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Pharmacological evaluation of *Alstonia scholaris*: Anti-tussive, anti-asthmatic and expectorant activities

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ABSTRACT

Ethnopharmacological relevance: Alstonia scholaris (Apocynaceae) was documented as an effective herb for the treatment of chronic respiratory diseases in "dai" ethnopharmacy historically, and its leaf crude extract, used for releasing tracheitis and cold symptom, was approved to be a commercial formulation by State Food and Drugs Administration of China (SFDA).
Aim of the study: The investigation evaluates the anti-tussive and anti-asthmatic activities of the ethanolic extract, fractions and main alkaloids of *Alstonia scholaris* leaf to provide experimental evidence for its traditional and modern clinical use. For our most interesting, is to reveal the active components for further new drug development.
Materials and methods: The leaf of *Alstonia scholaris* was extracted with ethanol and then separated into different fractions. Furthermore, alkaloids were isolated by phytochemical method. The anti-tussive activity was evaluated using three different models including ammonia or sulfur dioxide induced mice coughing, and citric acid induced guinea pigs coughing. The anti-asthmatic activity was investigated on guinea pigs bronchoconstraction induced by histamine. The expectorant activity was evaluated by volume of phenol red in mice's tracheas.
Results: The alkaloids fraction significantly inhibited mice's frequency of cough induced by ammonia, increased mice's latent period of cough induced by sulfur dioxide, and increased guinea pigs' latent period of cough and inhibited frequency of cough. Besides, the alkaloids fraction increased delitescence of convulsion, and tumble of guinea pigs in anti-asthmatic test, and enhanced tracheal phenol red output in expectorant evaluation. Moreover, the main alkaloid, picrinine exhibited anti-tussive and anti-asthmatic activities *in vivo*.
Conclusions: The alkaloids fraction was anti-tussive, anti-asthmatic and expectorant activities component of *Alstonia scholaris* leaf, and it may also be a valuable lead material for respiratory diseases drug development. Picrinine, the main anti-tussive and anti-asthmatic compound, could be applied in quality control of products from *Alstonia scholaris* leaf.

1. Introduction

Coughing is a symptom of respiratory illness that prevents talking and causes chest and thorax pain (Irwing and Madison, 2000). Mucolytics, expectorants, anti-tussives, bronchodilators and glucocorticoids can be used to treat cough (Pérez et al., 2008). Presently available therapies to treat cough are limited for lack of effective and safe medications, so coughing remains among the most common complaints for which patients seek medical attention (Zhang et al., 2009). In the same way, there are increasing demands for the use of traditional medicines in the therapy of asthma, because of little side effect compared to those of synthetic drugs to prevent and treat such chronic disease. In traditional Chinese medicines, many plants are recorded to treat respiratory complaints such as cough, asthma, bronchial affections, pneumonia and expectoration (Jiangsu New Medical College, 1977), which have been used for hundreds of years. However, they still cannot be accepted by most advanced countries as therapeutic agents, although many of today's new drugs come directly or indirectly from traditional medicines (Newman and Cragg, 2007). A major reason is lack of chemical and pharmacological investigation on them.

The leaf of *Alstonia scholaris* has been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases in the Yunnan province of the People's Republic of China (Compiling Group of Yunnan Traditional Chinese Medicine, 1977). The leaf extract, developed as a commercially available traditional Chinese

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medicine, has also been prescribed in hospitals and sold over the counter in drug stores (Ministry of Public Health, People's Republic of China, 1997). The available clinical efficiency stimulated us to carry on the phytochemical and pharmacological research on this plant. In our previous chemical study on *Alstonia scholaris*, a serial of monoterpenoid indole alkaloids, iridoids and terpenoids were isolated from different parts of plant (Cai et al., 2007, 2008a, 2008b; Du et al., 2007a, 2007b; Feng et al., 2008, 2009; Xu et al., 2009). The evaluation of the anti-tussive, anti-asthmatic and expectorant activities of different extracts and main alkaloids from *Alstonia scholaris* leaf is the subject of this paper.

2. Materials and methods

2.1. Plant materials

The leaves of *Alstonia scholaris* were collected in April 2006 in Simao of Yunnan Province, People's Republic of China, and identified by Dr. Chun-Xia Zeng, Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (Luo20060407) has been deposited in the herbarium of Kunming Institute of Botany Chinese Academy of Sciences (KUN).

2.2. Extract, fractions and alkaloids preparation

The dried and powdered leaves of *Alstonia scholaris* were extracted with EtOH under reflux conditions, and the solvent was evaporated *in vacuo* to afford the ethanolic extract. Part of the ethanolic extract was suspended with water and extracted with petroleum ether, EtOAc, successively. The other part of ethanolic extract was dissolved in 1% HCl, the residue was recognized as the non-alkaloid fraction, and the solution was subsequently basified using ammonia water to pH 9–10. The basic solution partitioned with EtOAc, afford the alkaloids fraction (EtOAc layer). The yields of the different fractions were expressed as the weight percentage of obtained extract in the total weight of plant material, specifically, 32%, 2.6%, 3.4%, 20.8%, 5.0%, and 1.0% for the ethanolic extract, petroleum ether fraction, EtOAc fraction, water fraction, non-alkaloid fraction, and alkaloids fraction respectively.

The alkaloids fraction was subjected to chromatography column on silica gel eluted with $CHCl_3$–MeOH (30:1–1:1) to afford 6 fractions (I–VI). Picrinine and vallesamine were isolated from fraction IV by column chromatography over silica gel ($CHCl_3$–acetone) (4:1–2:1), repeatedly. Scholaricine was isolated from fraction V by column chromatography over silica gel ($CHCl_3$–CH_3OH) (5:1–4:1).

2.3. Animals

ICR (Institute of Cancer Research) mice of either sex (19–24 g) and guinea pigs of either sex (280–345 g) were purchased from Kunming Medical College (licence number SYXK 2005-0001). All animals were housed at room temperature (20–25 °C) and constant humidity (40–70%) under a 12 h light–dark cycle in SPF (Specific Pathogen Free) grade laboratory. 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.

2.4. Anti-tussive effects against ammonia induced coughing

ICR mice of either sex weighing 21–24 g were divided randomly, 10 mice per group. The negative control of animals was treated with distilled water orally, and the positive control was treated with codeine phosphate, the remaining groups treated were with test samples respectively. Anti-tussive activity was investigated on a classical mouse cough model induced by ammonia liquor (Xu et al., 1991). Briefly, each mouse was placed in a 300 ml special glass chamber and exposed to 40 μl 25% NH_4OH. The cough frequency produced during 2 min exposure period was counted. In the second assay for alkaloids, cough frequency and latent period of cough were recorded.

2.5. Anti-tussive effects against sulfur dioxide induced coughing

ICR mice of either sex weighing 19–22 g were divided and treated as Section 2.4. The anti-tussive effects were measured according to literature (Miyagoshi et al., 1986). A burette containing concentrated sulfuric acid was fixed to a three necked flask containing aqueous saturated sodium hydrogen sulfite solution, and the acid was added to this solution to generate sulfur dioxide gas. After gastric perfusion 30 min, the mice were placed in a 300 ml special glass chamber and exposed for to 4 ml sulfur dioxide. The latent period of cough was recorded for 2 min.

2.6. Anti-tussive effects against citric acid induced coughing

To screen the sensitivity, guinea pigs were placed in a glass chamber and sprayed with 33% citric acid (w/v) for 2 min (Chen, 1993). The period from the start to the onset of cough (latent period of cough) and frequency of cough was recorded. The frequency of cough between 10 and 30 were selected for further anti-tussive test. After 24 h recovery, the selected sensitive animals were randomly divided into seven groups ($n = 10$), and were individually placed into a transparent Perspex airtight chamber. At 45 min after oral treatment test samples, each animal was exposed to 1 M citric acid aerosols for 30 s with a flow rate of 1 ml/min. During the aerosol exposure, the animal was continuously monitored, and the latent period and frequency of cough were observed for 5 min.

2.7. The anti-asthmatic effects in guinea pigs

To screen the sensitivity, each guinea pig was sprayed with 2 ml of the mixture of 0.1% histamine and 2% acetylcholine chloride (1:1, v/v) (Xu et al., 1991). The time to onset of respiratory distress and tumble (preconvulsive time) was recorded. The guinea pigs with preconvulsive time in 90 s were selected. After 24 h recovery, the eligible guinea pigs were allotted randomly for test samples. All groups were administered once 45 min before, and each animal was exposed to the mixture of 0.1% histamine and 2% acetylcholine chloride aerosols for 20 s before the measurement of preconvulsive time. The delitescence of convulsion and tumble for each guinea pig within 6 min were observed. Guinea pig without convulsion and tumble was record as 360 s.

2.8. Expectorant test

The procedures were performed as described previously (Engler and Szelenyi, 1984). Male and female mice were randomly allotted and treated with a single dose 30 min before intraperitoneal injection of phenol red solution (5% in saline solution, w/v, 0.1 ml/10 g body weight). Mice were sacrificed by cervical dislocation 30 min after application of phenol red. After dissected free from adjacent organs, the trachea was removed from the thyroid cartilage to the main stem bronchi and put into 2 ml normal saline immediately. After ultrasonic for 5 min, 0.1 ml of 1 M NaOH solution was added to the saline and optical density of the mixture were measured at 546 nm using 722 UV–vis spectrophotometer.