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Pharmacological evaluation of *Alstonia scholaris*: Anti-inflammatory and analgesic effects

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ABSTRACT

Ethnopharmacological relevance: Alstonia scholaris (Apocynaceae) has been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases. The leaf extract, developed as a commercially available traditional Chinese medicine, used to release tracheitis and cold symptom, has also been prescribed in hospitals and sold over the counter in drug stores.
Aim of the study: The investigation evaluated the anti-inflammatory and analgesic activities of the ethanolic extract, fractions and main alkaloids of *Alstonia scholaris* leaf to provide experimental evidence for its traditional and modern clinical use. Besides, to discover the active fraction and components for further better use in Chinese medicine is hopeful.
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 analgesic activities were investigated using acetic acid-induced writhing, hot-plate and formalin tests in mice. The anti-inflammatory activities were carried out *in vivo* and *in vitro*, including xylene-induced ear edema and carrageenan-induced air pouch formation in mice, and COX-1, -2 and 5-LOX inhibition.
Results: It has been exhibited that the EtOAc and alkaloid fractions reduced acetic acid-induced writhing response in mice, significantly. The ethanolic extract, EtOAc and alkaloid fractions remarkably inhibited xylene-induced ear edema. Further investigation was focused on the alkaloids fraction and three main alkaloids isolated from the alkaloids fraction, in different animal models. Alkaloids reduced acetic acid-induced writhing response, and xylene-induced ear edema in mice. In the hot-plate test, alkaloids did not increase the latency period of mice obviously. In the formalin test, alkaloids did not inhibit the licking time in first phase, but significantly inhibited the licking time in second phase of mice. Alkaloids increased significantly SOD activity and decreased levels of NO, PGE2 and MDA significantly, in air pouch mice model. Moreover, some alkaloids isolated from the leaf of *Alstonia scholaris* exhibited inhibition of COX-1, COX-2 and 5-LOX *in vitro* anti-inflammatory assay, which supported alkaloids as the bioactive fraction.
Conclusions: The alkaloids fraction of *Alstonia scholaris* leaf, three main alkaloids, picrinine, vallesamine and scholaricine, may produce the anti-inflammatory and analgesic effect peripherally based on several *in vivo* assays. In *in vitro* tests, alkaloids exhibited inhibition of inflammatory mediators (COX-1, COX-2 and 5-LOX), which is accordant with results on animal models. Besides, COX-2/5-LOX dual inhibitors found in the experiment, such as 16-formyl-5α-methoxystrictamine, picralinal, and tubotaiwine might be valuable for further attention.

1. Introduction

The leaves of *Alstonia scholaris* (L.) R. Br. (Apocynaceae) have been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases in the Yunnan Province PR China (Compiling Group of Yunnan Traditional Chinese Medicine, 1977). The leaf extract, developed as a commercially available traditional Chi-

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Table 1
Effect of the extract and fractions on acetic acid-induced writhing response.

Group	Dose (mg/kg)	Treatment	Number of writhing	Inhibition ratio (%)
Control	–	ig	33.1 ± 3.2	–
Aspirin	200	ig	9.3 ± 3.5**	71.9
Ethanolic extract	3200	ig	28.4 ± 3.6	14.2
Petroleum ether fraction	260	ig	28.7 ± 5.7	14.2
EtOAc fraction	340	ig	21.9 ± 3.6*	33.8
Water fraction	2080	ig	23.3 ± 3.9	29.6
Alkaloids fraction	100	ig	20.0 ± 2.0**	39.6
Non-alkaloid fraction	500	ig	28.1 ± 3.9	15.1

Values expressed as mean ± S.E.M. ($n = 10$).
The doses of test samples are equal to 10 g of plant materials.
* $p < 0.05$ compared with control.
** $p < 0.01$ compared with control.

nese medicine, used to release tracheitis and cold symptom, has also been prescribed in hospitals and sold over the counter in drug stores (Ministry of Public Health, People's Republic of China, 1997). Allergic asthma is a chronic inflammatory disease characterized by eosinophilic airway inflammation, mucus hyper-secretion, and bronchial hyper-responsiveness (Busse and Lemanske, 2001). The traditional and clinical uses are likely related to the anti-inflammatory and analgesic actions. The available clinical efficiency stimulated us to investigate the anti-inflammatory components from this plant. For this purpose, intensive phytochemical investigations were carried out. As a result, a series of monoterpenoids indole alkaloids, iridoids and terpenoids were isolated from different parts of plant (Cai et al., 2007, 2008a,b; Du et al., 2007a,b; Feng et al., 2008, 2009; Xu et al., 2009b). This paper focuses on the evaluation of the anti-inflammatory and analgesic activities in different animal models of extracts, fractions and main alkaloids from the leaf of *Alstonia scholaris*. Moreover, the *in vitro* anti-inflammatory evaluation of alkaloids from *Alstonia scholaris* against COX-1, -2 and 5-LOX was also reported.

2. Materials and methods

2.1. Plant materials

The leaves of *Alstonia scholaris* (L.) R. Br. 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, the Chinese Academy of Sciences (KUN).

2.2. Extracts and fractions 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 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 non-alkaloid fraction, and the solution was subsequently basified using ammonia water to pH 9–10. The basic solution partitioned with EtOAc, afford 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 ethanolic extract, the petroleum ether fraction, EtOAc fraction, water fraction, non-alkaloid fraction, and alkaloids fraction, respectively.

2.3. Alkaloids preparation

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). Akuammidine, leuconoxine, isogentialutine, picralinal and 5-methoxylstrictamine were isolated from fraction II by column chromatography over silica gel (petroleum ether–EtOAc) and RP_{18} (H_2O–CH_3OH), repeatedly. Cantleyine, corypalmine, strictamine, vallesiachotamine, scholarisines B and E were isolated from fraction III by column chromatography over silica gel (petroleum ether–Acetone) and RP_{18} (H_2O–CH_3OH), repeatedly. Crebanine, dicentrine, discretamine, picrinine, 3-epi-dihydrocorymine 17-acetate, 16-formyl-5α-methoxystrictamine, stephanine and salutaridine were isolated from fraction IV by column chromatography over silica gel ($CHCl_3$–Acetone) and RP_{18} (H_2O–CH_3OH), repeatedly. Nareline, scholaricine and tubotaiwine were isolated from fraction V by column chromatography over silica gel ($CHCl_3$–CH_3OH), repeatedly. Echitamine, 19-epi-scholaricine, 12-hydroxy-echitamidine N^b-oxide, N(4)-demethylechitamine, and strictosamine were isolated from fraction VI by column chromatography over silica gel ($CHCl_3$–CH_3OH) and RP_{18} (H_2O–CH_3OH), repeatedly.

2.4. Animals

ICR mice of either sex (18–22 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 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.

2.5. Chemicals

Carrageenan was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD) kits were purchased from Nanjing Jiancheng Bioengineering Institute (China). All other reagents were of the highest commercial grade available.

2.6. Acetic acid-induced writhing response in mice

The writhing test was carried out as described in literature (Nakamura et al., 1986). Each mouse was administered with aspirin, test samples or vehicle 30 min before an intraperitoneal injection of 0.6% acetic acid at 0.1 ml/10 g bodyweight. The number of stretching or writhing was recorded between 5 min and 20 min after acetic acid injection.