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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

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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.

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(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,

followed by placing one bottle of 1% sucrose and one bottle of tap water for 24 h. After adaptation, rats were deprived of water and food for 24 h, followed by the sucrose preference test. During the test, rats housed in individual cages had free access to two bottles with one containing 200 ml of 1% sucrose and the other 200 ml of tap water. After 2 h the consumption of sucrose and water (v) was measured and the sucrose preference was calculated as the sucrose preference (%) = sucrose consumption/(sucrose consumption + water consumption). The test was conducted 1 h after the last treatment of drugs between 7:00 a.m. and 11:00 a.m.

2.2.2.6. Open field test. The open field test was performed as described by Blokland et al. (1990). Briefly, the apparatus consisting of a black square cage with 100×100×40 cm was divided into 25×25 cm equal squares on the floor of the arena. A rat was placed in the center of the cage, and after 30 s of adaptation the number of ambulation (with at least three paws), the number of rears (standing upright on hind paws), and the frequencies of grooming (including face cleaning, paw licking, fur licking, head scraping, and rubbing) were counted manually for 5 min as described in recent studies (Brenes Saenz et al., 2006; Swiergiel and Dunn, 2007). During the interval of the test the apparatus was cleaned. The open field test was conducted 1 h after the last treatment of drugs between 7:00 a.m. and 11:00 a.m. in a quiet room dimly illuminated.

2.2.3. Measurements of plasma ACTH and serum CORT

After the behavioral tests, between 9:00 a.m. and 11:00 a.m., all the rats were decapitated, and the blood samples were collected in tubes with or without heparin for plasma and serum preparation, respectively. The samples were centrifuged for 10 min at 3000 rpm, and the upper layer of each sample was collected and stored at −30 °C for assays. Plasma ACTH and serum CORT levels were determined using corresponding radioimmunoassay kits.

2.3. Statistical analysis

Data were presented as mean ± SEM. Differences among groups were examined using one-way ANOVA, followed by LSD post hoc test (two-tailed), except for the analysis of weight change. Because the experimental design involved two within-subjects factors (time and treatment), a repeated measures ANOVA was performed. All the behavior tests were scored live. All statistical analyses were performed using SPSS software (v. 13.0). A p value of <0.05 was considered to be statistically significant.

3. Results

3.1. Preparation of FAE

With the method employed in the present study, the yield of extracts from Fructus Akebiae was about 0.5%. The content of hederagenin in the FAE was calculated through peak area ratio between its area in test solution and hederagenin area in the hederagenin standard solution according to the Chinese Pharmacopoeia (Table 1). HPLC analysis showed that the enrichment of hederagenin in the FAE was 69.48%.

3.2. Effect of acute treatment with FAE on the immobility time both in the FST and TST and locomotor activity

In order to determine if FAE can produce an acute effect in depression-related behavior in FST and TST, we conducted both the tests after the first treatment of mice with various dosages of FAE.

One-way ANOVA showed significant difference [$F(4,46) = 2.777$, $p<0.05$] in FST on the mice among experimental groups. FAE at 25 mg/kg ($p<0.05$), 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.01$) treated mice had significantly decreased immobility time compared with vehicle-treated mice in the FST. The effect of FAE was similar to that of ESC ($p<0.01$), an SSRI-type antidepressant drug served as positive control (Fig. 1A).

Table 1
Both peak area and concentration of hederagenin and FAE by HPLC analysis.

Sample	Concentration (mg/ml)	Peak area (mAU)
Hederagenin	0.8	7831 ± 45
FAE	0.2	2791 ± 36

Likewise, one-way ANOVA also showed significant difference [$F(4,38) = 11.450$, $p<0.001$] in TST on mice among experimental groups. After acute administration, FAE at 25 mg/kg ($p<0.01$), 50 mg/kg ($p<0.001$) and 100 mg/kg ($p<0.001$), as well as ESC at 6.25 mg/kg ($p<0.001$) treated mice had significantly decreased immobility time compared with vehicle-treated mice in the TST (Fig. 1B).

The result of locomotor activity was shown in Fig 1C after the first acute treatment. Statistical analysis by one-way ANOVA showed no

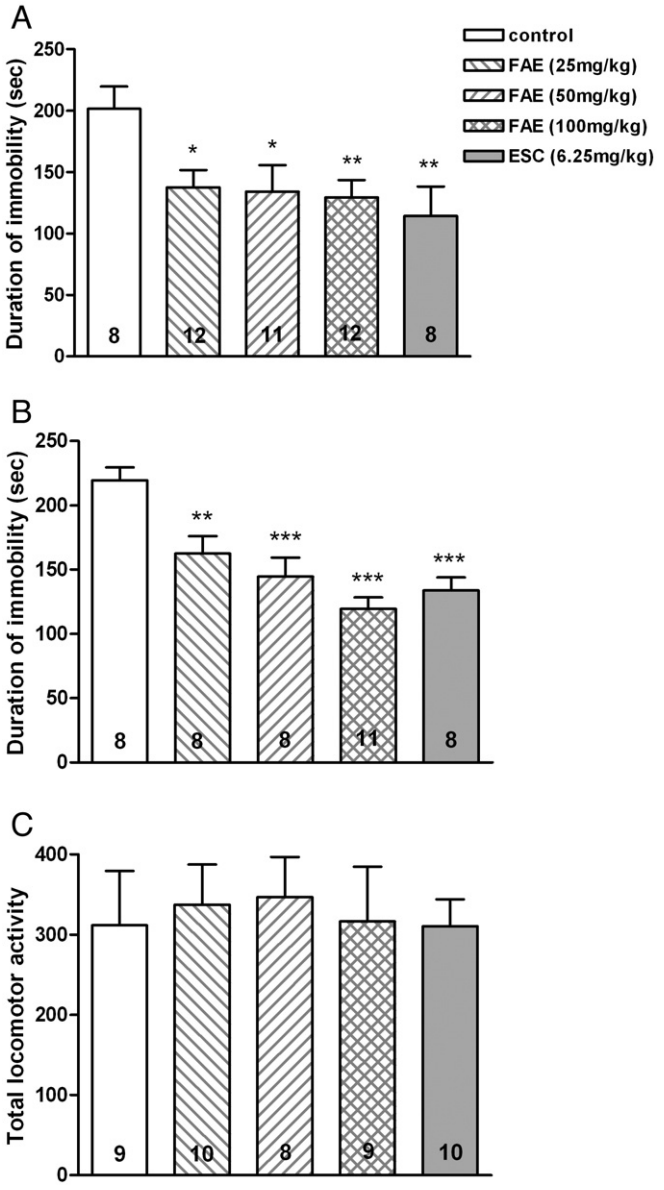

Fig. 1. The acute effect of FAE (dose range: 25–100 mg/kg) and ESC (6.25 mg/kg) on the time of immobility during the total 6-min testing period in the forced swimming test (A) and the tail suspension test (B), as well as on the locomotor activity (C) in mice. Results are expressed as mean ± SEM (n = 8–12/group). The drugs were administered via oral route 60 min prior to testing. For statistical significance, *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the vehicle control group (distilled water containing 0.5% CMC-Na).

significant difference [$F(4,41)=0.073$, $p>0.05$] in total locomotor activity among the groups. FAE produced no significant difference in the total locomotor activity number ($p>0.05$) compared with vehicle-treated mice in the test (dose range: 25–100 mg/kg).

3.3. Effect of repeated treatment with FAE on the immobility time both in the FST and TST and locomotor activity

In order to investigate if FAE can produce chronic changes in depression-related behavior in FST and TST, we treated mice with different dosages to mice via continuous oral administration for 7 days.

One-way ANOVA showed significant difference [$F(4,35)=6.499$, $p<0.01$] in FST on the mice among experimental groups. FAE at 25 mg/kg ($p<0.001$), 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.01$) produced a reduction in the immobility time. The effect of FAE was similar to that of ESC ($p<0.001$) (Fig. 2A).

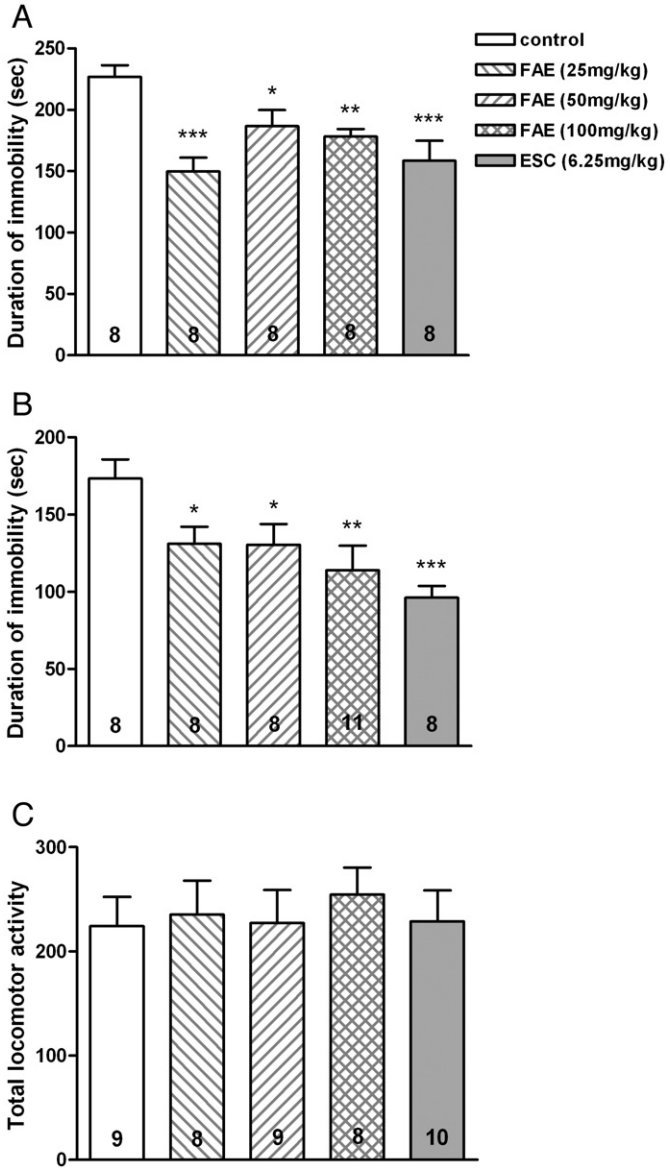

Fig. 2. The sub-chronic effect of FAE (dose range: 25–100 mg/kg) and ESC (6.25 mg/kg) on the time of immobility during the total 6-min testing period in the forced swimming test (A) and the tail suspension test (B), as well as on the locomotor activity (C) in mice. Results are expressed as mean ± SEM ($n=8$–12/group). The drugs were administered via oral route 60 min prior to testing. For statistical significance, *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the vehicle control group (distilled water containing 0.5% CMC-Na).

Likewise, one-way ANOVA also showed significant difference [$F(4,40)=5.600$, $p<0.01$] in TST on mice among experimental groups. After the 7-day administration, FAE at 25 mg/kg ($p<0.05$), 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.01$), as well as ESC at 6.25 mg/kg ($p<0.001$) also produced a reduction in the immobility time (Fig. 2B).

The result of locomotor activity was shown in Fig 2C after repeated treatment. Statistical analysis by one-way ANOVA showed no significant difference [$F(4,39)=0.157$, $p>0.05$] in total locomotor activity among the groups. FAE produced no significant difference in the total locomotor activity number ($p>0.05$) compared with vehicle-treated mice in this test (dose range: 25–100 mg/kg).

3.4. Effect of FAE on body weight and sucrose preference

A repeated measures ANOVA [4 sampling days = within-subjects; 6 sampling treatments = within-subjects] revealed significant days [$F(3,15)=329.69$, $p<0.001$], treatments [$F(5,25)=4.74$, $p<0.01$], days × treatments [$F(15,75)=3.763$, $p=0.001$] effects on mean body weight change. Rats in the vehicle group without stress treatment showed a significant increase in body weight on any of the 4 sampling days (Fig. 3) and also exhibited significant difference compared with any other stressed groups ($p<0.05$), while CUMS-treated rats exhibited a relatively small increases in body weight throughout the 5th, 10th, 15th and 20th days (Fig. 3). The magnitude of increases in body weight of rats was similar in all CUMS-treated groups ($p>0.05$).

For the sucrose and water intake, as well as total fluid consumption (Fig. 4A), one-way ANOVA showed significant difference in sucrose [$F(5,34)=16.896$, $p<0.001$] and water [$F(5,34)=9.351$, $p<0.001$] intake among experimental groups. However, there was no significant difference in total fluid consumption among experimental groups [$F(5,34)=1.756$, $p>0.05$].

For sucrose preference, one-way ANOVA showed significant difference among experimental groups [$F(5,34)=14.171$, $p<0.001$]. Compared to rats in the vehicle group, CUMS-treated rats showed a significant decrease in sucrose preference ($p<0.001$). After 21 days of administration, both FAE and ESC significantly increased rats' sucrose preference at all dosages used ($p<0.001$), and the effect of FAE showed a dose-dependent manner (Fig. 4B).

3.5. Effect of FAE on locomotor activity in open field test

Open field test is used to assess locomotor activity, exploration and anxiogenic-like behavior of rats or mice (Keeney and Hogg, 1999).

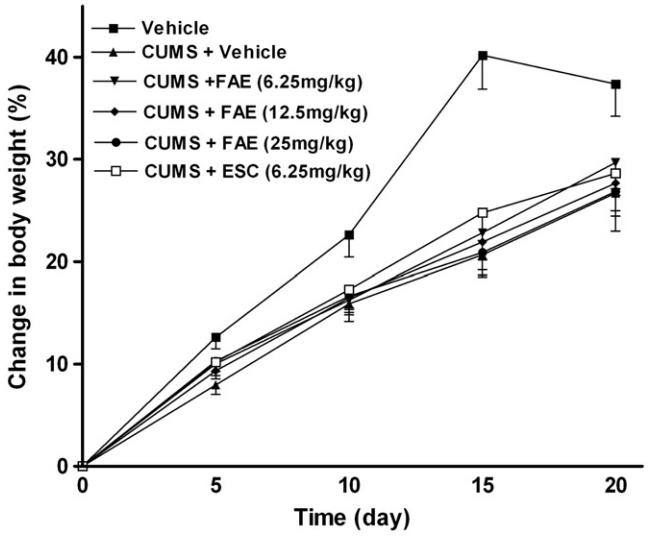

Fig. 3. The effect of FAE (dose range: 6.25–25 mg/kg) and ESC (6.25 mg/kg) on body weight change throughout the 5th, 10th, 15th or 20th days. Data are expressed as mean ± SEM ($n=6$–7/group).

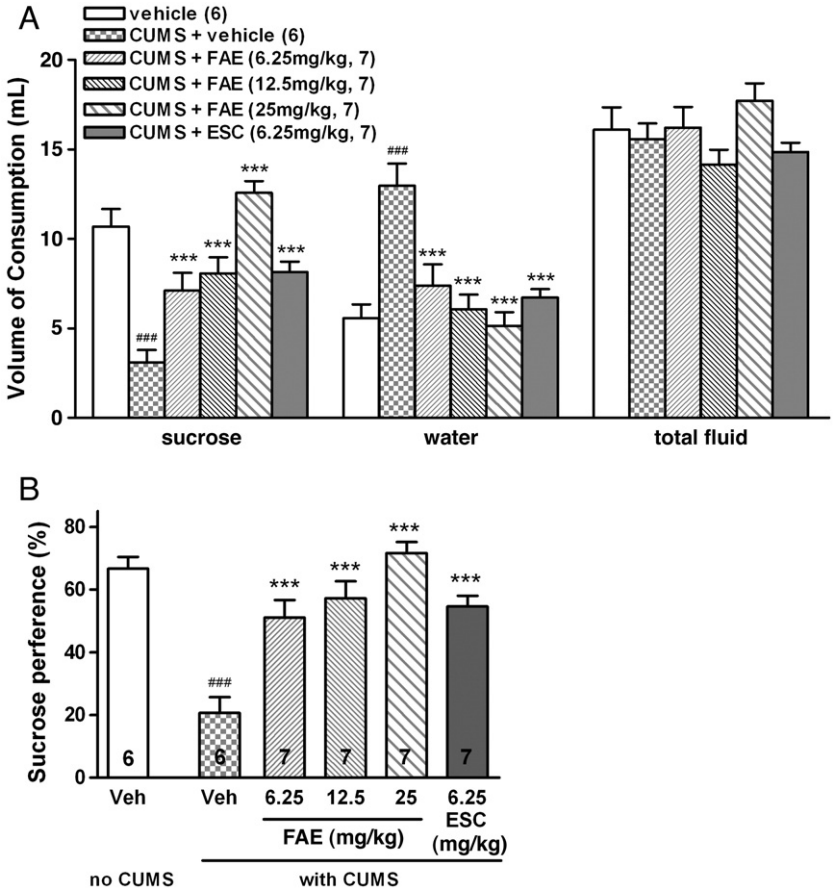

Fig. 4. The effect of FAE (dose range: 6.25–25 mg/kg) and ESC (6.25 mg/kg) on fluid consumptions (A) and sucrose preference (B) in rats exposed to CUMS during sucrose intake test. All rats were tested between 7:00 a.m. and 11:00 a.m. Data are expressed as mean ± SEM (n = 6–7/group). For statistical significance, $^{###}p<0.001$ compared to the vehicle (distilled water containing 0.5% CMC-Na) and $^{***}p<0.001$ compared to the CUMS vehicle group (distilled water containing 0.5% CMC-Na).

One-way ANOVA indicated that there were significant differences in ambulation [$F(5,34) = 5.318$, $p<0.01$], rearing [$F(5,34) = 3.616$, $p<0.05$] and grooming [$F(5,34) = 3.269$, $p<0.05$] among experimental groups. Compared to the vehicles, CUMS-treated rats showed a significant reduction in ambulation ($p<0.001$), rearing ($p<0.01$), and grooming ($p<0.01$) activities (Fig. 5). Compared to the CUMS-treated rats, 21-day treatment with FAE at various dosages or with ESC at 6.25 mg/kg did not significantly affect ambulatory, rearing, and grooming activities, though they all showed an increasing tendency on locomotor activity.

3.6. Effects of FAE on plasma ACTH and serum CORT levels

CUMS procedure markedly induced increase in plasma ACTH levels compared to rats in the vehicle control group ($p<0.001$). Statistical analysis by one-way ANOVA showed that there was significant difference in the plasma ACTH levels among the experimental groups [$F(5,32) = 19.343$, $p<0.001$]. After the 21-day treatment, FAE at 12.5 ($p<0.05$) and 25 mg/kg ($p<0.001$) significantly decreased the levels of plasma ACTH in CUMS-treated rats. The maximal effect was obtained by FAE at 25 mg/kg. FAE at 6.25 mg/kg ($p>0.05$) did not

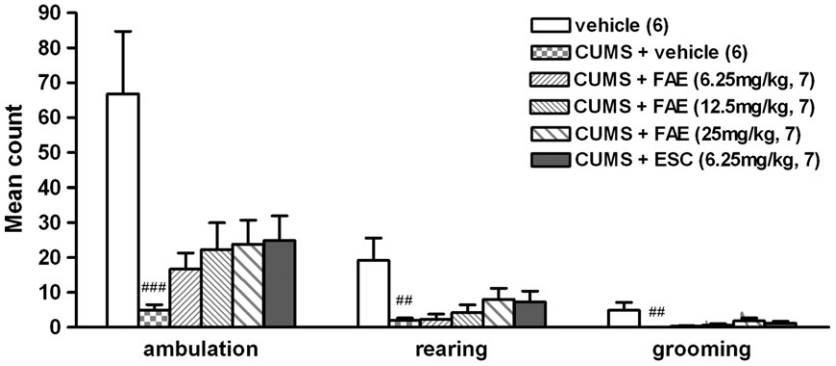

Fig. 5. The effect of FAE (dose range: 6.25–25 mg/kg) and ESC (6.25 mg/kg) on the number of ambulation, the number of rearing, and the frequencies of grooming in rats exposed to CUMS during the open field test. The test was conducted between 7:00 a.m. and 11:00 a.m. Data are expressed as mean ± SEM (n = 6–7/group). For statistical significance, $^{##}p<0.01$, and $^{###}p<0.001$ compared to the CUMS control group (distilled water containing 0.5% CMC-Na).

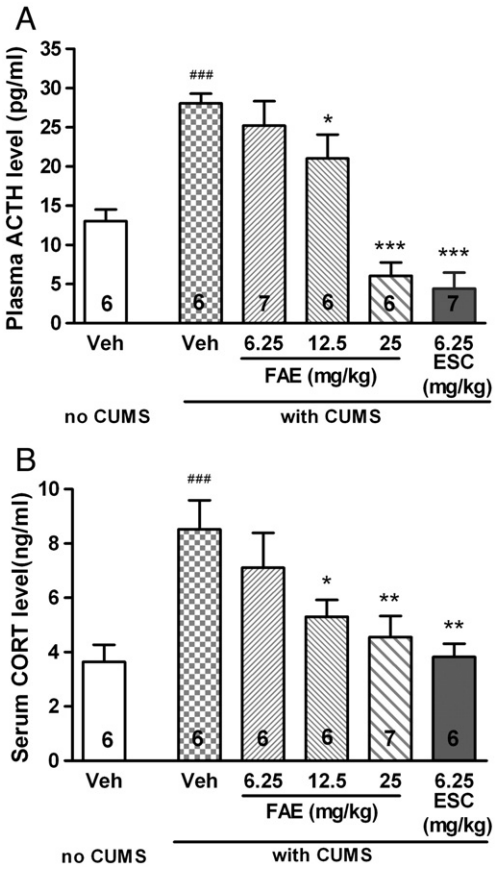

Fig. 6. The effects of FAE (dose range: 6.25–25 mg/kg) and ESC (6.25 mg/kg) on plasma ACTH (A) and serum CORT (B) levels in rats exposed to CUMS. Data are expressed as mean ± SEM (n = 6–7/group). For statistical significance, $^{###}p<0.001$ compared to the vehicle control group (distilled water containing 0.5% CMC-Na) and $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to the CUMS control group (distilled water containing 0.5% CMC-Na).

show a significant effect on plasma ACTH levels. As a positive control group, ESC at 6.25 mg/kg ($p<0.001$) significantly decreased ACTH levels in CUMS-treated rats (Fig. 6A).

Similarly, CUMS procedure also evoked a significant increase in rat's serum CORT levels compared with the vehicle control group ($p<0.001$). One-way ANOVA revealed that there was significant difference in rat's serum CORT levels among the experimental groups [$F(5,31)=5.124$, $p<0.01$]. After 21 days of treatment with FAE at 12.5 ($p<0.05$) and 25 mg/kg ($p<0.01$), the serum levels of CORT decreased significantly. FAE at 6.25 mg/kg did not change the serum CORT levels in CUMS-treated rats ($p>0.05$). ESC at 6.25 mg/kg ($p<0.01$) significantly decreased the serum CORT levels in CUMS-treated rats (Fig. 6B).

4. Discussion

In the present study, we found that FAE could normalize the immobility time in mice in FST and TST, as well as sucrose preference, ACTH levels, and CORT levels in plasma of rats exposed to CUMS. These findings confirm our hypothesis that FAE have an antidepressant-like effect.

Because of the insufficient availability and high cost of pure preparation of hederagenin, in the present study, we used FAE instead of pure hederagenin for the investigation of their antidepressant effect. Hederagenin was successfully enriched in FAE with a concentration of 69.48% by HPLC analysis, clearly showing that the major component of FAE used in our experiments was hederagenin.

The key issue in screening for new antidepressant drugs is to establish a valid paradigm, which must be capable of accurately identifying various antidepressant treatments (Willner, 1984). In this study, we used two animal models, FST and TST. Both the paradigms are widely accepted behavioral models for assessing pharmacological antidepressant activity (Bourin, 1990; Porsolt et al., 1977). Characteristic behavior scored in these tests is termed immobility, reflecting behavioral despair as seen in human depression (Steru et al., 1985; Willner, 1984). In addition, it is well known that many antidepressant drugs are able to reduce the immobility time in rodents (Porsolt et al., 1977). In the present study, we explored the acute and sub-chronic effects of FAE using both the animal models, respectively. After the first acute treatment FAE produced a marked reduction in immobility time at doses of 25 mg/kg, 50 mg/kg, 100 mg/kg in the mouse FST, with a profile comparable to that observed for the classical antidepressant drug ESC. Furthermore, the effect of FAE showed a dose-dependent manner. Likewise, in the mouse TST, both FAE and ESC acutely decreased immobility time at all dosages used, and the effect of FAE showed a dose-dependent manner. For purposes of avoiding the acute effect and investigating the sub-chronic effect of FAE, both the tests were performed 24 h after repeated treatment for 7 days. In the FST, the magnitude of immobility time reduction at a dosage of 25 mg/kg was greater than at 50 mg/kg or 100 mg/kg. However, in the TST, the intensity of immobility at 100 mg/kg was comparable and greater than at the other two dosages. From these results the mouse behavioral difference was rather inconsistent. Nevertheless, these contradictions were not surprising, since the mouse FST has not traditionally been viewed as a consistently sensitive model for detecting selective serotonin reuptake inhibitor activity, whereas these antidepressants are generally reported as active in the TST (Cryan et al., 2005). Moreover, TST is proposed to have a greater pharmacological sensitivity as compared with FST (Cryan et al., 2005; Thierry et al., 1986). In order to exclude the possibility of FAE exerting such a psychostimulant-like effect, we employed an additional locomotor activity test to check the motor stimulating activity of FAE after tests (Sakakibara et al., 2006; Zomkowski et al., 2006). However, FAE at any dosage resulted in no behavioral changes or motor dysfunction in the locomotor activity test after either the acute or repeated treatment. Our results demonstrated that not only the acute treatment, but also the repeated administration (7 days) with FAE produced a significant antidepressant-like response in both FST and TST.

In addition to using the acute and sub-chronic stressor animal models, we also employed a chronic stressor model CUMS to test the antidepressant effect of FAE (Willner et al., 1987). This paradigm has the advantage of excluding false positive effects caused by psychostimulant agents in acute stress-based models (De Pablo et al., 1989; Duncan et al., 1985; Papp et al., 1996; Steru et al., 1985). In spite of some initial disputes (Forbes et al., 1996), the validity of CUMS has been confirmed by numerous independent studies (Willner, 2005). In 1987, the rodent CUMS preparation was established by Willner and his colleagues for the major purpose of inducing anhedonia-like behavioral change, i.e. inability to experience pleasure, which is the core symptom of clinic human major depression (Willner, 1997; Willner et al., 1987). Anhedonia has been defined as decreased responsiveness to rewards, which is now measured by decreased preference of sucrose solution (Anisman and Matheson, 2005; Willner et al., 1987). In the present study, we first established a CUMS-induced rat model with depression-like behavioral changes by measuring the change of sucrose preference, locomotor activity, rearing, and grooming in the open field test. The CUMS procedure significantly reduced both the consumption and preference of sucrose intake, but did not to change the total fluid consumption in the present study. These data indicated that the reduced sucrose preference we observed here was not attributable to decreased thirst in general but rather due to anhedonia. These results further supported the successful experimental protocol of the CUMS procedure. Furthermore, administration of FAE over 21 days could reverse the decrease in sucrose preference. This effect was similar

to that of fluoxetine (Brenes and Fornaguera, 2009). FAE-treated rats showed higher intake and preference than stressed littermates. Moreover, all stressed groups showed similar body weight changes, indicating that difference in the sucrose intake and total fluid consumption induced by FAE was not a consequence of reduction in body weight changes.

Open field exploratory test is commonly used to evaluate exploratory behavior and response to the novel environment in experimental animals. The number of ambulation and rears, as well as the frequencies of grooming was measured in this test to evaluate the antidepressant behavior of rats (Wang et al., 2008). Some research show that CUMS is able to reduce the locomotor activity of rats in an open field test (D'Aquila et al., 2000a), which may mimic some aspects of human psychomotor retardation (Willner et al., 1987), an accompanying symptom of major depression in humans. Meanwhile, normal rats show increased rearing and grooming in a novel open field, which is driven by the instinct interests of rats to explore a novel environment. After CUMS, however, rats display decreased rearing and grooming in a novel open field (D'Aquila et al., 2000b; Katz et al., 1981), which may indicate a "refractory loss of interest" (Katz et al., 1981), another core symptom of human major depression. All those behaviors can be remarkably improved by some antidepressant drugs (Wang et al., 2008). In the present study, CUMS rats exhibited evident decrease behavior in ambulation, rearing movement and grooming activity. However, treatment with FAEs at all dosage or ESC failed to significantly reverse the altered open field behavior, even if they showed an increased trend. This was probably due to the delayed effect onset of antidepressants. If the administration time extends to 2 months or even longer, FAEs or ESC may show marked antidepressant effect in open field test. Therefore, from these results we cannot yet deny the antidepressant-like activity of FAEs.

The HPA axis plays a key role in the physiological response to various stressful situations (Szafarczyk et al., 1993). Continuous activation of the HPA axis, especially abnormally increased CORT and ACTH levels, leads to hormonal imbalance and even to more severe diseases such as depressive disorder both in rodents and humans (Asnis et al., 1987; Ayensu et al., 1995). A previous study has suggested a close association between depressive disorder and neuroendocrine alterations (Jozuka et al., 2003). In the present study, the endocrine changes in the HPA axis were observed following CUMS, mirroring those changes seen in human depression. Most antidepressant drugs can normalize the HPA axis hyperactivity (Bschor et al., 2002; Frost et al., 2003; Himmerich et al., 2007; Inder et al., 2001). The present results showed that the plasma ACTH and serum CORT levels were significantly elevated in the CUMS rats. Furthermore, FAE and ESC treatments significantly reduced the CUMS-induced increase in plasma ACTH levels to even lower than normal and reversed elevated serum CORT levels to the normal levels, with a tendency of decrease in a dose-dependent manner. These data strongly suggested that FAE exerted antidepressant activity, at least in part, by regulating ACTH and CORT levels, thus normalizing the HPA axis hyperactivity.

In summary, in the present study we prepared FAE containing about 70% of hederagenin from dry fruits of Fructus Akebiae. FAE reduced immobility time in the mouse FST and TST, and reversed CUMS-induced deficit in sucrose intake to prevent anhedonia in rats. Moreover, FAE modified neuroendocrine activity by significantly reducing plasma ACTH and serum CORT levels in CUMS animal models. These results suggest that both crude FAE and hederagenin possess potent antidepressant properties. Further studies are needed to verify the antidepressant effect of hederagenin and investigate the underlying mechanisms.

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