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# Protective effect of total alkaloids on lipopolysaccharide-induced acute lung injury

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ABSTRACT

*Background:* *Corydalis denticulato-bracteata Fedde* is used as a traditional herbal medicine for the treatment of pneumonia. However, there is no scientific evidence, which validate the use of total alkaloids of *denticulato-bracteata Fedde* in the literature.

*Materials and methods:* Male Kunming mice were randomly divided into seven groups ($n = 12$, each): control group, total alkaloids alone (200 mg/kg, intragastric gavage), LPS group, and three different doses (50, 100, and 200 mg/kg, intragastric gavage) for total alkaloids-treated groups, Dexamethasone (5 mg/kg, intraperitoneally) group. Corresponding drugs or vehicles were given 24 and 1 h before lipopolysaccharide (LPS) administration (5 mg/kg, intraperitoneally). The severity of pulmonary injury was evaluated 6 h after LPS challenge.

*Results:* As revealed by survival study, pretreatment with total alkaloids significantly reduced LPS-induced death. We also found that total alkaloids pretreatment markedly decreased the lung wet-to-dry weight ratios and significantly attenuated histopathologic changes. Moreover, total alkaloids decreased the production of the tumor necrosis factor α and nitric oxide in the serum and bronchoalveolar lavage fluid. Total alkaloids pretreatment also reduced LPS-induced inducible nitric oxide synthase and p65 nuclear factor kappa B protein expression in the lung.

*Conclusions:* This study indicates that total alkaloids may have a protective effect against LPS-induced acute lung injury. This protective effect of total alkaloids seems to result from inhibition of nuclear factor kappa B activation, which causes the reduction of inflammatory markers such as tumor necrosis factor α and inducible nitric oxide synthase.



## 1. Introduction

Acute lung injury (ALI), as a common clinical disorder, is a syndrome of acute respiratory failure defined by an intense pulmonary inflammatory response, involving neutrophil recruitment, interstitial edema, a disruption of epithelial integrity, and lung parenchymal injury [1]. Despite increasing insights into ALI pathobiology, it still presents the high mortality rate of approximately 40% [1]. Therefore, there remains an urgent need for the discovery of definitive and targeted drug therapies for ALI. Lipopolysaccharide (LPS), the major integral structural component of the outer membrane of gram-negative bacteria, has been most widely used to induce the animal models of ALI, which resemble lots of the characteristics of human ALI [2]. LPS induces expression of various proinflammatory mediators including tumor necrosis factor (TNF-α) [3] and nitric oxide (NO) produced by inducible NO synthase (iNOS) [4]. TNF-α, plays a key role in the process of

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inflammation. TNF-α, is the cardinal inflammatory mediator associated with various diseases, including rheumatoid arthritis, bacterial sepsis, and skin inflammation [5]. In addition, TNF-α can stimulate production of a host of other cytokines, such as COX-2 and iNOS [6,7]. NO, a short-lived small molecule, plays a significant role in a variety of physiological processes [8]. It is well known that NO deprived from the induction of iNOS may play a harmful role by directly inducing pathophysiology of ALI like tissue damage and through the formation of peroxynitrite and other inflammatory mediators [9,10]. Nuclear factor kappa B (NF-κB) is a transcription factor and binds to the κB motifs in the promoters of target genes, and thus, induces the transcriptions of iNOS, COX-2, and TNF-α [11]. NF-κB is activated in response to various inflammatory stimuli including bacterial LPS. Thus, the inhibition of the TNF-α, iNOS, and NF-κB excessive production stands as a central therapeutic goal.

*Corydalis denticulato-bracteata Fedde* is a tree of genus *Corydalis* (family Papaveraceae). The genus *Corydalis* comprises of 320 species distributed in the Northern hemisphere, and 70 species have been used as traditional medicine in China, Japan, and Korea [12]. *C denticulato-bracteata Fedde* is used as a traditional herbal medicine for the treatment of pneumonia. Isoquinoline alkaloids have been identified as major active secondary metabolites of *Corydalis* [13]. Alkaloids have been reported to possess anti-inflammatory activities [14]. We hypothesize that maybe total alkaloids of *denticulato-bracteata Fedde* has anti-inflammatory activity. However, there is no scientific evidence, which validate its use in the literature.

In our present study, we investigated the protective effect of *C denticulato-bracteata Fedde* total alkaloids against LPS-induced ALI in mice. Meanwhile, the possible mechanisms for its protective activity were also explored in this study.

## 2. Materials and methods

### 2.1. Plant material and chemicals

*C denticulato-bracteata Fedde* was collected from Lhasa in Tibet of China. Identity of the herbs was confirmed by Professor Xiaofeng Niu, Xi'an Jiaotong University, Shanxi Province, China. A voucher specimen of the plant was deposited at the herbarium of the Pharmacognosy Department of Xi'an Jiaotong University, China for further reference.

Tween80 (cloud point) was obtained from Tianjin Chemical Company. Dexamethasone (Dex) was purchased from Xi'an Lijun Pharmaceutical Company Limited (Shanxi, China). LPS (*Escherichia coli* serotype O55:B5) and Griess reagent were purchased from Sigma (St. Louis, MO). The enzyme linked immunosorbent assay kit for mouse TNF-α was purchased from R&D Systems (Minneapolis, MN). p65 NF-κB and iNOS polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA).

### 2.2. Preparation of total alkaloids

The air-dried whole plant was milled into fine powder. The powder (800 g) was macerated in 95% ethanol for 24 h (with occasional shaking), and then extracted with boiling 95% ethanol three times (2, 2, and 1 h). The solvent was mixed and then evaporated under reduced pressure to yield a residue (135 g). The residue was dissolved in 2% aqueous hydrochloric acid (vol/vol). The acidic solution was filtered and then exhaustively extracted with petroleum ether to remove the neutral materia. The acidic solution was subsequently basified with NaOH to pH 11 and extracted with chloroform several times to yield the total alkaloids (5 g).

### 2.3. Animals

Male Kunming mice with body weight 22–25 g were used in this study. They were obtained from the Experimental Animal Center at Xi'an Jiaotong University (Xi'an, China). Animals were maintained under standard conditions with a 12-h light–dark cycle and fed with standard pelleted food and water *ad libitum*. The animals were acclimatized to their environment for at least 1 wk before the experiments were started and were used only once throughout the pharmacologic experiments.

The care of animals in this study was conducted in accordance with the National Institute of Health guidelines.

### 2.4. Drug administration

The control group received vehicle (10 mL/kg, 0.5% Tween80 suspension in distilled water). The total alkaloids were administered in 50, 100, and 200 mg/kg doses after being suspended in vehicle. Dex in vehicle was used as reference drug. Three different doses of the samples were determined on the basis of pilot studies.

### 2.5. LPS-induced accumulative mortalities

To assess mortality rates, mice received an intraperitoneal injection of LPS 20 mg/kg with or without different doses of total alkaloids (50, 100, and 200 mg/kg, intragastric gavage [i.g.]) and Dex (5 mg/kg, intraperitoneally [i.p.]). Corresponding drugs or vehicle was administered 24 and 1 h before LPS stimulation. The mortality of mice was recorded every 12 h for 3 d after the LPS injection in each group ($n = 12$).

### 2.6. Experimental design

Male mice were divided into seven groups ($n = 12$/group) randomly. Group I considered as control received the 0.5% Tween80 (10 mL/kg, i.g.). Group II was i.g. administered with total alkaloids (200 mg/kg) only. Group III served as the LPS group (5 mg/kg, i.p.) [15]. Group IV–VI considered as alkaloids treatment groups (50, 100, and 200 mg/kg, i.g.; LPS, 5 mg/kg, i.p.). Group VII used as Dex group (5 mg/kg, i.p.; LPS, 5 mg/kg, i.p.). Corresponding drugs or vehicles were given 24 and 1 h before LPS administration [16]. The severity of pulmonary injury was evaluated 6 h after LPS challenge. In one set of experiments, six mice were chosen, blood samples were collected from the retro-orbital plexus, and then all animals were sacrificed. Bronchoalveolar lavage fluid (BALF) was performed through the lung. In the other separate set of experiments, six mice were chosen and sacrificed. The left lung was excised for analysis of lung wet-to-dry (W/D) weight ratio. The