Pharmacology, Biochemistry and Behavior 94 (2010) 488–495

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Antidepressant effect of the extracts from Fructus Akebiae

Dan Zhou, Hong Jin [1], Huan-Bing Lin, Xue-Mei Yang *, Yu-Fang Cheng, Feng-Jun Deng, Jiang-Ping Xu *

Department of Pharmacology, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, PR China

ARTICLE INFO

Article history:
Received 22 May 2009
Received in revised form 30 October 2009
Accepted 12 November 2009
Available online 17 November 2009

Keywords:
Fructus Akebiae
Hederagenin
Antidepressant
Chronic unpredicted mild stress
Behavior
Forced swim test
Tail suspension test
Sucrose consumption
Rodents

ABSTRACT

Fructus Akebiae is a common ingredient in many traditional Chinese medicine complex prescriptions for the treatment of mental disorders. Previous studies indicate that the main chemical compositions of Fructus Akebiae are triterpenoid saponins with hederagenin as their sapogenin. In the present study, we enriched hederagenin from the extracts of Fructus Akebiae with a purity of approximately 70%. Using behavioral tests sensitive to antidepressant drugs, we demonstrated that acute and sub-chronic administration of the extracts of Fructus Akebiae produced antidepressant-like effects, as evidenced by decreases in the duration of immobility in forced swim and tail suspension tests in mice and reversal of chronic unpredicted mild stress-induced inhibition of sucrose consumption in rats. In addition, the extracts decreased the levels of plasma adrenocorticotrophic hormone and serum corticosterone in rats exposed to chronic unpredicted mild stress. Both behavioral and biochemical effects of the extracts were mimicked by the proven antidepressant escitalopram. These results suggest that the extracts of Fructus Akebiae exert antidepressant activity. Administration of the extracts may be beneficial for patients with depressive disorders.

1. Introduction

Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world population (Schechter et al., 2005). Medications such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase A (RIMAs), and specific serotonin–noradrenaline reuptake inhibitors (SNRIs) are clinically employed for drug therapy (Fava, 2003). However, these drugs can impose a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder (Antai-Otong, 2004; Baldwin et al., 2006; Khurana and Baudendistel, 2003; Park et al., 2005). During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders. In particular, the antidepressant effects of many traditional Chinese medicines (TCM) such as Chinese St. John's Wort herb, morinda root, gingko, valerian, areca seed, as well as some TCM complex prescriptions have received great amount of attention (Dar and Khatoon, 2000; Deltito and Beyer, 1998; Hattesohl et al., 2008; Li et al., 2003; Tesch, 2003; Zhang et al., 2002). These TCM are popular in China, Korea, and Japan for treating stress-related disorders. However, the active chemical components and the exact pharmacological mechanisms of these TCM remain largely to be investigated.

Fructus Akebiae is the dry fruit of Akebiae quinata (THUNB.) DECNE., a well-known medicinal plant widely distributed in China. It is recorded in the Compendium of Materia Medica that Fructus Akebiae is the major ingredient in some complex prescriptions for treating mental disorders and cognitive and behavioral deficits, including insomnia and dreaminess, loss of memory, paraphasia, phobia, and depressive disorder etc. Previous studies reveal that the genus Akebiae contains more than thirty types of triterpenoid saponins, and most of these triterpenoid saponins comprise hederagenin (Jiang et al., 2006).

In the present study, we aimed to investigate the effect of Fructus Akebiae extracts (FAE) on the development of behavioral despair and depressive-related behavior, and to evaluate FAE's effect on the neuroendocrine system by measuring alterations in plasma adrenocorticotrophic hormone (ACTH) and serum corticosterone (CORT). Our results suggest that FAE has an antidepressant activity by improving motivational behavioral deficits.

2. Materials and methods

2.1. FAE preparation

2.1.1. Materials

Fructus Akebiae was obtained from the Beijing Tongrentang Medicine Facility, Beijing, China (batch #: 701001532-1). The plant was harvested from Jiangsu Province, China. Hederagenin standard preparation was obtained from Shanghai Tauto Biotech Co., Ltd, China

* Corresponding authors. Xu is to be contacted at the Department of Pharmacology, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, PR China. Tel./fax: +86 20 6164 8236. Yang, Department of Chemistry, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, PR China. Tel.: +86 20 6164 8196.
E-mail addresses: xujp@fimmu.com (X.-M. Yang), jpx@fimmu.com (J.-P. Xu).
[1] Equally contributed to this work.

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.11.003

(batch #: 08072522). All the reagents used for sample preparation were analytical pure or, in the case of HPLC, chromatographic pure.

2.1.2. Sample preparation

500 g of Fructus Akebiae dry fruit powder was defatted twice by ultrasonication in petroleum ether (1000 ml) at room temperature. The solvent was volatilized, and the coarse powder was extracted twice by recirculation for 1 h with 2 l of 80% ethanol (EtOH). The extract was concentrated under reduced pressure into 500 ml, and was placed overnight at room temperature before filtration. H_2O (800 ml) was added to the filtrate followed by extraction for 3 times with 800 ml of ethyl acetate (EtOAC) and for 4 times with 800 ml of H_2O-saturated n-butanol (n-BuOH). n-BuOH was recycled under reduced pressure and the general saponin was obtained. A total of 5 g of general saponin was degraded for 3 h with 60 ml of 2 mol/l HCl in 45% EtOH at 100 °C and filtered, followed by washing off acid with H_2O and drying in vacuo, resulting in crude crystal. The crude crystal was dissolved in 300 ml of hot 70% EtOH and decolored by heating-recirculation with 1 g of activated carbon for 0.5 h, followed by filtration at hot temperature. The filtrate was concentrated for 12 h at 4 °C under reduced pressure to obtain clustered crystal. The crystal was filtered, followed by washing off chloridion with H_2O and drying in vacuo into white powder. The yield of the final extract was approximately 0.5% (w/w). The powder was stored at 4 °C until analysis.

2.1.3. HPLC analysis

For HPLC analysis, 8 mg FAE was dissolved in 10 ml of methanol and filtered through 0.2 μm nylon membrane prior to injection into HPLC. The hederagenin standard solution was prepared by dissolving 2 mg hederagenin in 10 ml of methanol and filtered through nylon membrane.

HPLC analysis was carried out using Agilent 1100 series HPLC systems linked to both diode array and multiple wavelength detectors (Agilent, USA). Samples were separated using an Agela C18 column (4.6 mm × 250 mm, i.d. 5 μm, Agela Technologies, USA) which was maintained at 25 °C. The mobile phase was used according the following ratio: CH_3OH–H_2O–CH_3COOH–$(C_2H_5)_3N$ (87:13:0.04:0.02, v/v). For each run, 20 μl of sample solution was injected. The solvent flow was 0.8 ml/min, and the detection wavelength was 210 nm. The ChemStation software was used to control the instruments and for data acquisition and processing.

2.2. Animal tests

2.2.1. Animals and materials

Male Kunming mice (18–25 g) at the age of 7–9 weeks and male Sprague–Dawley rats (2 months old, 180–200 g) were obtained from the Laboratory Animal Centre of Southern Medical University (Guangzhou, China) and were acclimated to the facility for 1 week before use in the experiments. Mice were housed 8–12 per cage, and rats were singly housed in standard rat cages except for the control group. The animals were housed at 22 ± 1 °C with a 12:12 h light/dark cycle (lights on at 7:00 a.m.), and were given ad libitum access to water and food. All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Escitalopram (ESC) was kindly provided by Guangzhou Zuosen Biotechnol Co., Ltd, China (batch #: 20020325). ACTH and CORT radioimmunoassay kits were purchased from Beijing North Biotech Institute, China. FAE and ESC were suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) solution, and the dosage used in the experiments was determined based on reported studies (Sanchez et al., 2003).

2.2.2. Behavior despair study

2.2.2.1. Forced swim test. For the forced swim test (FST), mice were divided into five groups (n = 8–12/group): control (0.5% CMC-Na solution), 25 mg/kg FAE, 50 mg/kg FAE, 100 mg/kg FAE, and 6.25 mg/kg ESC. All the drugs were given via the oral route once a day at 8 a.m. for 1 week. FST was conducted 60 min after the first acute treatment and 24 h after repeated treatment for 7 days with drugs.

FST was performed according to the published procedure (Porsolt et al., 1977) with minor modifications. Mice were placed in a glass cylinder (12 cm diameter) filled with water (24 ± 2 °C) to a depth of 15 cm. The duration of immobility was measured during the total 6 min of the test. Immobile time was defined as the absence of active/escape directed movements (mouse floating in the water without struggling) and was scored in a blind manner by an observer (Dias Elpo Zomkowski et al., 2004; Zomkowski et al., 2005).

2.2.2.2. Tail suspension test. For the tail suspension test, mice were divided into five groups (n = 8–11/group): control (0.5% CMC-Na solution), 25 mg/kg FAE, 50 mg/kg FAE, 100 mg/kg FAE, and 6.25 mg/kg ESC. All the drugs were given via the oral route once a day at 8 a.m. for 1 week. TST was conducted 60 min after the first acute treatment and 24 h after repeated treatment for 7 days with drugs.

TST was performed based on the method of Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during the total 6-min test.

2.2.2.3. Locomotor activity. In order to determine whether FAE really has an antidepressant-like action, we have to find out whether FAE has significant action on the central nervous system by measuring spontaneous motor activity of mice after the FST and TST. For measuring locomotor activity, mice were divided into five groups (n = 8–10/group): control (0.5% CMC-Na solution), 25 mg/kg FAE, 50 mg/kg FAE, 100 mg/kg FAE, and 6.25 mg/kg ESC. All the drugs were given via the oral route once a day at 8 a.m. for 1 week. The test was conducted 60 min after the first acute treatment and 24 h after repeated treatment for 7 days with drugs.

In this test, mice were respectively placed in the five separated chambers of an autonomous movement instrument (Shandong Medical Academy of Science, China). The total locomotor activity (ambulation activity) number of mice was automatically recorded during the 30-min test. During the interval of the test the apparatus was cleaned.

2.2.2.4. Chronic unpredicted mild stress procedure. For the chronic unpredicted mild stress (CUMS) study, rats were divided into six groups (n = 6–7/group): vehicle control (0.5% CMC-Na, no CUMS), CUMS vehicle (0.5% CMC-Na, with CUMS), CUMS with 6.25 mg/kg FAE, CUMS with 12.5 mg/kg FAE, CUMS with 25 mg/kg FAE, and CUMS with 6.25 mg/kg ESC. All the drugs were administrated via the oral route once a day at 8 a.m. for 3 weeks. The CUMS procedure was simultaneously conducted with drug administration on rats.

The CUMS procedure, a variation of the method described by Ossowska et al. (2004), was designed to maximize the unpredictable nature of the stressors. Rats were exposed to the following 9 stressors in a random order: inversion of the light/dark cycle (12 h/12 h), restraining behavior (120 min), forced swimming in cold water (4 °C, 5 min), damp sawdust (24 h), hot environment (45 °C), water deprivation (24 h), food deprivation (24 h), nip trail (1 min), and footplate shock (50 mV, once for 10 s every 50 s, repeated for 30 times). Rats received one of these stressors per day randomly, and the same stressor was not applied for 3 consecutive days. The stress procedure lasted for 3 weeks prior to behavioral testing.

Rats were weighed on day 0 (before experiment, baseline), 5, 10, 15, and 20 during the CUMS experiment. The body weight change was calculated based on the baseline value as: changes in body weight (%) = (body weight − body weight at baseline) / body weight at baseline.

2.2.2.5. Sucrose preference test. This test was conducted 1 day after CUMS. According to a previous procedure (Willner et al., 1987), 72 h before the test, rats were trained to adapt to a 1% sucrose solution (w/v) by placing two bottles of 1% sucrose solution in each cage for 24 h,