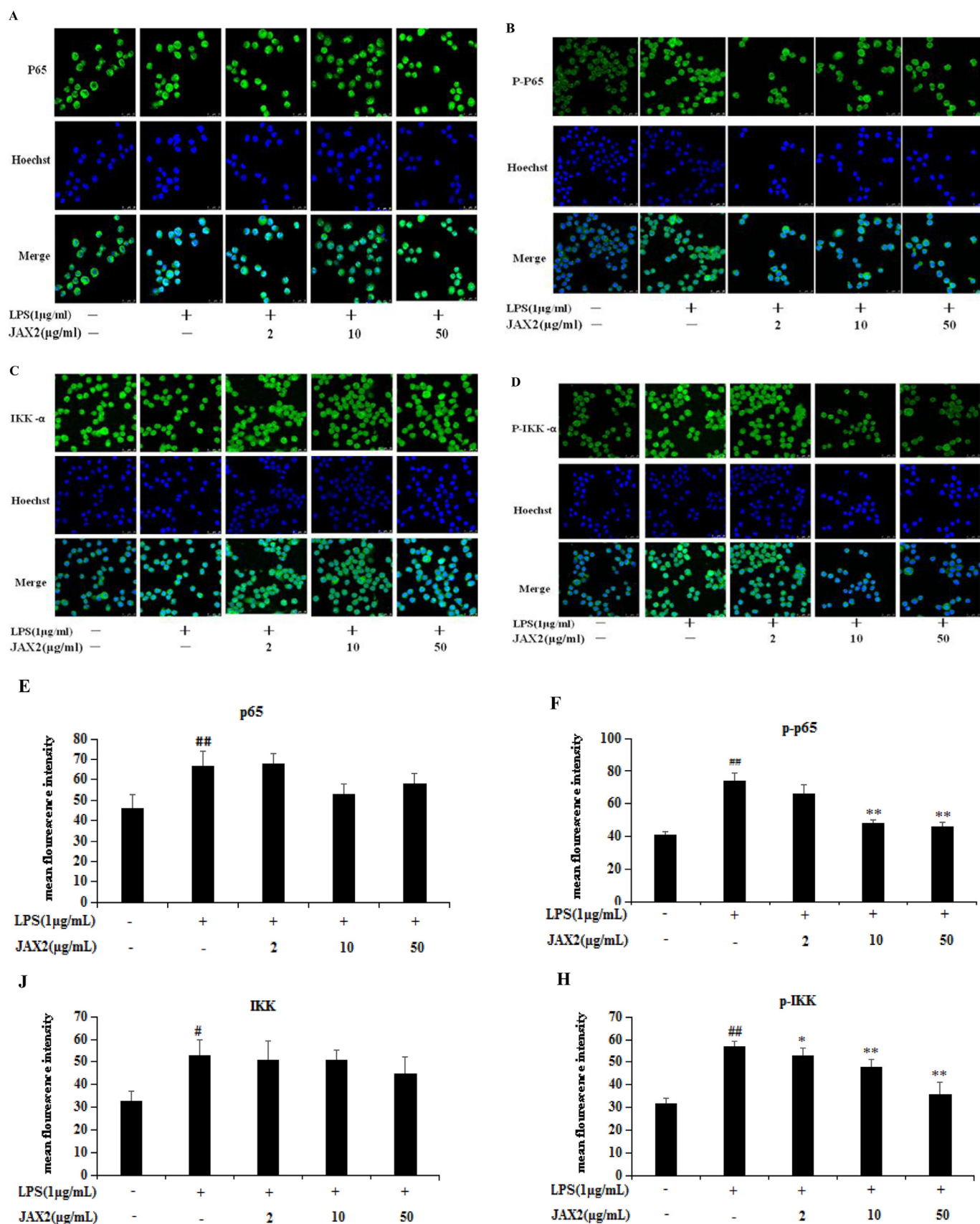


Fig. 9. Effect of JAX2 on translocation of NF- κ B protein. The immunofluorescence assay was used to assess the potential effect on translocation of NF- κ B. Cells were pretreated with JAX2 for 3 h, and then incubated with LPS (1 μ g/ml) for 30 min. Compared with the control group, intracellular p65 and Ikk translocated from cytoplasm to nucleus after LPS treatment. This nuclear migration was inhibited by JAX2 pretreatment (A–D). Fluorescence intensity demonstrated dose-dependency with increasing JAX2 concentration (E–H). ^{##} $p < 0.01$ compared with the control group; ^{*} $p < 0.05$, ^{**} $p < 0.01$ compared with the LPS group.

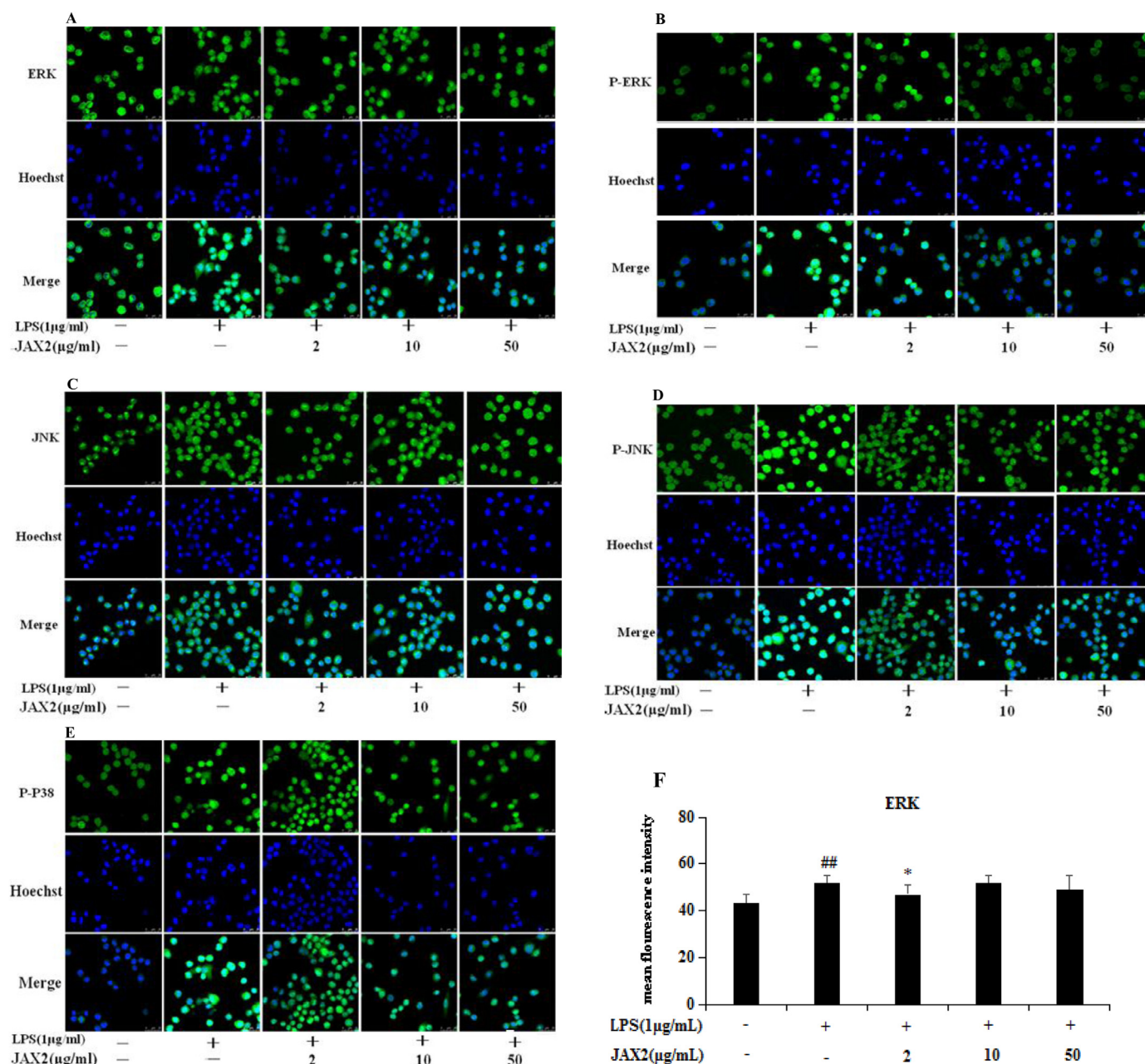


Fig. 10. Effect of JAX2 on translocation of MAPK protein. Immunofluorescence assay was used to assess the potential effect on translocation of p MAPK. Cells were pretreated with JAX2 for 3 h, and then incubated with LPS (1 μg/ml) for 30 min. Compared with the control group, intracellular ERK, JNK and p38 translocated from cytoplasm to nucleus after LPS treatment. This nuclear migration was inhibited by JAX2 pretreatment (A–E). Fluorescence intensity demonstrated dose-dependency with increasing JAX2 concentration (F–J). ^{##} $p < 0.01$ compared with the control group; ^{*} $p < 0.05$, ^{**} $p < 0.01$ compared with the LPS group.

(Li et al., 2013). It is a regulatory factor in the immune response, and in cell proliferation and differentiation (Gan et al., 2016). IL-6 is a proinflammatory cytokine produced after immune activation that can aggravate chronic inflammation (Cha et al., 2015; Yeom et al., 2015; Li et al., 2017). JAX2 can reduce the inflammatory response by inhibiting secretion of IL-17, TNF- α and IL-6 in alveolar lavage fluid and supernatant.

In conclusion, JAX2 can balance the Th1/Th2 ratio in rat BALF, and inhibit inflammatory cytokine release in BALF and cell supernatant. This means that JAX2 has an anti-inflammatory action that can potentially benefit asthma. Further research is needed into the underlying mechanisms for its anti-asthma effects. It is also important to establish whether JAX2 inhibits inflammatory mediator release by down-regulation of NF- κ B and MAPK gene expression.

Data availability

SPSS (version 4.0.100.1124; IBM Corporation, Armonk, NY, USA), ImageJ 1.48v (Java1.6.0_20; National Institutes of Health, USA).

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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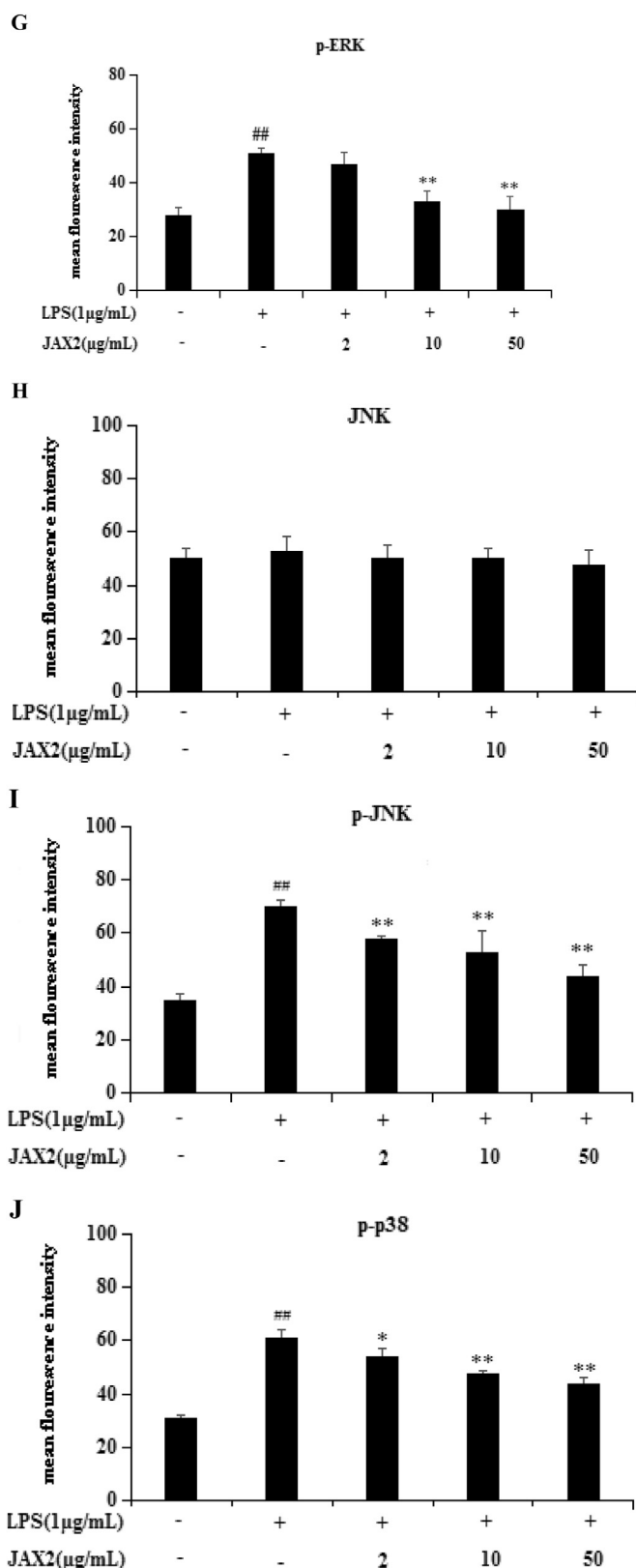


Fig. 10. (continued)

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