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Laetispicine, an amide alkaloid from *Piper laetispicum*, presents antidepressant and antinociceptive effects in mice

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Abstract

In the present work, we studied the effect of laetispicine, an amide alkaloid isolated from the stems of *Piper laetispicum* (*Piperaceae*), in forced swimming, open field, acetic acid writhing and formalin tests in KM mice to assess antidepressant and antinociceptive effects. A significant and dose-dependent decrease in the immobility time, as evaluated by the forced swimming test, was observed after laetispicine administration (38.18, 39.79, 58.77 and 67.28% decreased at the doses of 5, 10, 20, 40 mg/kg, respectively), suggesting an antidepressant effect. Furthermore, in the open field test, laetispicine at the given doses did not alter the number of crossings and rearing, as compared to controls. Results from writhing and formalin tests showed that laetispicine reduced the number of writhing in mice in a dose-dependent manner, attenuated the licking and spiting time of the injected paw in the first phase of formalin test. The antinociceptive effect of laetispicine was not affected by pre-treatment (i.p.) with naloxone (2 mg/kg). In conclusion, we showed that laetispicine possessed significant antidepressant and antinociceptive properties, making this drug potentially useful in depression and pain.

Keywords: *Piper laetispicum*; Amide; Laetispicine; Antinociceptive activity; Antidepressant activity

Introduction

Piper is widely distributed in the tropical and subtropical regions of the world and is highly commercially, economically and medicinally important in different countries. Phytochemical investigation revealed the presence of amides, lignans, flavones and terpenes (Parmar et al. 1997). Amide alkaloids are characteristic constituents of *Piper* family, which can be classified as isobutyl, pirrolydine, pirydonil and piperydines (Cícero Bezerra Felipe et al. 2007).

Piperine is the first amide isolated from *Piper* species (*Piper longum and Piper nigrum*), and displays antipyretic, analgesic, insecticidal, anti-inflammatory (Parmar et al. 1997; Kumar et al. 2007), immunomodulatory, antitumor (Sunila and Kuttan 2004) and antidepressant activities (Li et al. 2007). Reported beneficial effects of some other isolated amides include antifungal, antiplatelet, anxiolytic and antidepressant activities (Navickiene et al. 2000, 2003; Park et al. 2007; Cícero Bezerra Felipe et al. 2007).

Piper laetispicum C.DC. (*Piperaceae*), popularly known in folk as Xiao Chang-feng, Shan Hu-jiao, Ye Hu-jiao, is an endemic climbing, glabrous plant available in the southern part of China. As a folk medicine, this plant enjoys vast uses for invigorating circulation

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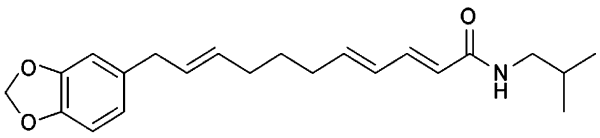

Fig. 1. Structure of laetispicine.

and reducing stasis, detumescence and analgesic (Zhonghua Herbals Editorial Committee 1999). Laetispicine (*N*-isobutyl-(3, 4-methylendioxyphenyl)-2*E*, 4*E*, 9*E*-undecatrienoamide) is a novel alkaloid amide from *P. laetispicum* (Pan et al. 2005). The structure of laetispicine was given in Fig. 1.

Results from studies in our group demonstrated that the *P. laetispicum* extract showed antinociceptive and antidepressant activities in several behavioral models. Although the literature is plenty of studies on the biological properties of the isolated amides from *Piper* family, almost none is found on laetispicine. Thus, the main aim of the present study was to assess the antidepressant and antinociceptive effect of laetispicine by behavioral models and investigated the mechanisms of action.

Materials and methods

Animals

KM mice (18–22 g) of either sex were obtained from Laboratory Animal Center of the College of Medicine, Fudan University. Animals were housed in standard environmental conditions with free access to food and water. The animals were used only once throughout the study. They were allowed to acclimatize to the laboratory 7 days before pharmacological tests. The experiment procedures were conducted in compliance with the National Institutes of Health Guide for Care and Use of the laboratory Animals and were approved by the Local Bioethics Committee (College of Pharmacy, Fudan University, China).

Drugs

Acetic acid, formaldehyde, acetylsalicylic acid (ASA) and tween80 were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), morphine hydrochloride from Shenyang First Pharmaceutical Company (Shenyang, China), naloxone hydrochloride from Beijing Sihuan Pharmaceutical Co., Ltd (Beijing, China), clomipramine from Hunan Dongting Pharmaceutical Co., Ltd. All other drugs were of analytical grade.

All drugs were dissolved in saline, except laetispicine (dissolved in saline with 2% Tween 80) and formaldehyde (dissolved in distilled water). Drugs and vehicle were administered by intragastric (i.g.) or intraperitoneal (i.p.) route 60 min before the forced swimming, open field, acetic acid writhing and formalin tests. Appropriate vehicle-treated groups were also assessed simultaneously.

Plant material and isolation of laetispicine

The stems of *P. laetispicum* were collected in 2006, from Hainan Province, China. The plant was identified by Prof. Sheng-li Pan, School of Pharmacy, Fudan University, where a voucher specimen (No. 060812) of the plant material has been deposited for further reference.

For the isolation of laetispicine, the methods of Pan et al. (2005) were used. Briefly, the air-dried stems of *P. laetispicum* (1 kg) were powdered and extracted with 95% hydroethanol (6 l) for 2 h under reflux, cooled and filtered. The residual material was extracted twice in 95% hydroethanol (4 l) for 2 h under reflux, cooled and filtered. The filtrate was combined with the former, concentrated under reduced pressure to give a dark brown colored semisolid extract (50 g). The hydroethanolic extract of the stems was dissolved in water and fractionated with petroleum ether, benzene, chloroform and ethyl acetate, respectively. The ethyl acetate extract was chromatographed on a silica gel column by a gradient elution using mixtures of cyclohexane and ethyl acetate to afford six fractions. Fraction 4 was subjected to silica gel column chromatography and eluted gradiently with petroleum ether and acetone, to give four subfractions, 4-1, 4-2, 4-3 and 4-4. Laetispicine (300 mg) was separated from subfraction 4-3 by repeated chromatography on silica gel column, eluted with petroleum ether and acetone.

Pharmacological tests

Forced swimming test (FST)

The forced swimming test adopted here is a modification of the method described by Porsolt et al. (1977). Briefly, mice were individually forced to swim for 15 min in glass cylinders (height: 20 cm, diameter: 14 cm), containing 10 cm of water at 25 °C, which is a pre-test, and then mice were removed and dried before being returned to cages. Twenty-four hours later, mice were placed in the cylinders again for a 6-min test in the same system depicted above. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

Open field test

In order to detect any association of immobility in the FST with changes in motor activity, the activities of animals treated with laetispicine were tested in an open