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Anti-amnesic effect of extract and alkaloid fraction from aerial parts of *Peganum harmala* on scopolamine-induced memory deficits in mice

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ABSTRACT

Ethnopharmacological relevance: Aerial parts of *Peganum harmala* Linn (APP) is used as traditional medical herb for treatment of forgetfulness in Uighur medicine in China. But, the active ingredients and underlying mechanisms are unclear.

Aim of the study: The present study was undertaken to investigate the improvement effects of extract and alkaloid fraction from APP on scopolamine-induced cognitive dysfunction and to elucidate their underlying mechanisms of action, and to support its folk use with scientific evidence, and lay a foundation for its further researches.

Materials and methods: The acetylcholinesterase (AChE) inhibitory activities of extract (EXT), alkaloid fraction (ALK) and flavonoid fraction (FLA) from APP were evaluated in normal male C57BL/6 mice. The anti-amnesic effects of EXT and ALK from APP were measured in scopolamine-induced memory deficits mice by the Morris water maze (MWM) tasks. The levels of biomarkers, enzyme activity and protein expression of cholinergic system were determined in brain tissues.

Results: The AChE activity was significantly decreased and the content of neurotransmitter acetylcholine (ACh) was significantly increased in normal mice cortex and hippocampus by treatment with donepezil at dosage of 8 mg/kg, EXT at dosages of 183, 550, 1650 mg/kg and ALK at dosages of 10, 30, 90 mg/kg ($P < 0.05$), and the AChE activity and the content of ACh were not significantly changed in cortex and hippocampus after treatment with FLA at dosages of 10, 30, 90 mg/kg ($P > 0.05$). In the MWM task, scopolamine-induced a decrease in both the swimming time within the target zone and the number of crossings where the platform had been placed were significantly reversed by treatment with EXT at dosages of 550, 1650 mg/kg and ALK at dosages of 30, 90 mg/kg ($P < 0.05$). Moreover, the activity and protein expression of AChE was significantly decreased and the content of neurotransmitter ACh was significantly increased in cerebral cortex of scopolamine-induced mice by treatment with EXT at dosages of 183, 550, 1650 mg/kg and ALK at dosages of 10, 30, 90 mg/kg ($P < 0.05$), compared with scopolamine-treated group.

Conclusions: EXT and ALK from APP exert beneficial effect on learning and memory processes in mice with scopolamine-induced memory impairment. APP is an effective traditional folk medicine and the ALK fraction is proved to be the main effective components for the treatment of forgetfulness. The ALK may be valuable source for lead compounds discovery and drug development for treatment of memory impairment such as in Alzheimer's disease.

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1. Introduction

As the most common type of dementia, Alzheimer's disease (AD) is progressive neurodegenerative disorder that results in memory impairment and cognitive dysfunction (Grutzendler and Morris, 2001). The immoderate reduction of acetylcholine (ACh) hydrolyzed by acetylcholinesterase (AChE) in the cerebra of AD patients appears to be one of the critical elements in producing dementia. The loss of cholinergic cells in the basal forebrain is frequently accompanied by a reduction of the neurotransmitter ACh. One approach is to inactivate AChE activity, a key enzyme that cleaves synaptic cleft ACh and terminates neuronal signaling (Chuong et al, 2014; He et al., 2015a). Since 1997, the clinical application of the first cholinesterase inhibitor, most clinicians and probably most patients were considered the cholinergic drugs, donepezil, galantamine and rivastigmine, as the first-line pharmacotherapy for moderate AD (Birks, 2006; Yang et al., 2015).

Peganum harmala Linn (Zygophyllaceae) is a perennial herb spontaneously growing in north-west China, India, the Middle East, Africa, the southern United States, Mexico and South America (Cheng et al., 2010; Farouk et al., 2008; Zhao et al., 2011). In China, the seeds and aerial parts of *P. harmala* have been used as folk medicine to treat various ailments, including stroke hemiplegia forgetfulness, cold, asthma, malaria, rheumatism, lumbago, hemiplegia, and some skin diseases (Cheng et al., 2010; Chinese Pharmacopoeia Committee, 1998; Liu et al., 2015a; Zheng et al., 2009). Previous studies have proved that the total alkaloids and the active ingredient harmine from seeds of *P. harmala* can improve the learning and memory abilities in chemical induced dementia mice models and in vascular dementia rat models (He et al., 2015a, 2015b; Zhang et al., 2015; Fu et al., 2011). The beta-carboline alkaloids harmaline, harmine among others are the main active ingredients with strong cholinesterases inhibitors *in vitro* (Liu et al., 2014; Zhao et al., 2013; Zheng et al., 2009, 2011), and harmine can increased the ACh level in cerebral cortex by inhibiting cholinesterases *in vivo* (He et al., 2015a). However, previous studies indicated that there are some differences in chemical composition between the seeds and aerial parts (stems and leaves) of *P. harmala*. The quinazoline alkaloids (vasicine, deoxyvasicine and their analogs) and flavonoids (deacetylpeganetin and peganetin) are the main ingredients in APP, and beta-carboline alkaloids (harmaline, harmine) are the main chemical ingredients in seed of *P. harmala* (Duan et al., 1998; Wen et al., 2014; Zhao et al., 2010). It has been reported that vasicine, deoxyvasicine and their analogs alkaloids are strong cholinesterases inhibitors *in vitro* (Liu et al., 2014; Zhao et al., 2013; Zheng et al., 2009, 2011), and they have the potential improvement learning and memory effects *in vivo*. In addition, some flavonoids, such as luteolin, oroxylin A and daidzin among others, have been proven with potential anti-amnesic effect (Yoo et al., 2013; Kim et al., 2007, 2010). Therefore, these exciting results prompted us to perform a series of experiments to evaluate the improvement learning and memory effects of the total extract and two mainly fractions of total alkaloids and total flavonoids from APP. The aim of the present study is to confirm its traditional function of APP and to provide valuable scientific research data for the discovery of new anti-amnesic drugs from APP.

2. Materials and methods

2.1. Reagents and materials

The aerial parts of *P. harmala* (APP, composed of stems, leaves and a small amount of flowers) 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. The voucher specimen with voucher number of PH-XJ1104 was deposited at the Herbarium of Shanghai R & D Center for Standardization of Traditional Chinese, Shanghai, China.

Scopolamine hydrobromide and donepezil hydrochloride monohydrate were purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China). Acetylcholine (ACh) chloride, choline (Ch) chloride, chlormequat (internal standard, IS) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Titanium dioxide (TiO_2) was obtained from Shanghai Dyestuffs Research Institute Co., Ltd (Shanghai, China). The standard compounds of vasicine, deoxyvasicine, harmaline, harmine, deoxypeganetin, peganetin were isolated from 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. Preparation and chemical analysis of plant material

The preparation procedures of total extract (EXT) from APP, the alkaloid fraction (ALK) and flavonoid fraction (FLA) by macroporous resin column chromatography were described in previous report (Liu et al., 2015a). Dried APP (2500 g) was sheared into segments and extracted with 50 L of 50% ethanol (v/v) thrice in reflux, each for 2 h. Extracts was combined, filtered, and concentrated under reduced pressure at 45 °C to afford 10 L concentrated extract of APP. A portion of the concentrated extract (3 L) was dessicated in vacuum to afford EXT (187.5 g, extract yield was 25% from APP). The residuary concentrated extract (7 L, approximately 437.5 g) was separated and prepared ALK and FLA by macroporous resin column chromatography, 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 (379.3 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 (24.2 g, extract yield was 5.53% from EXT) mainly contains alkaloids, and the 80% ethanol fraction (23.8 g, extract yield was 5.44% from EXT) mainly contains flavonoids.

The chemical compositions of EXT, ALK, and FLA were analyzed by using ultra-performance liquid chromatography on Waters ACQUITY™ UPLC system (Milford, MA, USA). The separation was conducted on an ACQUITY UPLC HSS T3 column (100 mm×2.1 mm, 1.8 μm) maintained at 40 °C. The mobile phase was consisted of A (methanol) and B (aqueous 0.1% formic acid) at a flow rate of 0.3 mL/min and eluted with gradient elution: 0–2 min (2% A), 2–10 min (2–10% A), 10–20 min (10–30% A), 20–26 min (30–60% A), 26–29 min (95% A), 29–30 min (2% B). The injection volume was 5 μl. The typical chromatographic fingerprints of different fractions of EXT, ALK, FLA from APP and mixture reference standards of vasicine, deoxyvasicine, harmaline, harmine, deoxypeganetin, and peganetin were deposited in Fig. 1. The contents of targeted markers vasicine, deoxyvasicine, harmaline, harmine, deacetylpeganetin, and peganetin in EXT were determined as 2.58%, 1.43%, 0.02%, 0.07%, 0.90% and 1.25%, respectively. The contents of vasicine and deoxyvasicine were 52.47% and 28.71% in ALK, and the contents of deacetylpeganetin and peganetin were 17.00% and 23.45% in FLA with rare harmine (0.32%), respectively.

Adequate doses of EXT, ALK and FLA fractions were suspended and diluted with 0.5% carboxylmethylcellulose (CMC-Na) solution and used for oral administration. The administrated doses of EXT (183 mg/kg, the low dosage) in present animal study were extrapolated from human equivalent dose of APP (6 g/day) by a simple conversion based on body weight and combined with the extract yield of EXT from APP, and the administrated doses of ALK and FLA fractions were extrapolated from the administrated doses of EXT with their extract yield from EXT. A preliminary experiment was performed and the results indicated that these doses of animal experiment were not showed any