Journal of Ethnopharmacology 162 (2015) 79–86

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

In vivo evaluation of the antitussive, expectorant and bronchodilating effects of extract and fractions from aerial parts of *Peganum harmala* linn

Wei Liu [a], Xuemei Cheng [a,b], Yongli Wang [a,b], Shuping Li [a], Tianhui Zheng [a], Yingying Gao [a], Guofeng Wang [a], Shenglan Qi [a], Jingxin Wang [a], Jiayi Ni [a], Zhengtao Wang [a,b], Changhong Wang [a,b,*]

[a] *Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, The MOE Key Laboratory for Standardization of Chinese Medicines and The SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, 1200 Cailun Rood, Shanghai 201203, China*
[b] *Shanghai R&D Centre for Standardization of Chinese Medicines, 199 Guoshoujing Road, Shanghai 201210, China*

ARTICLE INFO

Article history:
Received 15 June 2014
Received in revised form
2 December 2014
Accepted 23 December 2014
Available online 31 December 2014

Keywords:
Peganum harmala L.
Antitussive
Expectorant
Bronchodilating
Alkaloids
Flavonoids

ABSTRACT

Ethnopharmacological relevance: Aerial parts of *Peganum harmala* Linn (APP) is used as traditional medical herb in Uighur medicine in China, and it is traditionally used for treatment of cough and asthma. The aim of the present study is to evaluate the antitussive, expectorant and bronchodilating effects of extract and fractions (alkaloids and flavonoids) from APP, and to support its folk use with scientific evidence, and lay a foundation for its further researches.
Materials and methods: APP was extracted with 50% ethanol by reflux, extracts were concentrated in vacuum to afford total extract of APP (EXT). EXT was separated to provide alkaloid fraction (ALK) and flavonoid fraction (FLA) by macroporous resin. Antitussive evaluations were carried out with cough models in mice and guinea pigs induced by ammonia liquor, capsaicin, and citric acid. Phenol red secretion experiments in mice were performed to evaluate the expectorant activity. Bronchodilating activities were evaluated with a bronchoconstrictive challenge induced by acetylcholine chloride and histamine in guinea pigs.
Results: In all the three antitussive tests, the EXT and ALK could significantly inhibit the frequency of cough, and prolong the cough latent period in animals. High dose of EXT (1650 mg/kg) and ALK (90 mg/kg) in mice and in guinea pigs created therapeutic activities as good as that of codeine phosphate (30 mg/kg). EXT could significantly increase phenol red secretion in mice for 0.64, 1.08 and 1.29 fold averagely at dosages of 183, 550, and 1650 mg/kg, ALK for 0.63, 0.96, 1.06 fold averagely at dosages of 10, 30, and 90 mg/kg, and ammonium chloride (1500 mg/kg, standard expectorant drug) for 0.97 fold, comparing with control group. Aminophylline could dramatically prolong the preconvulsive time for 162.28% in guinea pigs, EXT for 67.34%, 101.96% and 138.00% at dosages of 183, 550, and 1650 mg/kg, ALK for 55.47%, 97.74% and 126.77% at dosages of 10, 30, and 90 mg/kg, and FLA for 84.69%, 95.94% and 154.52% at dosages of 10, 30, and 90 mg/kg, comparing with pretreatment.
Conclusions: APP is an effective traditional folk medicine for the treatment of cough with potent antitussive, expectorant and bronchodilating activities. The alkaloid fraction is proved to be the most effective components of APP. The alkaloids from APP may be valuable lead compounds for drug development of respiratory diseases.

1. Introduction

Coughing is one of common symptoms associated with many respiratory diseases such as asthma, chronic bronchitis, pneumonia and so on (Irwing and Madison, 2000; Ge et al., 2009). At present, the antitussives, expectorants, mucolytics, bronchodilators, and glucocorticoids can usually be used to treat cough (Pérez et al., 2008). However, available therapies to treat cough are limited for lack of effective and safe medications and coughing remains among the most common complaints for which patients seek medical attention (Zhang et al., 2009; Wang et al., 2012). In traditional Chinese medicines, many medicine herbs are used for hundreds of years to treat respiratory complaints such as cough, asthma, expectoration,

* Corresponding author at: The Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China. Tel.: +86 21 51322511; fax: +86 21 51322519.
E-mail address: wchcxm@hotmail.com (C. Wang).

http://dx.doi.org/10.1016/j.jep.2014.12.046
0378-8741/

bronchial inflammatory, and pneumonia, and have shown less or no side effects as being compared to synthetic drugs (Jiangsu New Medical College, 1977; Shang et al., 2010). 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. A major reason is lack of chemical and pharmacological investigation on them (Newman and Cragg, 2007; Shang et al., 2010). So it is worthy to search for effective medicines from traditional medicines for treatment of cough (Akah et al., 2003; Chu et al., 2007; Yang et al., 2008).

Peganum harmala Linn (Zygophyllaceae) grows spontaneously in the arid and semiarid areas north-west China, and also distributed in North Africa and the Middle East (Farouk et al., 2008; Cheng et al., 2010). The seeds and aerial parts of *Peganum harmala* have been commonly used as traditional folk medicine to treat various ailments, including cough, asthma, rheumatism, hypertension, diabetes and jaundice in the Xinjiang Uygur and Mongolian Autonomous Regions of China for a long time (Chinese Pharmacopoeia Committee, 1998; Zheng et al., 2009). It is also a well-known and effective herbal medicine in Turkey, Iran, Algeria and Morocco (Kartal et al., 2003; Hemmateenejad et al., 2006; Farouk et al., 2008; Bensalem et al., 2014). *Peganum harmala* is used as an effective herb to treat cough and asthma in the folk medicine, there are only a few of preliminary studies on the seeds of *Peganum harmala* in the past years (Hider et al., 1981; Nie et al., 2004). But, rare investigation has been conducted on the aerial parts of *Peganum harmala* (APP). Therefore, a series of experiments are designed to evaluate the antitussive, expectorant and bronchodilating effects of the extract and two mainly fractions (alkaloids and flavonoids) from APP. The aim is to confirm its traditional function of APP and to provide scientific evidence for the discovery of new antitussive, expectorant; and bronchodilating drugs from APP.

2. Materials and methods

2.1. Reagents

Codeine phosphate, phenol red, ammonium chloride and ammonia liquor were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Capsaicin, aminophylline, acetylcholine (ACh) chloride, histamine phosphate were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The standard compounds of vasicine, harmaline, harmine, deoxypeganetin, peganetin were isolated previously from the APP in our laboratory and characterized by NMR and mass spectral data and comparison with literature values. The purities of these compounds were determined to be more than 98% by HPLC analysis.

2.2. Collection and preparation of plant material

APP was collected in Urumqi, Xinjiang Uygur Autonomous Region, China in August 2011 and authenticated by Professor Changhong Wang, the Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine. Fresh herbs were dried in shade for a week. The voucher specimens (Voucher number: PH-XJ1104) were deposited at the Herbarium of Shanghai R&D Center for Standardization of Traditional Chinese, Shanghai, China.

Dried APP (2500 g) was sheared into segments, extracted with 50L of 50% ethanol (v/v) thrice in reflux conditions by 100L-extraction tank, each for 2 h. Extracts was combined, filtered, and concentrated under reduced pressure at 45 °C to afford 10L concentrated extract of APP. A portion of the concentrated extract (3L) was dessicated in vacuum to afford extract of APP (EXT, 187.5 g). And the other concentrated extract (7 l) was separated and prepared alkaloid fraction (ALK) and flavonoid fraction (FLA) by macroporous resin column chromatography (15×150 cm; 4 l), being eluted with a gradient system of water–ethanol (100:0, 80:20, 20:80). Finally, three different fractions (water, 20% ethanol, 80% ethanol) of the eluted solutions were concentrated under reduced pressure at 45 °C and dessicated in vacuum. The water fraction (388.1 g) mainly contains polysaccharides, hydrosoluble pigment, tannin and so on (the fraction may not be the active part of APP, and no follow studies), the 20% ethanol fraction (35.6 g) mainly contains alkaloids, and the 80% ethanol fraction (34.5 g) mainly contains flavonoids. The contents of alkaloids (vasicine, harmaline and harmine) and flavonoids (deacetylpeganetin and peganetin) of prepared EXT, ALK and FLA were determined by previous method (Wen et al., 2014).

2.3. Animals and drug administration

All animals, including ICR mice of either sex (body weight 20–25 g) for ammonia or capsaicin induced mice cough studies, ICR mice of either sex (body weight 20–25 g) for phenol red secretion study, and guinea pigs of either sex (body weight 200–300 g) for citric acid induced guinea pig cough experiment and bronchodilating tests were purchased from Experimental Animal Center, Shanghai University of Traditional Chinese Medicine, China.

All animals were housed with free access to food and water. The animals were maintained on a 12 h light–dark cycles (light on from 7:00 to 19:00) at environmental temperature (22–24 °C) and 60–65% relative humidity for 7 days. Before the experiments, all animals were fasted for 12 h and fed with water. All animal experimental protocols were in accordance with the regulations of experimental animal administration issued by the State Committee of Science and Technology of People's Republic of China on November 14th, 1988.

Indicated doses of extract (EXT) and fractions (ALK and FLA) were suspended and diluted with 0.5% carboxylmethylcellulose (CMC-Na) solution and administrated orally. The animal dose was extrapolated from human daily dose by a simple conversion based on body weight. And these doses did not show any toxicity properties by a preliminary experiment (Data not show). CMC-Na solution (0.5%) was taken as control.

2.4. Antitussive activity against ammonia induced cough in mice

The test of ammonia liquor induced cough in mice was carried out as described before (Wang et al., 2012) with slight modification. Mice (110) were numbered and individually placed into a 500 ml special glass chamber which was placed upside down, and exposed to 0.1 ml 25% NH_4OH (loaded in a glass plate, diameter 28 mm, height 10 mm) for 45 s. The latent period (the period from the start to the onset of cough) and the frequency of cough within 2 min were recorded by a trained observer. During observation, only typical cough reflection, characterized by obvious contraction of the abdominal cavity and successively distinctive opening of mouth was counted. Then, the latent period (the period from the start to the onset of cough) and the frequency of cough within 2 min were recorded before treatment. After 24 h recovery, these mice were divided into 11 groups of 10 each randomly. Animals in Group 1 were administrated with 0.5% CMC-Na, animals in group 2 (positive control) were received 30 mg/kg of codeine phosphate, animals in groups 3–5 were treated with 183.3, 550, 1650 mg/kg of EXT, animals in groups 6–8 were treated with 10, 30, 90 mg/kg of ALK, and animals in groups 9–11 were treated with 10, 30, 90 mg/kg of FLA. One hour after administration, mice were exposed to ammonia again as described above. The latent period and the frequency of cough before and after treatment were compared.