



Effect of total alkaloids from *Alstonia scholaris* on airway inflammation in rats



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ABSTRACT

Ethnopharmacological relevance: *Alstonia scholaris* (Apocynaceae) have been traditionally used for treatment of respiratory diseases in “dai” ethnopharmacy for hundreds years, especially for cough, asthma, phlegm, chronic obstructive pulmonary disease and so on. The formulas including the leaf extract have also been prescribed in hospitals and sold over the retail pharmacies.

Aim of the study: *A. scholaris* is used as a traditional herbal medicine for the treatment of respiratory tract inflammation. However, there is no scientific evidence to validate the use of total alkaloids of *A. scholaris* in the literature. Here, we investigated the protective activity of total alkaloids (TA), extracted from the leaves of *Alstonia scholaris*, against lipopolysaccharide (LPS)-induced airway inflammation (AI) in rats. **Materials and methods:** 200 µg/µL LPS was instilled intratracheally in each rat, and then the modeling animals were divided into six groups (n=10, each) randomly: sham group, LPS group, Dexamethasone [1.5 mg/kg, intra-gastricly (i.g.)] group, and three different doses (7.5, 15, and 30 mg/kg, i.g.) of total alkaloids-treated groups. Corresponding drugs or vehicles were orally administered once per day for 7 days consecutively. The concentration of albumin (ALB), alkaline phosphatase (AKP), lactate dehydrogenase (LDH), and the number of inflammatory cells in bronchoalveolar lavage fluid (BALF) were determined by fully automatic biochemical analyzer and blood counting instrument. Nitric oxide (NO) level, malondialdehyde (MDA) content, and superoxide dismutase (SOD) activities were examined by multiskan spectrum, and histological change in the lungs was analyzed by H.E. staining. The levels of inflammatory cytokine tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) were measured using ELISA.

Results: Total alkaloids decreased the percentage of neutrophil, number of WBC, levels of ALB, AKP and LDH in the BALF, while increased the content of ALB in serum. It also improved SOD activity and increased NO level in the lungs, serum and BALF, and reduced the concentration of MDA in the lungs. Total alkaloids also inhibited the production of inflammatory cytokines TNF-α and IL-8 in the BALF and lung. Finally, histopathological examination indicated that total alkaloids attenuated tissue injury of the lungs in LPS-induced AI.

Conclusions: Total alkaloids have an inhibitory effect against LPS-induced airway inflammation in rats.

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Abbreviations: AI, airway inflammation; TA, total alkaloids; DXM, dexamethasone; LPS, Lipopolysaccharides; BALF, bronchoalveolar lavage fluid; TNF-α, tumor necrosis factor-α; IL-8, Interleukin-8; ALB, albumen; COPD, Chronic Obstructive Pulmonary Disease; AKP, alkaline phosphatase; LDH, Lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase; NO, nitric oxide; WBC, white blood (cell) count; AM, alveolar macrophage; N, neutrophil; M, monocyte; L, lymphocyte; ELISA, enzyme-linked immunosorbent assay

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1. Introduction

Airway inflammatory disease including chronic obstructive pulmonary disease (COPD), asthma and acute lung inflammatory and variety of other symptoms, is one of severe respiratory diseases suffering the patients. Some of the common characteristics are: maximal expiratory flow limitation, bronchial hyper-responsiveness as well as physiological airway remodeling, and

inflammation of the airways, disruption of epithelial integrity, and lung parenchymal injury pathologically. Asthma is a chronic inflammatory disease of the airways associated with polygenic inheritance, evoked by nonspecific stimuli such as environmental pollutants, allergens, exhaust gases, infections, cold air, and strenuous exercise. Each of these stimuli evokes a variety of effector cells, including inflammatory cells, epithelial cells, neurons, and narrows the airway indirectly through the release of mediators. Some investigation implicated that pro-inflammatory mediators released from mast cells and eosinophils adjusted by T cells were identified as being particularly relevant to the progress of asthma (Holgate et al., 1999). Nowadays, the role of inflammatory cells and secretory factors are the key subjects of substantial studies examining the pathogenesis, diagnosis and treatment of COPD. Most of researches persist that airway inflammation is a typical characteristic of COPD when worsen beyond the stationary phase. Crucial inflammatory cells, such as neutrophils, macrophages and T lymphocytes, are overspread. Consequently, neutrophils and macrophages infiltrate the lumen, and lymphocytes infiltrate the tracheal wall (Tetley, 2005). Various chemokines and cytokines, including interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), leukotriene B4 (LTB4), interleukin-8 (IL-8), growth factors (GF), reactive oxygen species (ROS), proteinases and so on, are produced by neutrophils (Chung, 2005). The extracellular matrix of lung injury induced by these cytokines and chemokines accentuate inflammation, leading to pulmonary parenchymal inflammation reactions. Therefore, improving the airway inflammation plays a pivotal role in the treatment of airway inflammatory diseases. Traditional Chinese Medicines with basic information of safety and efficiency might be resources of interest for searching of natural products to treat airway inflammation.

A. scholaris called 'tang-jiao-shu' in Chinese, is distributed wild throughout in deciduous, evergreen forests and even in plains widely over the tropical regions of Africa and Asia (Li et al., 1995). Its leaves has long been used in "dai" ethnopharmacy for the treatment of respiratory diseases (Medicine, 1977). Alkaloids showed an antitussive, anti-asthmatic, expectorant (Shang et al., 2010a), analgesic and anti-inflammatory effects (Shang et al., 2010b) *in vivo*. It activates β_2 adrenergic receptor (β_2 AR) (Hou et al., 2012a), and inhibits the nuclear factor- κ B (NF- κ B) (Hou et al., 2012b) bioactivities *in vitro* in our previous pharmacological studies on this plant. Moreover, the methanolic extract of *A. scholaris* exhibited anti-inflammatory and antioxidant activities against carrageenan induced rat paw oedema (Subraya and Gupta, 2012). Above results indicated that total alkaloids of *A. scholaris* leaf may produce the anti-inflammatory and analgesic effect peripherally. Based on the pre-clinical studies on *A. scholaris* leaf, total alkaloids have been registered as investigational new botanical drug (No. 2011L01436), and was approved for clinical trials (phase I and II) by China Food and Drug Administration (CFDA). A mono-center, randomized, double-blind, and placebo phase I clinical trial has been finished, and the results may support further phase II clinical trial. The evaluation of the protective effect on airway inflammation induced by LPS in rats was carried out which would hopefully provide remedy principle for phase II clinical application.

2. Materials and methods

2.1. Plant materials

The leaves of *A. scholaris* were collected in June 2013 in Pu'er of Yunnan Province, People's Republic of China, and identified by Dr. Xiao-Dong Luo, Kunming Institute of Botany, Chinese Academy of

Sciences. A voucher specimen (Luo20130601) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

2.2. Total alkaloids preparation

The dried and powdered leaves of *A. scholaris* (1000 g) were extracted with 90% EtOH under reflux condition (3 h $\times$ 4), and the solvent was evaporated *in vacuo* to afford the ethanolic extract. Ethanolic extract was dissolved in 0.3% HCl solution, and the residue was recognized as non-alkaloid fraction. Then the acidic water soluble materials, adjusted to pH 9–10 with 10% ammonia solution, was extracted with EtOAc to give total alkaloids fraction (10 g).

2.3. Chemicals

Lipopolysaccharide (LPS; *Escherichia coli*) was purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA reagents sets for TNF- α and IL-8 were purchased from R&D Systems (Minneapolis, MN, USA). The malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO) determination kits were purchased from the Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China). Biochemical reagents sets for ALB, AKP and LDH were obtained from Beckman Coulter, Inc. (CA, USA). All other chemicals and solvents were of highest grade and purity available.

2.4. Animals

Pathogen-free male Sprague-Dawley (SD) rats, weighing approximately 190–220 g, were purchased from Kunming Medical University (licence number SYXK 2011–0004). All animals were housed at room temperature (20–25 °C) and constant humidity (40–70%) under a 12 h light-dark cycle in SPF grade laboratory. Food and water were supplied *ad libitum*. Animals were acclimated for one week before treatment. The animal study was performed according to the international rules considering animal experiments and the internationally accepted ethical principles for laboratory animal use and care.

2.5. Experimental design

The experiment was carried out as described in literature (Dong et al., 2013). Rats were anesthetized by intraperitoneal injection of sodium pentobarbital and placed in a supine position with the head elevated. The trachea was intubated with a cannula (80 mm length and 2.76 mm outer diameter), and 200 μ L of sterile saline containing 200 μ g lipopolysaccharide was instilled, meanwhile, sham group was instilled 200 μ L saline. On the day of 22ed, the rats were randomly assigned to 6 groups and treated as follows: (1) Sham group (not received any intervention); (2) LPS group (0.5% carboxymethylcellulose, i. g.); (3) Positive control group (dexamethasone, 1.5 mg/kg bwt, i. g.); (4) total alkaloids (7.5 mg/kg bwt, i. g.); (5) total alkaloids (15 mg/kg bwt, i. g.); (6) total alkaloids (30 mg/kg bwt, i. g.). Each group contained ten rats.

2.6. Serum collection

On the 28th day, animals were euthanized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg, bwt) after overnight food deprivation. Then blood was harvested by carotid artery intubation for the determination of serum enzyme levels, cytokine and biochemical analysis. The serum samples were obtained by centrifuging blood samples at 4 °C, 4000 $\times$ g for 15 min, and serum samples were prepared and stored at –80 °C

until analyzed (Lemos et al., 2014; Hollander et al., 2007).

2.7. Bronchoalveolar LAVAGE fluid (BALF) and cell enumeration

At the end of the experiment, the right lungs were ligated at the right main bronchus. Then, the left lungs were lavaged with 2 ml of autoclaved PBS for five times. The recovery ratio of the fluid was about 50–80% (5–8 ml) and the BALF was immediately centrifuged at 4 °C, 300 × g for 10 min, and the supernatants were stored at –80 °C for subsequent tests. The cell pellets were resuspended in PBS, and the total cell number was counted using blood counting instrument. The total number of each cell type was determined by multiplying the percentage by the total number of cells. The results were expressed as the number of each cell population in 1 ml of BALF (Wang et al., 2010; Zheng et al., 2009).

2.8. Lung homogenate

Lung tissue supernatants were prepared as described previously (Tsai et al., 2014). After BALF collection, 50 mg lungs were homogenized in 0.45 ml of ice-cold PBS, the assay mixture was cooled on ice, followed by centrifugation at 4 °C, 1000 g for 10 min, to obtain the supernatant, and stored at –80 °C for subsequent analysis.

2.9. ELISA: Quantification of TNF- α and IL-8

TNF- α and IL-8, in serum, BALF and tissue homogenates, were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN). ELISA was performed according to the manufacturer's specifications. The lower limit of detection was 20, 3.75 pg/mL for respectively.

2.10. Biochemical analysis

The content of albumin (ALB), alkaline phosphatase (AKP) and lactate dehydrogenase (LDH) in serum, BALF supernatants and tissue homogenates was measured by fully automatic biochemical analyzer (Beckman coulter Co. USA) using the method of (Inoue et al., 2010).

2.11. Antioxidant detection

In order to determine the antioxidant defenses, we have measured the enzymatic defense activities in the BALF, lung tissue and blood (da Cunha et al., 2011). The levels of nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD) in serum, BALF and tissue homogenates were determined using the MDA, NO and SOD kits (Nanjing Jiancheng Bioengineering Institute), with one step (NO), xanthinoxidase (SOD) and TBA (MDA) chromometry, with enzyme-labelled meter at 550 nm (NO, SOD) and 532 nm (MDA) wave length, according to the manufacturer's instructions.

2.12. Lung histopathology

A histological examination was used to further assess the progress of the disease. With the rats under terminal anesthesia, the right upper lobes of lung were removed, washed in phosphate buffer, fixed in 4% formaldehyde buffered with a phosphate solution (0.1 M, pH 7.4) at room temperature. Organ fragments were dehydrated in graded concentrations of ethanol, and then embedded in paraffin. From each tissue, 3 μ m thick sections were made and stained with hematoxylin and eosin to evaluate the

general morphology (Ichiki et al., 2005; Imaoka et al., 2007; Okamoto et al., 2002). Each successive field was individually assessed for the severity of lung injury using the semi-quantitative grading system (Alireza et al., 2013). The grading was based on: Grade 0 (–), normal lung; grade 1 ($\pm$), the structural lesions of bronchial mucosa and alveolar wall were mild; Grade 2 (+), focal lesions of the bronchial mucosa and alveolar wall structures; Grade 3 (++) , the lesions of bronchial mucosa and alveolar wall were patchy; Grade 4 (+++), the lesions of bronchial mucosa and alveolar wall were less than 1/2 lobectomy; and Grade 5 (++++) , the lesions of bronchial mucosa and alveolar wall were more than 1/2 lobectomy. Pathological scores of lung damage evaluated according to the above criteria were recorded as 0, 1, 2, 3, 4, and 5 points (El-Agamy, 2011; Li et al., 2011).

2.13. Statistical analyses

Results were presented as mean $\pm$ SEM ($n=10$). Comparisons between 2 groups were made by using a 2-tailed Mann-Whitney test. Multiple comparisons were made by using 1-way ANOVA with the LSD (Least Significant Difference) test or Kruskal Wallis analysis with the Dunn post-test, in which nonparametric analysis were appropriate. Analyses used Graph Pad Prism Software (San Diego, Ca). A P value of less than 0.05 was considered as significant for all analyses.

3. Results

3.1. Observations on viability and clinical signs

In the period of modeling, all rats were wheezing, coughing, breathing hardly, bristling, being settled, and feeding less. They generally lost weight except for the sham group. In the administration period, no significant improvement observed in LPS group, but the symptoms of the treatment group were alleviated when compared to the LPS group, and no dose-response relationships were observed.

3.2. Effects of TA on LPS-induced inflammatory cell counting in BALF

Lungs were lavaged at the end of experiment, and differential cells in BALF were counted to investigate the effect of TA on inflammatory cells influx. We found that the total cell number, neutrophil counts in BALF of LPS group were greater than that in sham group significantly. Treatment with 7.5 mg/kg, 15 mg/kg and 30 mg/kg of TA reduced the number of total cells in BALF ($p < 0.05/0.01$), and which was similar with DXM at 1.5 mg/kg (Fig. 1A), and the effect on total cell number between different dosages of TA group was not dose dependent. Results from cell classification in BALF indicated that in LPS group, the number of neutrophil in the BALF increased as compared with sham group ($p < 0.01$), the number of alveolar macrophage, lymphocyte and monocyte decreased significantly as compared with sham group ($p < 0.01$). Treatment with 7.5 mg/kg, 15 mg/kg and 30 mg/kg of TA remarkably lowered the neutrophil ($p < 0.01$), raised the number of alveolar macrophage and monocyte ($p < 0.05/0.01$) in BALF as compared to the sham group. Dexamethasone at the rate of 1.5 mg/kg could prominently decrease the number of neutrophils ($p < 0.01$), while increase the number of alveolar macrophage, lymphocyte and monocyte ($p < 0.01$) in BALF, and the effect on neutrophil or alveolar macrophage, lymphocyte and monocyte influx exhibits no significant difference between different dosage groups of TA, and which was similar with that of DXM at the rate of 1.5 mg/kg (Fig. 1B).

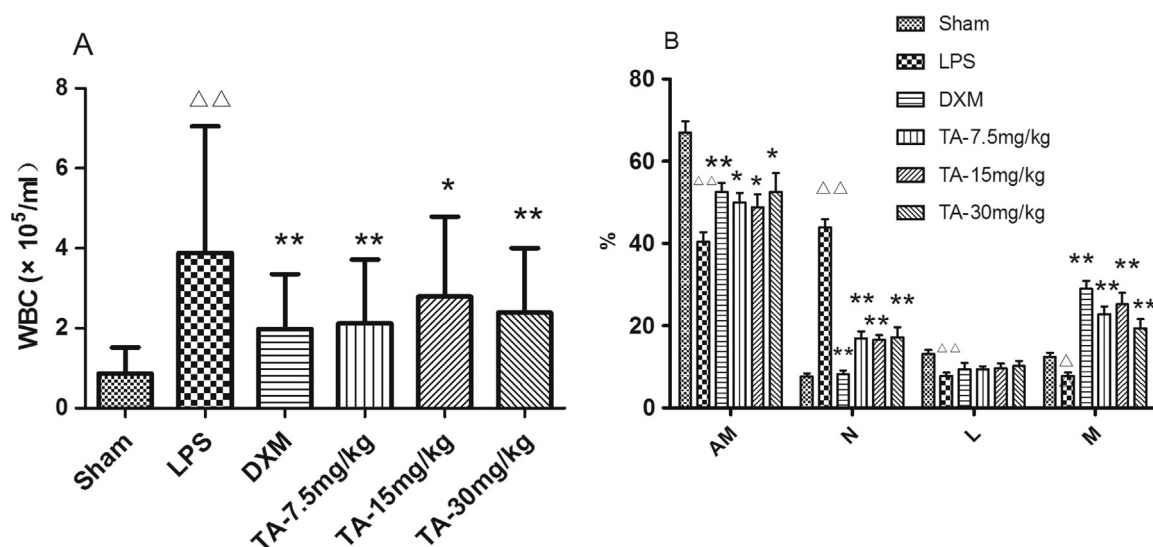


Fig. 1. TA inhibited inflammatory cell levels in BALF. Data was shown as means $\pm$ SEM values. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n=10$ rats per group). (A) the numbers of total leukocytes. (B) The percentage of various types of cells. The total white blood cells (WBC) were counted by using blood counting instrument. Each population of inflammatory cells including alveolar macrophages (AM), neutrophils (N), lymphocytes (L) and monocytes (M).

3.3. Effect of TA on TNF- α and IL-8 production in BALF, serum and lung tissues

The effect of TA on TNF- α and IL-8 production in BALF, serum and lungs was analyzed by ELISA (Fig. 2). The LPS significantly improved TNF- α and IL-8 production compared with the sham group ($p < 0.01$). TA at 7.5 mg/kg, 10 mg/kg and 30 mg/kg depressed TNF- α production in the BALF and homogenate ($p < 0.05/0.01$) (Fig. 2). The three doses of TA could also remarkably degrade IL-8 production in the BALF ($p < 0.05/0.01$) (Fig. 3) while DXM inhibited TNF- α and IL-8 production as positive control ($p < 0.05/0.01$).

3.4. Effect of TA on LPS-induced NO, SOD and MDA activities in BALF, serum and lung tissue

Oxidative stress plays an important role in the development of LPS-induced airway inflammation. To evaluate the effects of TA on oxidative stress, NO, SOD and MDA activities in the serum, BALF

and lungs were determined. As shown in (Figs. 4, 5 and 6), LPS group resulted in marked drooping of NO, SOD activities (Figs. 4 and 5), and significantly improving of MDA level compared to the sham group ($p < 0.05/0.01$) (Fig. 6). Interestingly, TA at 7.5, 15 and 30 mg/kg meaningfully promoted NO, SOD activities ($p < 0.05/0.01$) (Figs. 4 and 5), and rebated LPS-induced MDA level in the three samples ($p < 0.05/0.01$) (Fig. 6). DXM also significantly raised NO, SOD activities and descended MDA level in the previous samples ($p < 0.05$).

3.5. ALB, AKP and LDH levels in BALF and serum

The LPS administration induced an upgraded ALB, AKP and LDH concentrations in the BALF as shown in (Fig. 7). TA at the rate of 7.5, 15 and 30 mg/kg degraded the ALB (Fig. 7A), AKP (Fig. 7B) and LDH content (Fig. 7C), when compared to the LPS group ($p < 0.05/0.01$). DXM also notably diminished ALB, AKP and LDH levels in the BALF ($p < 0.01$). However, in serum a significant reduction in ALB (Fig. 8A), and enhancement in LDH ($p < 0.01$) (Fig. 8C) in the LPS

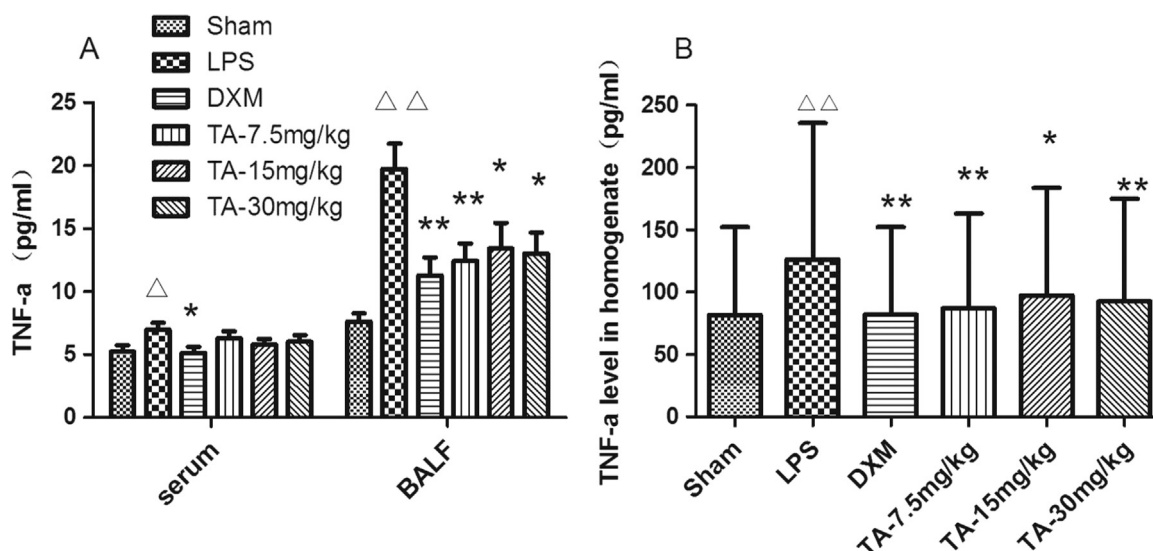


Fig. 2. Levels of TNF- α after intratracheal LPS instillation. Contents of TNF- α in serum BALF and homogenate were determined by ELISA. Data was shown as means $\pm$ SEM values. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n=10$ rats per group). (A) TNF- α level in serum and BALF. (B) TNF- α level in homogenate.

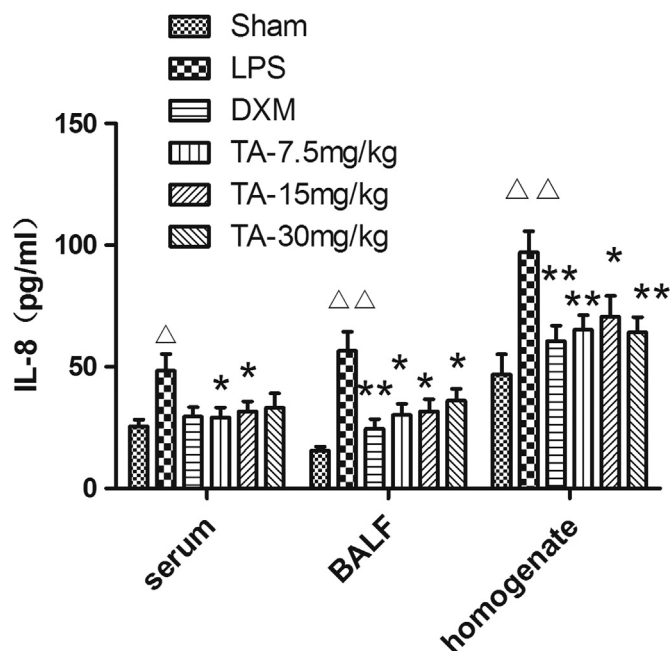


Fig. 3. TA regulated IL-8 production in serum, BALF and homogenate. Level of IL-8 was measured by ELISA. Data was shown as means $\pm$ SEM values. [△] $p < 0.05$, ^{△△} $p < 0.01$ vs. sham, ^{*} $p < 0.05$, ^{**} $p < 0.01$ vs. LPS ($n = 10$ rats per group).

3.6. Lung histopathology

Lung sections were prepared and hematoxylin-eosin (H.E.) staining was performed to investigate the effects of various treatments on LPS-induced lung injury, the pulmonary morphologic changes were observed under a light microscope. As shown in (Fig. 9), the lung section from sham group after H.E. staining shows a normal bronchoalveolar structure with few inflammatory cells infiltration. In contrast, lung tissues from the LPS group were significantly damaged, manifesting as interstitial pneumonia, thickening of the alveolar wall, narrowing of the alveolar cavity, and infiltration of inflammatory cells, especially neutrophils into the peribronchiolar and perivascular connective tissues as compared with the sham. Histologic damage was less pronounced in TA (7.5, 15, and 30 mg/kg) treated animals as compared with LPS group. DXM at 1.5 mg/kg treatment, used as the reference drug, also remarkably attenuates the pulmonary histologic changes. Moreover, a similar inhibitory effect was noted in semiquantitative analysis, which facilitated the measurement of severity and the extent of inflammatory change. As shown in (Table 1), there was no lesion score in lung tissues from the sham group (0). The microscopic lesion score for the LPS group was 3.9 ± 0.7 , which was significantly higher than the score for the sham group ($p < 0.01$), but there was marked difference between the TA and LPS groups ($p < 0.05/0.01$). The results suggested that TA attenuated many of the symptoms of LPS-induced in rats.

4. Discussion and conclusion

The key elements of building a successful airway inflammation model are the choice of LPS sensitive animals and dose, in which a perfect animal model need to keep the features of human disease. Based on literatures analysis, we find that exposure of lung with lipopolysaccharide (LPS) or endotoxin, a major constituent of the outer membrane of gram-negative bacteria and a pivotal inducer of pulmonary inflammation, may cause AI with an inflammatory response, alveolar macrophage activation and massive tissue neutrophil infiltration (Kitamura et al., 2001; Saluk-Juszczak and Wachowicz, 2005). In the present manuscript, biochemical evidence and histopathological evaluation confirmed that a successful animal model of AI was established by intratracheal instillation of LPS in rats, since the etiologies and mechanisms of AI have been extensively investigated in numbers of experimental models. Glucocorticoids with anti-inflammatory and anti-allergic are the most potent therapeutic agents, which are used to treat chronic respiratory diseases such as COPD (Barnes, 2013), and to alleviate respiratory failure caused by neutrophilic granulocyte and alveolar macrophages in their metabolically activated states (Caramori and Adcock, 2003). Then, dexamethasone (DXM) was selected as a positive control to evaluate the anti-inflammatory efficiency of total alkaloids (TA) in LPS-evoked inflammation. The results indicated that TA may be effective in alleviating airway inflammation in rats.

In airway inflammation, excessive amounts of neutrophils may exacerbate injury and amplify inflammation, including the injuring lung (Haslett, 1997), so neutrophils are the predominant inflammatory cells and considered to be central to the pathogenesis of AI (Barnes, 2013; Cepkova and Matthay, 2006). As expected, the animals exposed to endotoxin exhibited a massive recruitment of inflammatory cells including neutrophils in the airways in our experiment. Then, TA inhibited the endotoxin-evoked raising the number of total cells and neutrophils, and increased the number of alveolar macrophage, monocyte in the BALF of all tested groups. There was substantial infiltration of inflammatory cells in rats with LPS-induced AI consistent with histological examination of the

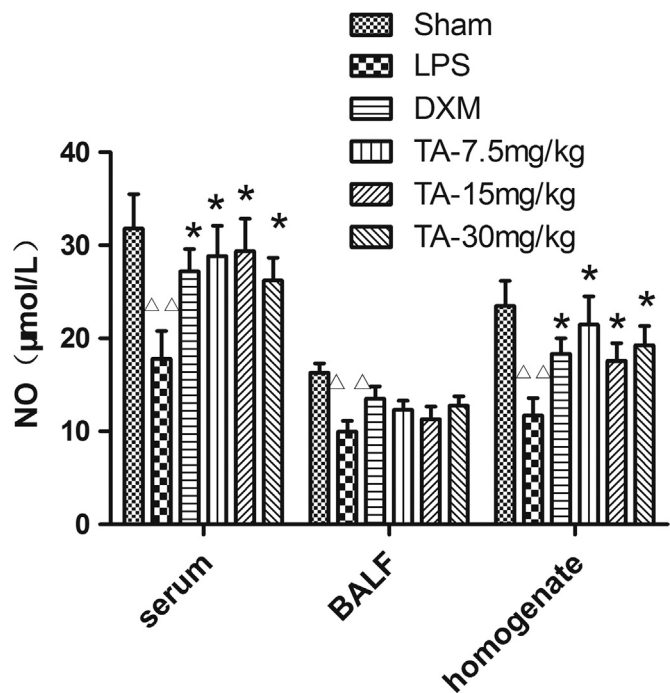


Fig. 4. TA regulated NO production in serum, BALF and homogenate. Data was shown as means $\pm$ SEM values. [△] $p < 0.01$ vs. sham, ^{*} $p < 0.05$, ^{**} $p < 0.01$ vs. LPS ($n = 10$ rats per group).

group were demonstrated when compared to the sham group. TA at 7.5, 15 and 30 mg/kg significantly elevated ALB level ($p < 0.05$) (Fig. 8A), and decreased LPS-induced LDH activity in serum ($p < 0.05$) (Fig. 8C), but the AKP level of serum was normal (Fig. 8B) ($p > 0.05$). DXM also significantly advanced ALB activity and abated AKP and LDH activities ($p < 0.01$) when compared to the LPS group (Fig. 8).

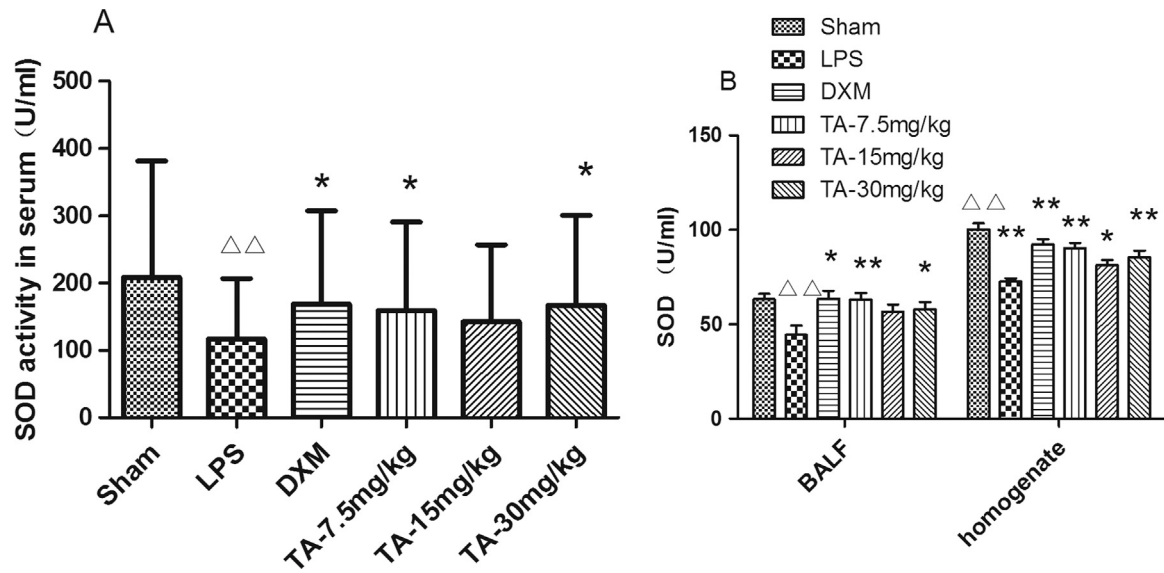


Fig. 5. TA inhibited the decrease of SOD activity in serum, BALF and homogenate. Data was shown as means $\pm$ SEM values. $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n = 10$ rats per group). (A) SOD activity in serum. (B) SOD activity in BALF and homogenate.

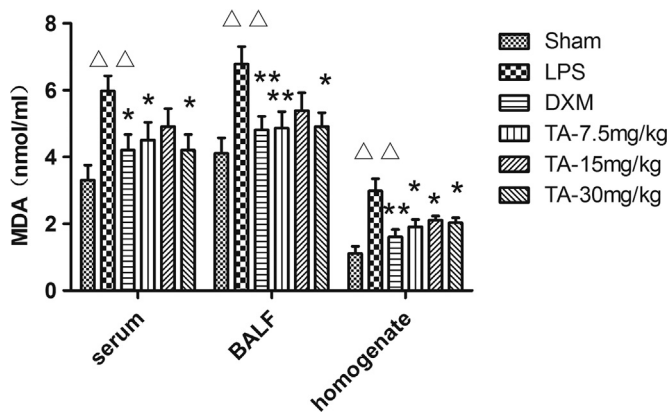


Fig. 6. TA decreased MDA content in serum, BALF and homogenate. Data was shown as means $\pm$ SEM values. $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n = 10$ rats per group).

lung tissue, while TA successfully alleviated lung inflammation and reduced the tissue neutrophilia, in accordance with our observation in the BALF. Predominantly protective role of TA against the development of LPS-evoked pulmonary inflammation might be related to an attenuation of inflammatory cell sequestration and migration into the lung tissue.

LPS led to the secretion of several inflammatory and chemotactic cytokines, and overexpressed pro-inflammatory cytokines TNF- α and IL-8 might cause lung inflammation more serious in the pathophysiology of the early phase of AI (Liaudet et al., 2002), and might give rise to the successive complications associated with AI (Ward, 1996), and high production of cytokines referred above in the BALF has been noted in patients (Minamino and Komuro, 2006). In our investigation, large amounts of TNF- α and IL-8 were produced in the BALF, serum and lung tissues of animals of all groups by endotoxin induced, except sham. Indeed, rats treated with TA intragastrically down-regulated TNF- α and IL-8 production after LPS challenge, which might protect against LPS-induced AI by cutting down the production of these pro-inflammatory cytokines.

It has been reported that oxidative stress and injury are common in most experimental models of lung injury and are confirmed by studies of patients with upper respiratory infection (Matthay et al., 1999). SOD is an enzyme that exists in cells for removing oxyradicals, whose activity variation may represent degree of tissue injury. Extracellular SOD might modulate neutrophil inflammation by reducing cytokine released from macrophages, suggest that extracellular SOD is an anti-inflammatory enzyme as well as a major anti-oxidant (Mates, 2000). Previous studies showed that a MDA concentration increase in the blood,

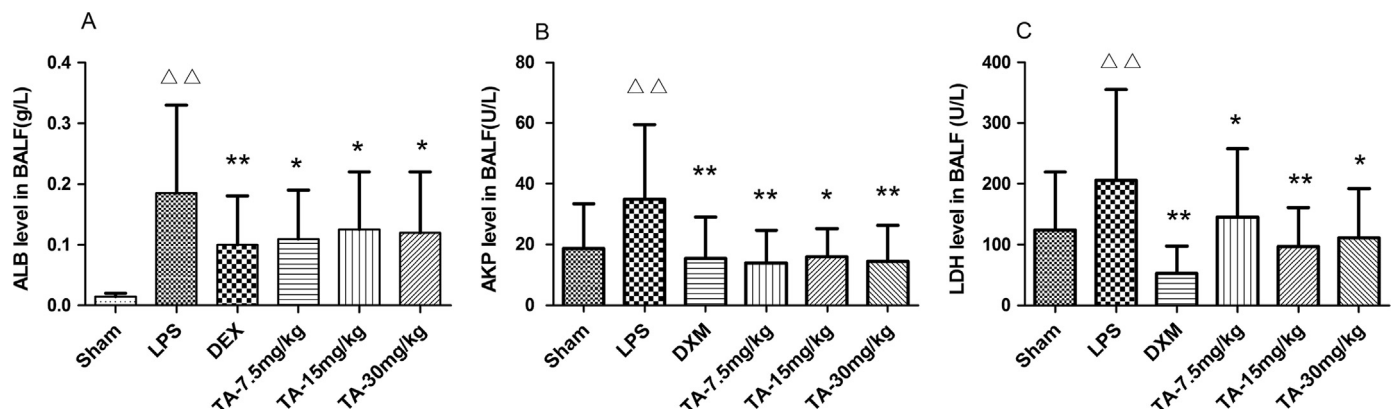


Fig. 7. TA regulated ALB, AKP and LDH level in BALF. These items were analyzed by fully automatic biochemical analyzer. Data was shown as means $\pm$ SEM values. $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n = 10$ rats per group). (A) ALB level. (B) AKP level. (C) LDH level.

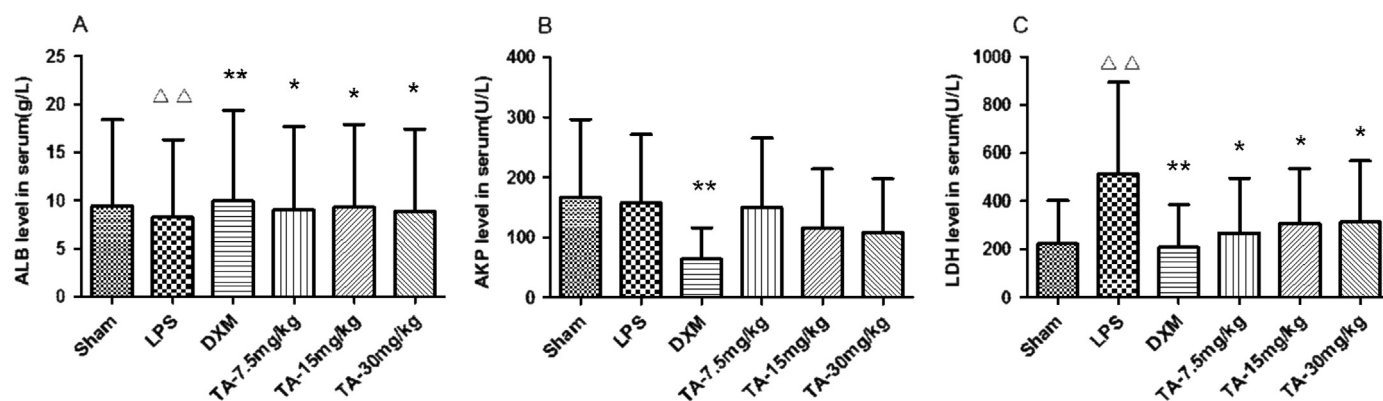


Fig. 8. TA regulated ALB, AKP and LDH level in serum. These items were analyzed by fully automatic biochemical analyze. Data was shown as means $\pm$ SEM values. $^{\Delta\Delta}p < 0.01$ vs. sham, $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS ($n = 10$ rats per group). (A) ALB level. (B) AKP level. (C) LDH level.

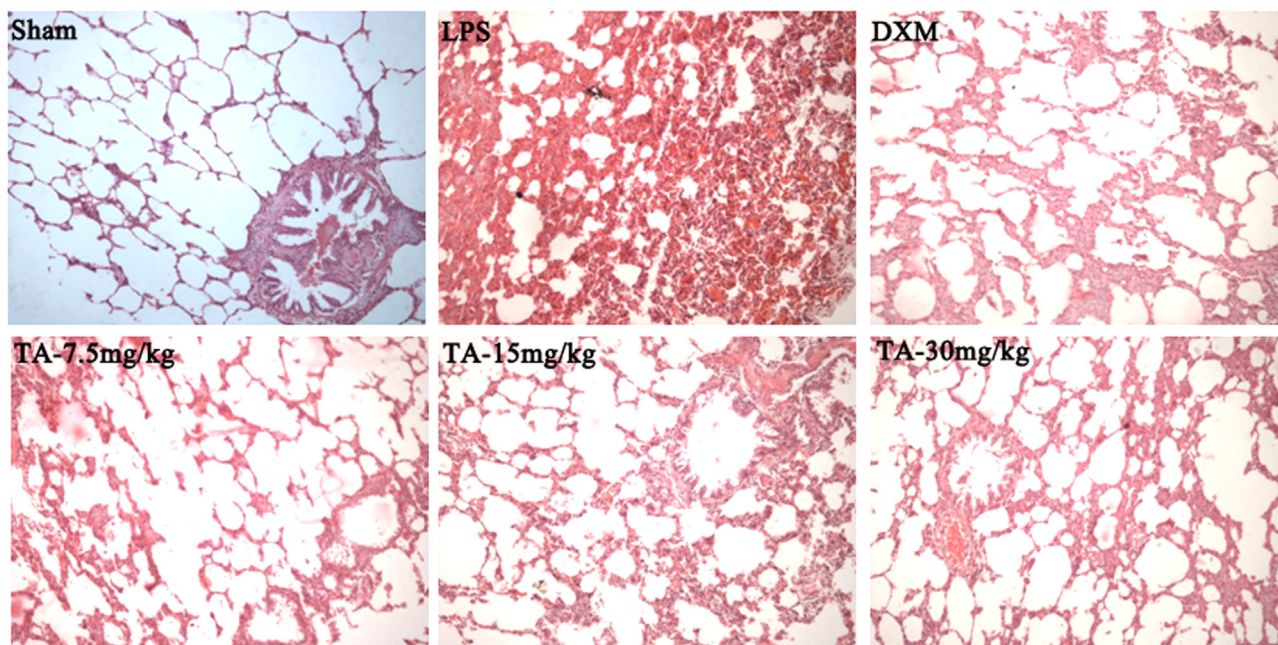


Fig. 9. Qualitative effects of TA on airway inflammation (images, Original magnification $\times 20$). At the end of the experiment, rats were subjected to being harvested for the lung, and the right upper lobes of lung was fixed with 4% neutralized formalin until the paraffin sections were prepared and stained with Hematoxylin and Eosin (H&E). The inflammatory cell infiltration was examined by light microscopy. A double-blind method was used in the pathology, the results were qualitatively identical, and representative results were shown.

Table 1
Pathological changes after intratracheal LPS instillation.

Group	Dose (/kg)	Pathological changes						Scores
		–	±	+	++	+++	++++	
Sham	–	9	1	0	0	0	0	0.1 $\pm$ 0.1
LPS	10 ml	0	0	0	3	5	2	3.9 $\pm$ 0.2 $^{\Delta\Delta}$
DXM	1.5 mg	0	0	4	5	1	0	2.7 $\pm$ 0.2 **
TA	7.5 mg	0	0	4	5	1	0	2.8 $\pm$ 0.2 **
	15 mg	0	1	3	3	2	1	2.9 $\pm$ 0.4 *
	30 mg	0	1	4	2	2	1	2.8 $\pm$ 0.4 *

Note: Semi-quantitative analysis of inflammatory cell infiltration in lung sections were performed as described in Materials and Methods. Lung tissues were fixed, sectioned at $3 < \mu\text{m} > \text{m}$, and stained with H&E for tissue neutrophils infiltration. Data are expressed as mean $\pm$ SEM. Statistical differences are represented as

$^*p < 0.05$

$^{**}p < 0.01$ vs. LPS ($n = 10$ rats per group).

$^{\Delta\Delta}p < 0.01$ vs. sham

BALF and pulmonary tissues after LPS administration (Shen et al., 2009), which was also observed in our experiment. NO is synthesized from L-arginine, and recognized as a mediator and regulator of inflammatory response. Although, the depressed production of NO and iNOS in respiratory tract inflammation are thought to contribute importantly to its pathophysiology, but still this role remains controversial (van der Vliet et al., 1999). In the current study, we analyzed the anti-oxidant parameters (SOD, MDA and NO) of blood, BALF and lung tissues, and found that the SOD and NO activities were run-down in all LPS-treated groups significantly, but recovered in animals treated with TA remarkably. Meanwhile, the promoted MDA in LPS-treated animals could be reduced by TA.

In our research, alkaline phosphatase (AKP), a specific marker of the degree of pneumocyte type II cell stimulation, differentiation and damage (Drent et al., 1996; Edelson et al., 1988; Henderson et al., 1995), and lactate dehydrogenase (LDH), a marker of lung cytotoxicity (Drent et al., 1996), as well as albumin (ALB), a marker of inflammation or physical lesion (Clarke et al., 1999), in the BALF were raised rapidly after the LPS challenge,

while they were alleviated after TA treated. However, ALB content was rebated, and LDH activity was enhanced markedly, while AKP was not changed in serum after the LPS instillation. The results supported that ALB was brought into the airway through alveolar epithelial injury-capillary barrier in airway inflammation (Clarke et al., 1999), while the AKP and LDH activities were elevated by the injured type II alveolar epithelial cell in BALF (Drent et al., 1996; Edelson et al., 1988; Henderson et al., 1995), whereas airway inflammation can cause the leakage of ALB and the enhancement of LDH in blood as well (Long et al., 1996).

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