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Original article

Total alkaloids from *Thesium chinense* inhibit lipopolysaccharide-induced respiratory inflammation by modulating Nrf2/NF-κB/NLRP3 signaling pathway

Guohui Li[a,b], Yueqin Guan[c], Lintao Xu[a], Guangcheng Peng[a], Qingtong Han[a], Tian Wang[a], Zhenpeng Xu[a], Xuesen Wen[a], Hongxiang Lou[a,*], Tao Shen[a,*]

[a] *Key Lab of Chemical Biology (MOE), School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, Jinan 250012, China*
[b] *Department of Pharmacy, Jinan Maternity and Child Care Hospital Affiliated to Shandong First Medical University, Jinan 250218, China*
[c] *Jiuhua Huayuan Pharmaceutical Co., Ltd., Chuzhou 239011, China*

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ABSTRACT

Inflammation plays a pivotal role in the etiology and progression of various diseases. In traditional Chinese medicine, the whole plants of *Thesium chinense* Turcz. and its preparations (e.g. Bairui Granules) have been employed to manage inflammatory conditions. While flavonoids were previously considered the primary anti-inflammatory components, other potentially active constituents have been largely overlooked and not thoroughly investigated. This study presents a novel finding that the total alkaloids of *T. chinense* (BC-Alk) are potent active substances underlying the traditional and clinical applications of *T. chinense* and Bairui Granules as anti-inflammatory agents. UPLC-MS/MS analysis identified the composition of BC-Alk as quinolizidine alkaloids. The anti-inflammatory efficacy of BC-Alk was evaluated using a lipopolysaccharide (LPS)-induced lung inflammation model in mice. Results demonstrated that BC-Alk significantly mitigated LPS-induced lung inflammation, attenuated the overproduction of IL-1β and the overproduction of inflammatory factors (TNF-α), and ameliorated lung tissue hyperplasia in mice *in vivo*. Mechanistic studies *in vitro* revealed that BC-Alk upregulated the expression of Nrf2 and its downstream proteins NQO1 and glutamate-cystine ligase and modifier subunit (GCLM), inhibited NF-κB phosphorylation, and suppressed NLRP3 activation. Collectively, these findings indicate that BC-Alk exerts potent inhibitory effects against lung inflammation by modulating Nrf2, NF-κB, and NLRP3 pathways. This study provides new insights into the anti-inflammatory constituents of *T. chinense* and Bairui Granules.

1. Introduction

The whole plants of *Thesium chinense* Turcz., a member of the Santalaceae family, are commonly utilized in traditional Chinese medicine for treating inflammatory conditions such as upper respiratory infections, laryngopharyngitis, and tonsillitis. A comprehensive clinical investigation on the efficacy of *T. chinense* whole plants in combating inflammation-related ailments (including acute tonsillitis, gingivitis, acute mastitis, pulmonitis, and upper respiratory tract infections) has been systematically conducted, revealing an overall effectiveness rate exceeding 90% [1].

Owing to its remarkable therapeutic efficacy, preparations of *T. chinense* [such as Bairui Granules] have been developed, demonstrating anti-infective properties in human upper respiratory tract diseases [2, 3]. Given its notable inhibitory effect on lung inflammation, Bairui Granules were incorporated into the official drug catalog issued by the Beijing government for the treatment of Corona Virus Disease 2019 (COVID-19). Phytochemical investigation of chemical constituents from *T. chinense* commenced in 1976. To date, thirty-four compounds have been isolated, including flavonoids, alkaloids, terpenoids, organic acids, and other substances [3].

Acute and chronic inflammation contribute to numerous human diseases, including respiratory disorders, cancer, diabetes, cardiovascular diseases, and neurodegenerative conditions [4-6]. The human respiratory tract is particularly vulnerable to direct exposure from pathogenic microorganisms (such as influenza virus A and SARS-CoV-2), particles, and toxicants. These agents can lead to oxidation of phospholipids, DNA, and proteins, as well as structural damage to the respiratory system [7, 8]. Simultaneously, these exogenous substances activate epithelial cells and inflammatory cells, triggering the release of inflammatory mediators [such as interleukins (ILs) and inducible nitric oxide synthase (iNOS)], which can subsequently induce the development of inflammatory lung diseases, including chronic obstructive pulmon-

* Corresponding author.
E-mail addresses: louhongxiang@sdu.edu.cn (H. Lou); shentao@sdu.edu.cn (T. Shen)

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ary disease (COPD) and COVID-19 [9].

Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a crucial role in modulating oxidative stress and inflammation [10]. Its activation inhibits the release of interleukins (ILs), such as IL-6 and IL-1β, and promotes the protein expression of glutamate-cystine ligase and modifier subunit (GCLM), thereby alleviating inflammation and oxidative damage [11]. NOD-like receptor 3 (NLRP3) inflammasome and nuclear transcription factor-κB (NF-κB) are involved in the regulation of inflammatory responses. The activation of NF-κB and NLRP3 contributes to the overproduction of cyclooxygenase-2 (COX-2), ILs, iNOS, and other factors, consequently exacerbating the inflammatory response [12, 13]. Therefore, activation of Nrf2 and inhibition of NLRP3 inflammasome and NF-κB can effectively reduce inflammation, presenting valuable strategies for treating inflammation-related respiratory diseases.

Previous phytochemical investigations of *T. chinense* have primarily focused on flavonoids as the dominant bioactive constituents. In this study, the total alkaloids of *T. chinense* (BC-Alk) were first identified as potential agents against inflammation-related diseases, and their anti-inflammatory effects were evaluated *in vivo*. Moreover, the targeting of Nrf2, NF-κB, and NLRP3 inflammasome signaling pathways was validated as a potential mechanism for BC-Alk in mitigating lung inflammation.

2. Materials and Methods

2.1. Plant material

The entire plants of *Thesium chinense* Turcz. were collected in July 2019 from Fuyang City, Anhui Province, China. Authentication was performed by Xuesen Wen (one of the authors), and a voucher specimen (No. 20190909-10-BRC) has been deposited in the Laboratory of Pharmacognosy, School of Pharmaceutical Sciences, Shandong University.

2.2. Preparation of extracts of T. chinense

The dried 20.0 g of *T. chinense* herb underwent reflux extraction with water for 3 h to yield a water extract (BC-H_2O, 4.86 g, yield 24.3%). Subsequently, 50.0 g of dried *T. chinense* herb was subjected to 3 h of reflux extraction in 75% EtOH solution, producing an EtOH extract (BC-EtOH, 6.75 g, yield 13.5%). This EtOH extract was dissolved in distilled water and partitioned sequentially with petroleum ether, dichloromethane, and ethyl acetate, resulting in crude extracts of petroleum ether (BC-EtOH-P, 0.06 g, yield 0.12%), dichloromethane (BC-EtOH-C, 0.43 g, yield 0.86%), ethyl acetate (BC-EtOH-E, 0.27 g, yield 0.54%), and water (BC-EtOH-H, 5.99 g, yield 11.98%). Additionally, 30.0 g of T . chinense herb was extracted with 0.5 mol·L^{-1} HCl solution for 3 h, followed by chlorophyll removal using methylene chloride. After adjusting the pH to 10.0, total alkaloids (BC-Alk, 0.072 g, yield 0.24%) were obtained through methylene chloride extraction. Bairui Granules (5.0 g), produced by Anhui Jiuhua&Huayuan Pharmaceutical, were ground into powder and extracted with methanol via ultrasonic treatment for 1 h, then filtered to obtain the methanol extract of the preparation (G-MeOH, 0.67 g, yield 13.4%). The complete extraction process is summarized in Fig. 1A.

2.3. UPLC-MS/MS analysis of BC-Alk

UPLC-LTQ-Orbitrap-MS (Waltham, MA, USA) analysis was employed to determine the composition of BC-Alk. The mobile phase consisted of a 0.1% formic acid solution with a flow rate of 0.3 mL·min^{-1}. Analyte separation was achieved using a UPLC BEH Shield RP_{18} column (Waters, USA). The ESI ion source operated in positive ion mode. Data acquisition occurred in Full MS/dd-MS^2 modes, with the Fourier high-resolution scanning range encompassing 100 to 1500 *m/z*. Subsequent data analysis was conducted using Thermo Xcalibur 4.0 software.

2.4. Cell culture

Beas-2B cells, RAW 264.7 murine macrophages, and J774 A.1 murine macrophages were obtained from American Type Culture Collection (Manassas, VA, USA). RAW 264.7 and J774 A.1 cells were cultured in DMEM, while Beas-2B cells were maintained in RPMI 1640 medium. Both media were supplemented with 10% fetal bovine serum (FBS). All cell lines were incubated at 37 °C in an atmosphere containing 5% CO_2.

2.5. Nitric oxide (NO) production assay

The cells were seeded in a 96-well plate and treated with various constituents, with or without LPS, for 24 hours. Griess reagent was subsequently added to the supernatant. Following incubation at room temperature (RT) for 15 minutes, the absorbance was measured at 570 nm. The nitrite concentration was determined by comparison to a standard curve of sodium nitrite.

2.6. Cell viability assay

The 3-(4,5-Dimthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to assess cell viability. Beas-2B cells were cultured in a 96-well plate and subsequently exposed to various components for 24 or 48 hours. Following a 3-hour incubation with MTT at 37 °C, cellular growth responses were evaluated using a Synergy 2 plate reader (BioTeK) at a wavelength of 570 nm.

2.7. Antibodies and immunoblot analysis

Cells underwent treatment with specified components, followed by homogenization in lysis buffer. The resulting proteins were separated using 10% SDS-PAGE and transferred onto nitrocellulose membranes (Millipore). These membranes were incubated with 5% skimmed milk for 1.5 h. Subsequently, the membranes were exposed to primary antibodies (1∶1000 dilution; Proteintech Group, Inc and ABclonal Technology Co., Ltd.) overnight at 4 °C, followed by incubation with corresponding secondary antibodies (1∶5000 dilution) for 2 h. The protein bands were visualized using the Bio Rad ChemiDoc XRS+ system.

2.8. ELISA assay

Cells were seeded in a D35 dish and stimulated with LPS (1 μg·mL^{-1}) for 3 h, followed by exposure to the test components for 10 h. The concentrations of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the cell supernatant were quantified using an enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions.

2.9. Immunofluorescence assay

The cells were immobilized using a 1∶1 mixture of acetone and methanol. Subsequently, the coverslips were exposed to Nrf2 antibodies overnight. Following a washing step, they were incubated with ALEX 594 secondary antibody. The samples were then incubated with DAPI in dark conditions for 10 minutes. Fluorescence signals were detected using an Olympus BX53 fluorescence microscope (Tokyo, Japan).

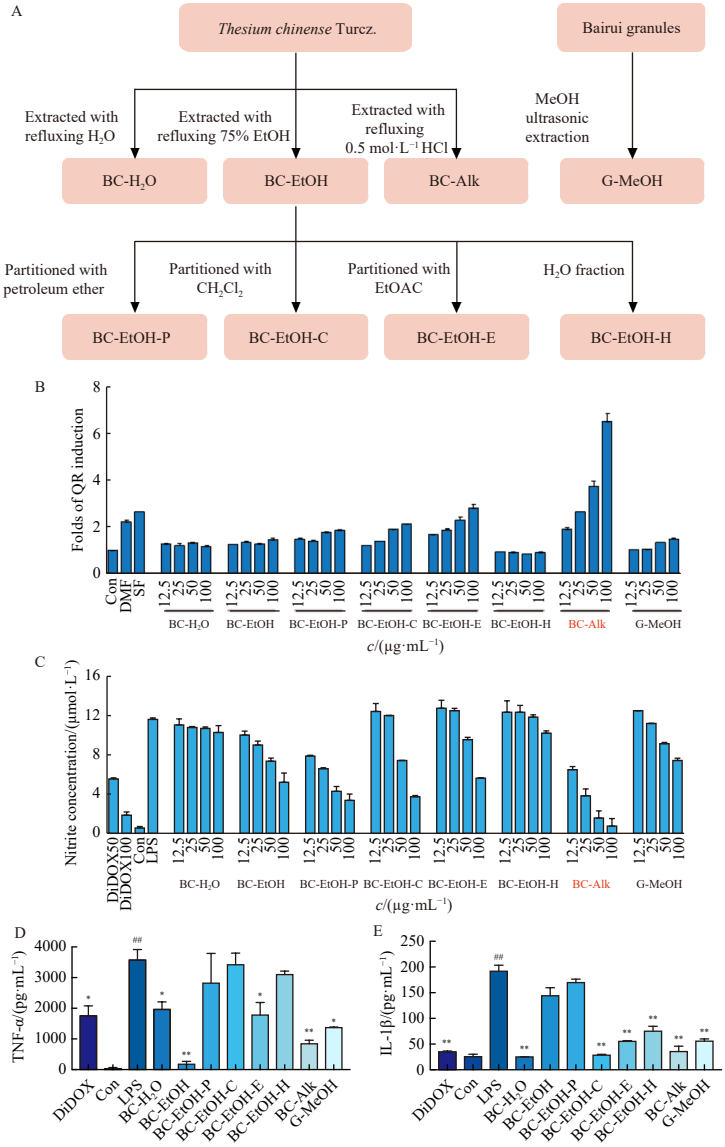


Fig. 1 Discovery of total alkaloids to be the most active anti-inflammatory substances of *T. chinense*. (A) Preparation of eight extracts of *T. chinense* and Bairui Granules. (B) Relative QR activity. Cells were treated with eight extracts for 24 h, and QR inducing activity was determined. (C) Relative NO level. Cells were treated with eight extracts and 1 μg·mL^{-1} LPS for 24 h, and the NO level was detected. (D) and (E) The levels of TNF-α or IL-1β. Cells were stimulated with LPS for 3 h, then treated with different doses of eight kinds of extracts for 0.5 h or incubated with nigericin (3 μmol·L^{-1}) for 0.5 h. The levels of TNF-α or IL-1β were detected by Elisa assay. The results were expressed as mean ± SD (n = 3), $^{\#\#}P < 0.01$ *vs* Con group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* LPS group.

2.10. Flow cytometry assay

Cells were seeded in D35 culture dishes and exposed to specified doses of the test components. Following this, $NaAsO_2$ (2.5 μmol·L^{-1}) was added, and incubation continued for 8 h. A FACSCalibur flow cytometer (BD Biosciences) was utilized to measure ROS levels after treatment with DCFH-DA.

2.11. Mice and treatment

Thirty C57BL mice (male, 22–26 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. and initially subjected to a two-week adaptive feeding period. The mice were then randomly assigned to six groups. Groups 1 and 2 received 200 μL of normal saline *via* gavage, while groups 3–6 were administered 20, 40, 50 mg·kg^{-1} G-MeOH, and 1 mg·kg^{-1} DEX, respectively (all agents dissolved in saline solution). Four hours post-treatment, 10 mg·kg^{-1} LPS was atomized into the trachea of mice in groups 2–6 (Shanghai Yuyan Instruments Co., Ltd.). All animal experiments were conducted in accordance with the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the Ethics Committee of Shandong First Medical University (No. W2021030033).

2.12. Hematological analysis

The collected blood samples were analyzed for the following hematological parameters: white blood cell count (WBC), basophils, and neutrophil-to-leukocyte ratio. These parameters were quantified using an automated blood cell analyzer (Mindray BC-6800).

2.13. Lung histological analysis

The isolated lung tissues were fixed in 4% paraformaldehyde at room temperature (RT) for 24 hours. Following paraffin embedding, sections were stained with hematoxylin-eosin (HE). The sections were then dried and examined using a BX53 + DP73 microscope system.

2.14. Quantitative real time reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total mRNA from isolated lung tissue was extracted using Trizol reagent, and cDNA synthesis was performed using the PrimeScript TM RT kit. Quantitative real-time PCR (qRT-PCR) was conducted using Perfect Start Green qPCR SuperMix. The primer sequences for the tested inflammatory mediators are listed in the Supplementary materials (Supplementary Table 1).

2.15. Statistical analysis

All results are presented as mean ± SD. The *t* test method, using GraphPad Prism 9 software, was employed to assess differences between two groups. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Discovery of total alkaloids to be the most active anti-inflammatory substances of T. chinense

To identify the bioactive components, *T. chinense* was fractionated into seven parts, and the Bairui Granules extract was prepared (Fig. 1A). The antioxidant activity of these fractions was assessed using the QR assay, while their anti-inflammatory activity was evaluated through the NO release assay. As illustrated in Fig. 1B, the alkaloid partition of *T. chinense* (BC-Alk) and BC-EtOH-E partition demonstrated significantly stronger QR-inducing activity compared to the positive control, sulforaphane (SF). BC-Alk (100 μg·mL^{-1}) exhibited remarkable antioxidant activity, with QR-inducing activity approximately seven times higher than the control group. Both BC-EtOH and BC-Alk showed dose-dependent inhibition of NO production, with BC-Alk demonstrating the most potent effect, surpassing the positive control (Fig. 1C). Additionally, BC-Alk strongly suppressed TNF-α and IL-1β production (Figs. 1D and 1E). These findings strongly indicate that BC-Alk possesses anti-inflammatory and antioxidant properties *in vitro*.

3.2. Identification of chemical constituents of alkaloids partition

The UPLC-HR-MS/MS chromatographic fingerprints of BC-Alk revealed four prominent peaks (Fig. 2A). Peak 4 was identified as sophocarpine (Fig. 2B), a compound previously isolated from *T. chinense* [3]. Peaks 1 and 3 were identified as reduction products and their isomers (matrine or sophoridine), while peak 2 was determined to be sophoramine, a product resulting from further oxidation of sophocarpine. These identifications were made by comparing the MS spectra with previously reported data.

3.3. BC-Alk alleviated lung inflammation in LPS-induced mice

To assess the potential effects of BC-Alk on respiratory inflammation, an LPS-induced respiratory inflammation model was employed. The LPS group exhibited characteristic features of inflammation, including pneumocyte hyperplasia, thickening of alveolar septa, and prominent leukocyte infiltration, as revealed by HE staining. Treatment with 40 mg·kg^{-1} BC-Alk effectively reversed these changes, demonstrating an effect comparable to that of 1 mg·kg^{-1} DEX (Fig. 3A). Moreover, 40 mg·kg^{-1} BC-Alk treatment significantly reduced the overproduction of cellular inflammatory cytokines TNF-α and IL-1β in LPS-stimulated lung tissues (Figs. 3B and 3C). Compared to the LPS group, a substantial decrease in neutrophil count was observed after treatment with 40 mg·kg^{-1} BC-Alk, while basophils showed a downward trend without significant difference (Figs. 3D and 3E). Furthermore, treatment with 40 mg·kg^{-1} BC-Alk successfully reversed the LPS-induced elevation of MDA levels (Fig. 3F). Collectively, these findings suggest that BC-Alk mitigates LPS-induced lung inflammation.

3.4. BC-Alk activated Nrf2, and inhibited LPS-stimulated activation of NF-κB and NLRP3 inflammasome in vivo

To further elucidate the mechanism by which BC-Alk treats pulmonary inflammation, we initially investigated its impact on LPS-stimulated activation of NF-κB and its downstream target iNOS. The results demonstrated that BC-Alk treatment effectively mitigated the LPS-stimulated upregulation of *p*-NF-κB and iNOS (Figs. 4A–4C). Additionally, BC-Alk suppressed the gene expression of NF-κB, COX-2, and iNOS Figs. 1A–1C). Furthermore, we observed that BC-Alk treatment significantly inhibited the activation of Caspase-1 and NLRP3 induced by LPS stimulation (Figs. 4D and 4F). Similarly, BC-Alk inhibited *NLRP3* mRNA levels Fig. 1D). We also examined the protein levels of Nrf2 and GCLM in lung tissues. The protein expression of Nrf2 and GCLM increased dose-dependently with BC-Alk treatment (Figs. 4G–4I). These findings suggest that BC-Alk regulates Nrf2, NF-κB, and the NLRP3 inflammasome *in vivo.*

3.5. BC-Alk activated Nrf2 in vitro

We initially investigated the effect of varying BC-Alk concentrations on the protein expression levels of Nrf2, GCLM, and NQO1. The results revealed a dose-dependent increase in the levels of Nrf2, GCLM, and NQO1 within the range of 3.13 to 25 μg·mL^{-1} (Figs. 5A–5D). Redox imbalance, closely associated with intracellular ROS, was crucial for lung inflammation[14]. After 8 h exposure

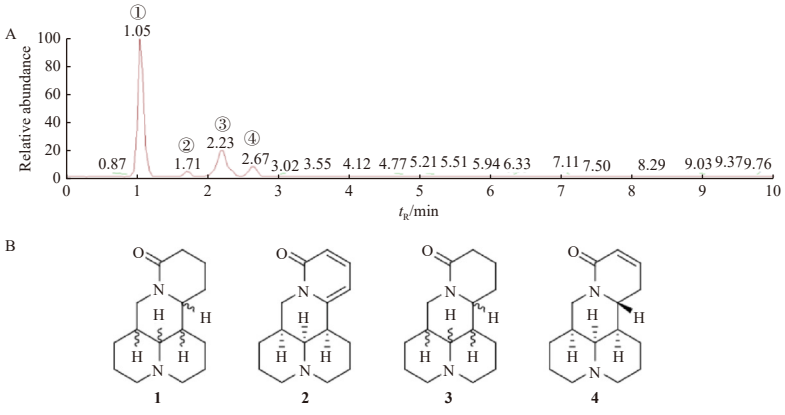


Fig. 2 UPLC-MS/MS analysis of BC-Alk. (A) The UPLC-MS chromatographic fingerprints of BC-Alk and the HRESIMS spectrum of the major compounds. (B) The possible structures of the major compounds from BC-Alk.

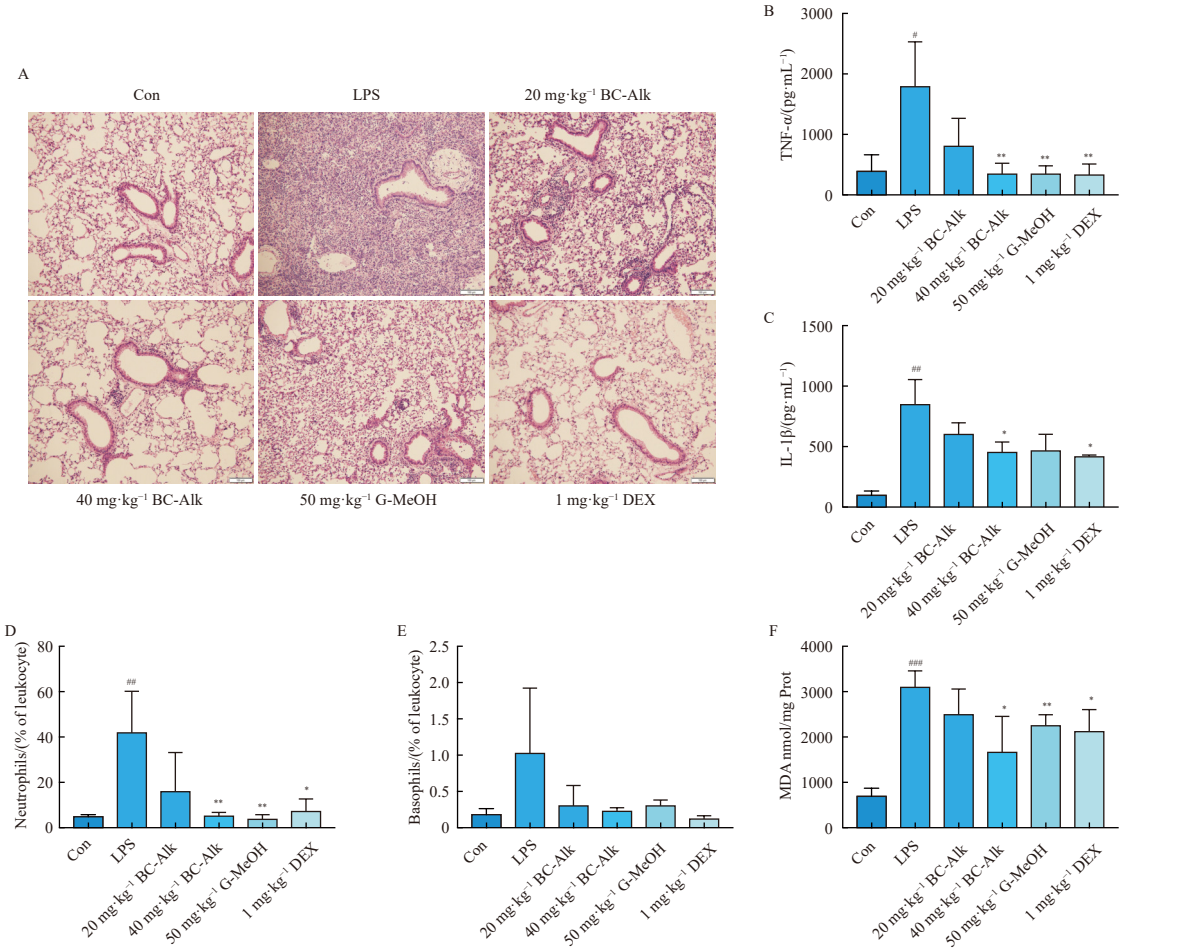


Fig. 3 BC-Alk attenuated LPS-induced lung inflammation in mice. (A) HE staining of lung tissue of mice. (B–C) The levels of TNF-α and IL-1β in the homogenate of lung tissue. (D–E) The ratios of neutrophils and basophils. (F) The levels of MDA in the homogenate of lung tissue. The results were expressed as mean ± SD (n = 5). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* Con group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* LPS group.

to arsenic (As), the ROS level increased. However, pre-treatment with BC-Alk demonstrated superior efficacy in reducing the ROS level compared to As alone (Figs. 5E and 5F). Additionally, intracellular GSH, an indicator of antioxidant capacity, showed a significant decrease in cells treated with arsenic. Notably, this reduction was reversed upon treatment with BC-Alk (Fig. 5G).

To elucidate the potential mechanism underlying BC-Alk's induction of Nrf2, we further examined its effect on Nrf2 translocation. Upon translocation into the nucleus, Nrf2 interacts with the antioxidant response element (ARE) sequence, thereby enhancing the transcription of GCLM and NQO1 [15, 16]. Immunofluorescence analysis revealed that BC-Alk facilitated the translocation of Nrf2 from the cytoplasm into the nucleus (Fig. 5H). Collectively, these findings provide compelling evidence for the anti-inflammatory and anti-oxidant properties of BC-Alk *in vitro*.

3.6. BC-Alk inhibited NF-κB in vitro

Typically, a complex of NF-κB and IκB predominantly resides in the cytoplasm. Upon phosphorylation and subsequent translocation into the nucleus, NF-κB regulates the downstream proteins iNOS and COX-2. Our results indicate that BC-Alk effectively counteracted the LPS-induced reduction in IκB-α levels and the consequent decrease in *p*-NF-κB levels (Figs. 6A–6C). Furthermore, BC-Alk diminished the protein levels of iNOS and COX-2 in a dose-dependent manner (Figs. 6D–6F). Additionally, BC-Alk treatment resulted in suppression of the gene expression levels of *p*-NF-κB and iNOS (Figs. 6G and 6H). Immunofluorescence analysis demonstrated that BC-Alk promoted the translocation of *p*-NF-κB from the cytoplasm to the nucleus (Fig. 6I). Moreover, BC-Alk significantly attenuated the LPS-induced elevation in *P*-NF-κB protein levels in J774 A.1 murine macrophage cells (Supplementary Fig. 2A). Collectively, these findings strongly suggest that BC-Alk effectively inhibits NF-κB activation.

3.7. BC-Alk inhibited the activation of NLRP3 inflammasome in vitro

The overactivation of the NLRP3 inflammasome is implicated in the pathogenesis of various inflammation-related diseases [17]. This study investigated whether BC-Alk could regulate the activation of NLRP3 inflammasome in nigericin-induced J774A.1 macrophages. The data demonstrated that BC-Alk significantly reduced the elevated levels of IL-1β and Caspase-1 following nigericin stimulation (Figs. 7A–7E). Additionally, BC-Alk notably decreased the NLRP3 inflammasome and pro-Caspase-1 in LPS-induced J774A.1 macrophages (Figs. 7F–7H). Furthermore, BC-Alk treatment led to a decrease in the gene expression of NLRP3 and Caspase-1 (Figs. 7I and 7J). Moreover, BC-Alk effectively suppressed the gene expression of IL-1β (Supplementary Fig. 2B). In summary, BC-Alk inhibits the activation of the NLRP3 inflammasome. By reducing the release of IL-1β and the activation of Caspase-1, BC-Alk demonstrates potential as a modulator of NLRP3 inflammasome activation.

4. Discussion

Exposure of lung tissue to environmental toxins (such as pathogenic microorganisms, atmospheric particulate matter, ci-

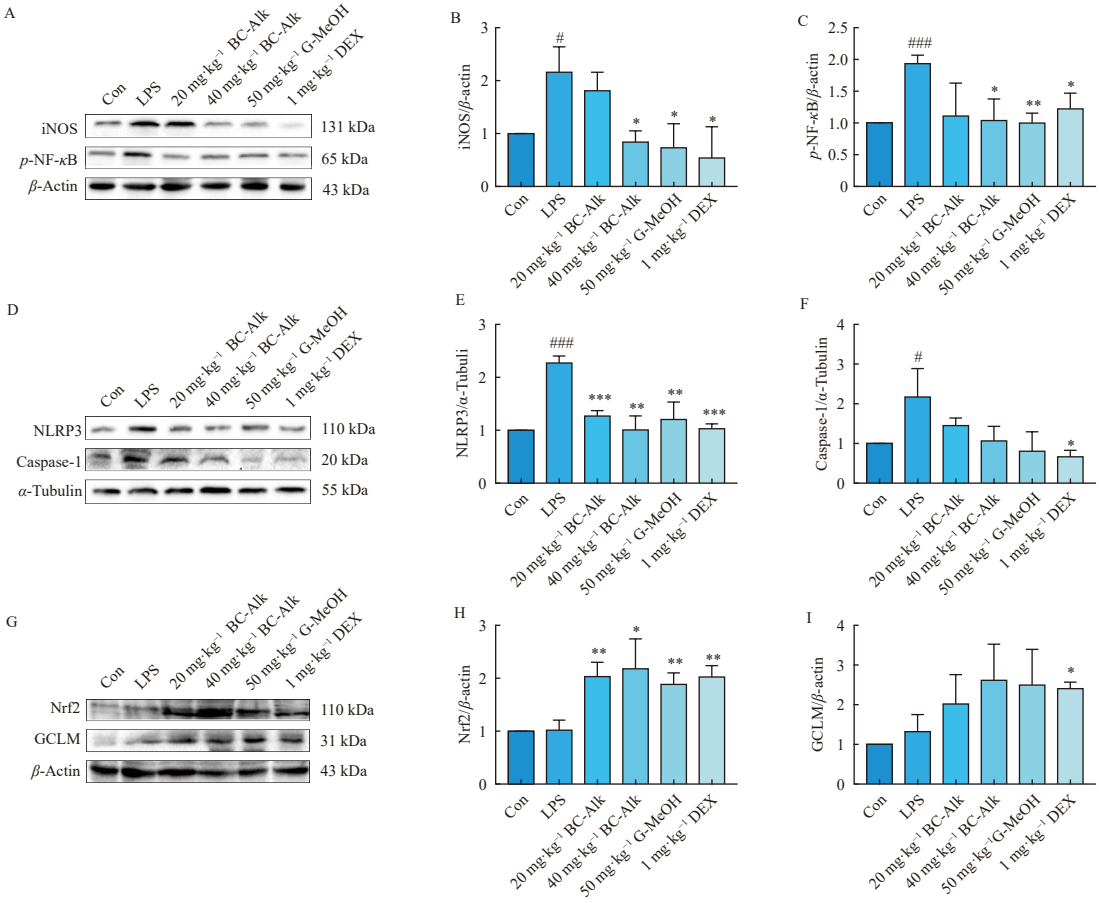


Fig. 4 The regulation of BC-Alk on Nrf2, NF-κB and NLRP3 *in vivo*. (A–C) BC-Alk inhibited NF-κB and iNOS. (D–F) BC-Alk inhibited activation of NLRP3 inflammasome. (G–I) BC-Alk upregulated Nrf2 and GCLM. The results were expressed as mean ± SD (n = 3). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs* Con group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* LPS group.

garette smoke, *etc.*) can readily trigger inflammatory lesions, resulting in inflammatory lung diseases including COPD, asthma, and pneumonia [18, 19]. While antibiotics and corticosteroids are commonly prescribed in clinical practice, they often lead to adverse reactions, including pathogen resistance, steroid tolerance, and limitations in long-term usage [20]. Consequently, the advantages of traditional Chinese medicine (TCM) in treating respiratory diseases are gaining recognition, and TCM-derived compounds represent a significant source for developing new drugs and innovative therapeutics [21, 22]. Alkaloids, a class of natural products with high drug potential, have attracted considerable attention and undergone extensive research [23]. Many naturally-derived alkaloids have been developed into innovative drugs, such as taxol, colchicine, and vincristine, demonstrating potent cytotoxic and anti-inflammatory properties [24-26].

The whole plants of *T. chinense* and Bairui Granules are widely used in traditional Chinese medicine for treating respiratory ailments. Previously, flavonoids, including kaempferol and kaempferol-3-*O*-glucorhamnoside, were considered the primary anti-inflammatory compounds and were thus selected as quality control markers for the crude drugs and Bairui Granules [3, 27]. In this study, we investigated the potential anti-inflammatory effects of various *T. chinense* herb extracts, including seven *T. chinense* herb extracts and Bairui Granules extract, by assessing their inhibition of NO production and inflammatory factors, as well as QR induction (Fig. 1). Our findings revealed that the total alkaloids exhibited remarkable anti-inflammatory and antioxidant activities, surpassing those of flavonoids. Additionally, we validated their therapeutic efficacy in treating LPS-induced lung inflammation in mice (Fig. 3). Mechanistically, the inhibition of LPS-stimulated inflammation was achieved through the regulation of the NLRP3 inflammasome, Nrf2, and NF-κB signaling pathways (Fig. 8).

UPLC-ESI-HRMS/MS analysis of BC-Alk identified four quinolizidine alkaloids, which are sophocarpine and its analogues. Among these, sophocarpine (Peak 4) has been previously isolated from *T. chinense* [28]. The structures of the other three compounds, particularly their stereochemical configurations, require further elucidation. The anti-inflammatory effect of sophocarpine has been investigated. It demonstrates anti-inflammatory properties by inhibiting the overproduction of inflammatory factors (TNF-α, IL-1β and IL-6) and modulating NF-κB, MAPKs, and TLR4 signaling pathways in LPS-induced acute lung injury (ALI) in mice [29]. Our data suggest that these compounds, being quinolizidine alkaloids, may play a crucial role in the anti-inflammatory effects of BC-Alk.

Nrf2 is a widely recognized transcription factor that aids organisms in responding to external stimuli and managing stress. When exposed to oxidative stress, inflammation, or other forms of damage, Nrf2 promotes the production of a series of antioxidant and detoxifying enzymes, thereby protecting cells from harm [30]. Nrf2 effectively scavenges ROS, and thus suppresses the activation of AP-1, NF-κB, and other transcription factors sensitive to oxidative stress [31, 32]. Furthermore, Nrf2 inhibits inflammation-related genes, making Nrf2 activators a promising avenue for identifying anti-inflammatory agents [11, 33]. Several natural products, such as curcumin and astaxanthin, target the Nrf2 pathway and have already advanced to clinical trials [34].

NF-κB is a critical inflammatory pathway that regulates inflammatory mediators. BC-Alk treatment effectively reduced NF-κB activation by increasing IκB-α levels, subsequently reversing the elevated expression of iNOS and COX-2. Furthermore, BC-Alk impeded the nuclear translocation of NF-κB. Recent studies have

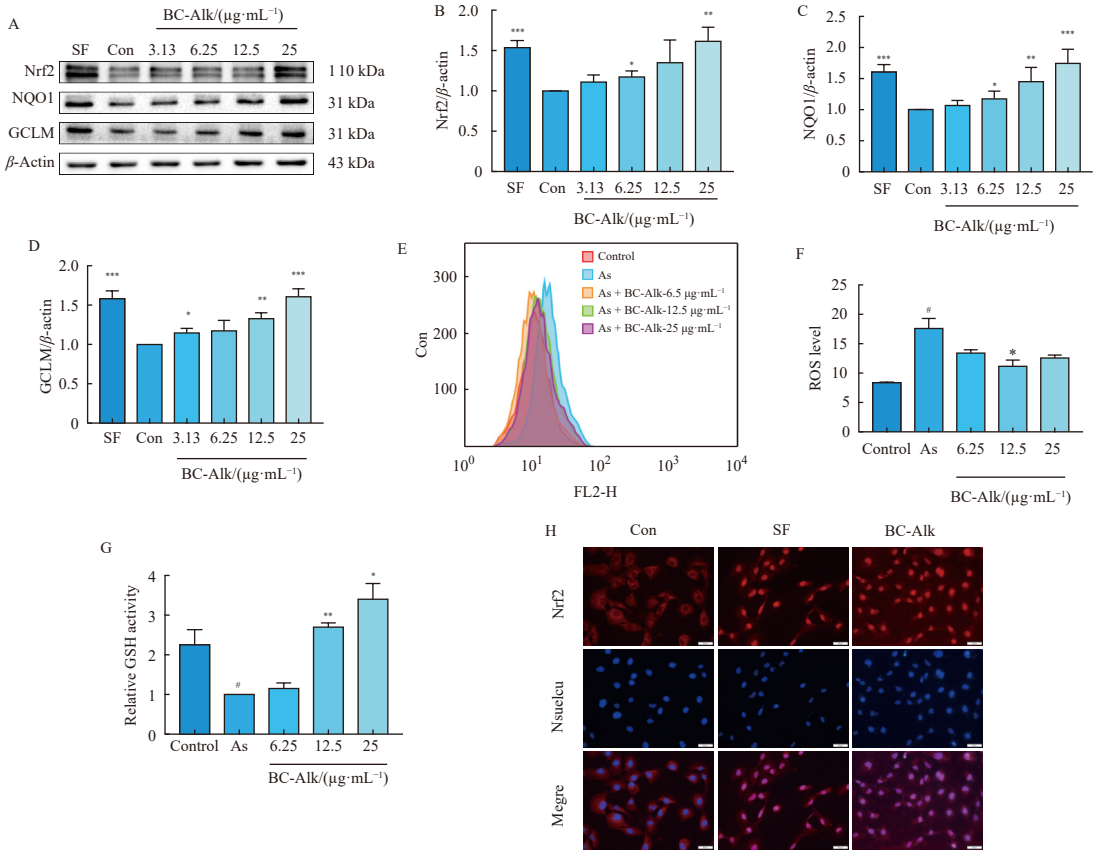
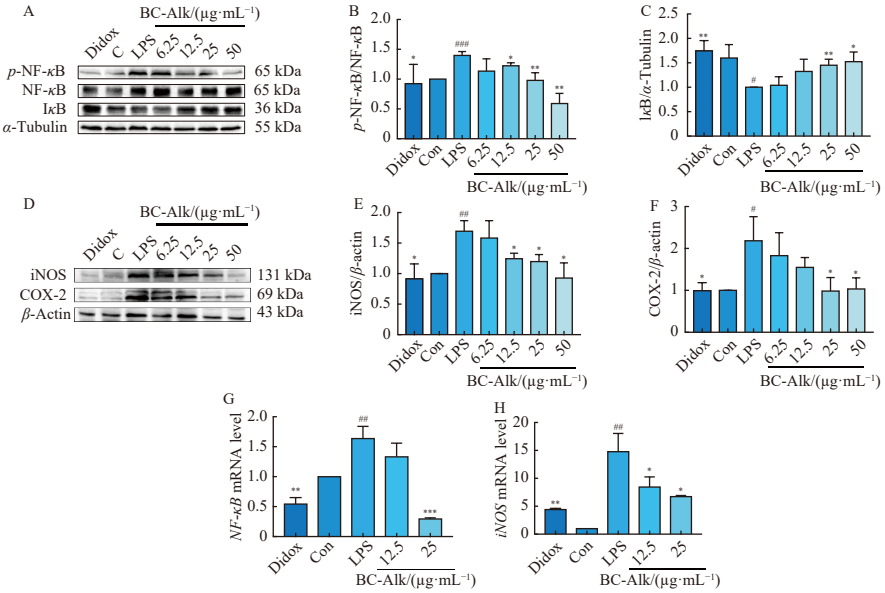


Fig. 5 BC-Alk enhances intracellular antioxidant capacity by activating Nrf2 in Beas-2B cells. (A–D) BC-Alk upregulated Nrf2 and the downstream proteins. The results were expressed as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* Con group. (E–F) BC-Alk attenuated As (III)-induced overproduction of ROS. (G) BC-Alk reversed the reduction of GSH induced by As (III). Results are expressed as mean ± SD (n = 3). $^{\#}P < 0.05$ *vs* Con group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* As (III). (H) BC-Alk induced Nrf2 nuclear translocation. The scale bar was 20 μm.

demonstrated anti-inflammatory activity through NF-κB inhibition in various alkaloid extracts, including those from *M. cochinchinensis* [23], *Corydalis saxicola* Bunting [35], Fuzi [36], and aloperine isolated from *Sophora alopecuroides* [37]. Our research corroborates these findings, revealing that BC-Alk inhibits NF-κB activation.

The NLRP3 inflammasome, a protein complex comprising NLRP3, ASC, and caspase-1 [38,39], functions as a critical intracellular inflammatory signaling activation mechanism [40]. Upon cellular damage, infection, or other stimuli, the NLRP3 inflammasome is activated, leading to ASC aggregation and inactive caspase-1 recruitment [41]. The activated NLRP3 inflammasome promotes the release of pro-inflammatory cytokines within the cell[13]. Excessive or sustained activation of the NLRP3 inflammasome may be associated with various diseases, including inflammatory disorders [42]. Several natural compounds have shown promise in treating inflammatory diseases by inhibiting NLRP3 inflammasome activity, such as curcumin, rutin, and dihydromyricetin [43,44].

Bairui Granules, derived from the single medicinal herb *T. chinense* Turcz., have been incorporated into clinical medication guidelines and recommended for various respiratory conditions, including pneumonia, upper respiratory tract infections, chronic



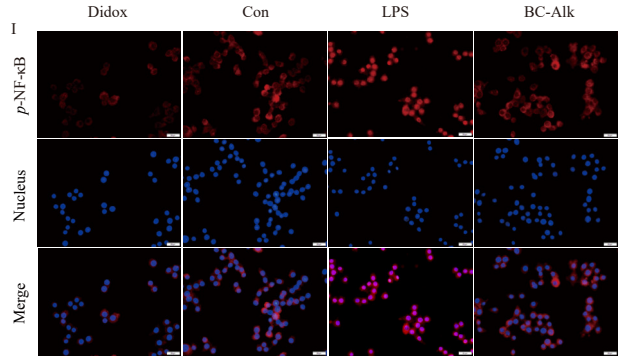


Fig. 6 BC-Alk inhibits LPS-stimulated inflammatory response in RAW 264.7 cells. (A–C) BC-Alk reverted LPS-stimulated activation of *p*-NF-*κ*B, and IκB in the protein levels. (D–F) BC-Alk reverted LPS-stimulated activation of iNOS and COX-2 in the protein levels. (G–H) BC-Alk blocked LPS-stimulated increase of proinflammatory mediators in mRNA levels. The results were expressed as mean ± SD (n = 3). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* Con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* LPS group. (I) BC-Alk induced *p*-NF-*κ*B nuclear translocation. The scale bar was 20 μm.

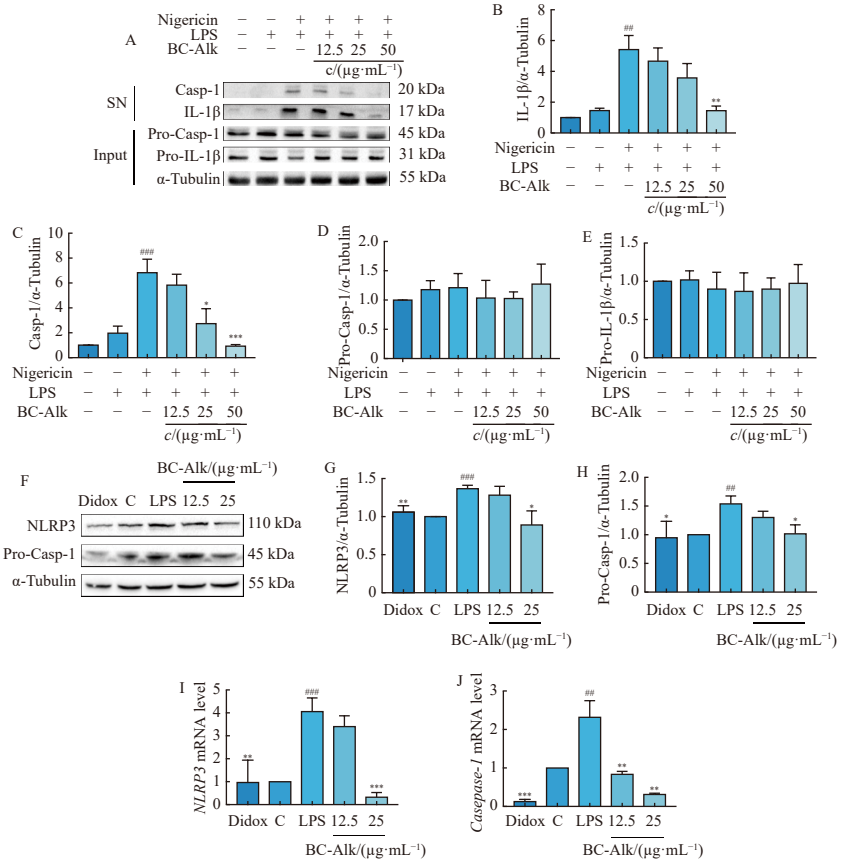


Fig. 7 BC-Alk inhibited the activation of NLRP3 inflammasome. (A–E) BC-Alk inhibited the of maturation and release of Caspase-1 and IL-1β. Results were expressed as mean ± SD (n = 3). $^{*}P < 0.05$ *vs* LPS-Nigericin group. (F–H) BC-Alk inhibited the activation of NLRP3 inflammasome induced by LPS in J774A.1 macrophages. (I–J) BC-Alk blocked LPS-stimulated increase of *NLRP3* and *Caspase-1* in mRNA levels in J774A.1macrophages. Results were expressed as mean ± SD (n = 3). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* Con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* LPS group.

bronchitis, tonsillitis, and pharyngitis. Substantial clinical evidence has demonstrated the high efficacy of Bairui preparations in treating respiratory inflammatory diseases, with reported success rates exceeding 94%. Notably, recent research has revealed the presence of alkaloids in Bairui Granules (Supplementary Fig. 3), suggesting a potential synergistic effect between flavonoids and alkaloids in clinical applications. Further investigation into the coordination and underlying mechanisms of this interaction is warranted to fully elucidate its therapeutic potential.

5. Conclusion

In conclusion, this study has identified the primary chemical constituents of BC-Alk and elucidated its potential mechanism for combating pulmonary inflammation in mice. BC-Alk demonstrated a significant capacity to mitigate the release of TNF-α and IL-1β in LPS-induced lung inflammation. The anti-inflammatory mechanism appears to be associated with the inhibition of NF-*κ*B and NLRP3, as well as the activation of the Nrf2 signaling pathway (Fig. 8). These findings provide scientific support for the traditional application of *T. chinense* and its derivative, Bairui Granules, in treating respiratory ailments. In essence, the total alkaloids of *T. chinense* represent a promising therapeutic agent for the treatment and prevention of pulmonary inflammation, establishing a chemical and biological foundation for further development of *T. chinense*.

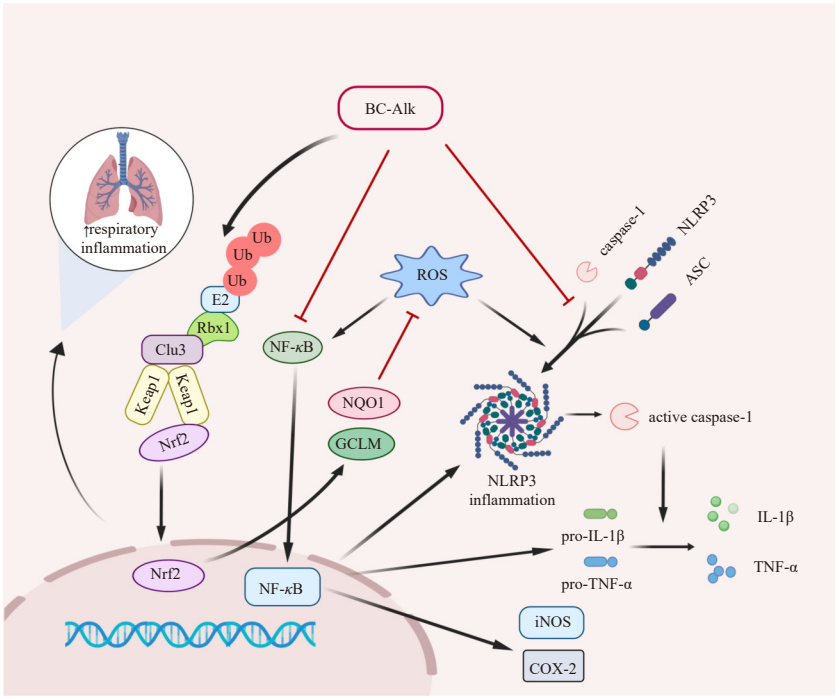


Fig. 8 Schematic depiction of BC-Alk inhibited LPS-induced respiratory inflammation by modulating Nrf2, NF-κB and NLRP3.

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Supporting Information

Supporting data can be requested by sending E-mail to the corresponding authors.

Declaration of Competing Interest

These authors have no conflict of interest to declare.

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