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Antidepressant-like effects of liquiritin and isoliquiritin from *Glycyrrhiza uralensis* in the forced swimming test and tail suspension test in mice

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Abstract

Two classic animal behavior despair tests—the Forced Swimming Test (FST) and the Tail Suspension Test (TST) were used to evaluate the antidepressant activity of liquiritin and isoliquiritin from *Glycyrrhiza uralensis* in mice. It was observed that both liquiritin and isoliquiritin at doses of 10, 20 and 40 mg/kg significantly reduced the immobility time in the FST and TST in mice 30 min after treatment. Measurement of locomotor activity indicated that liquiritin and isoliquiritin had no central nervous system (CNS)-stimulating effects. The main monoamine neurotransmitters and their metabolites in mouse brain regions were also simultaneously determined by HPLC-ECD. It was found that these two compounds significantly increased the concentrations of the main neurotransmitters 5-HT and NE in the hippocampus, hypothalamus and cortex. Liquiritin and isoliquiritin also significantly reduced the ratio of 5-HIAA/5-HT in the hippocampus, hypothalamus and cortex, slowing down 5-HT metabolism compared with mice treated with vehicle+stress. In conclusion, liquiritin and isoliquiritin produced significant antidepressant-like effects, and their mechanism of action may be due to increased 5-HT and NE in the mouse hippocampus, hypothalamus and cortex.

Keywords: Antidepressant; Forced swimming test; Isoliquiritin; Liquiritin; Neuro-transmitters; Tail suspension test

1. Introduction

Depression is one of the major mental disorders. Symptoms of depression include lowered mood and reduced interest and pleasure. According to WHO's prediction, depression will be the second most common disease in 2020 (Murray and Lopez, 1996). A metabolic disorder of monoamine neurotransmitters in the CNS is believed to be the main biochemical symptom of depression.

Liquorice, which mainly includes three species: *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza glabra* L., and *Glycyrrhiza inflata* Bat., is a famous medicinal plant with a long history of pharmaceutical use in China. *G. uralensis* has been reported to have a number of pharmacological activities including anti-virus (Nakashima et al., 1987), antitumor (Tsutomu et al., 1988) and anti-inflammatory (Kakegawa et al., 1992). It has also been reported that an aqueous extract of *G. glabra* L. showed significant antidepressant-like activity in mouse immobility tests (Dhingra and Sharma, 2006). Glycyrrhizin was thought to be the main constituent causing this activity as it was reported to have antidepressant effects (Dhingra and Sharma, 2005).

A previous study from our laboratory found that a flavone compound, liquiritin, isolated from *G. uralensis* demonstrated an antidepressant effect on chronic stress depressed rats (Zhao et al., 2006). With more attention being paid to the antidepressant

Abbreviations: CNS, central nervous system; DA, Dopamine; DHBA, 3,4-Dihydroxybenzylamine; DMSO, dimethyl sulfoxide; FST, forced swimming test; 5-HIAA, 5-Hydroxyindoleacetic acid; HPLC-ECD, high performance liquid chromatography coupled with electro chemical detector; 5-HT, 5-Hydroxytryptamine; NE, Norepinephrine; ODS, octadecylselyl; OSA, Octenyl succinate; SSRIs, selective serotonin reuptake inhibitor antidepressants; TST, tail suspension test; WHO, World Health Organization.

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activity of flavonoids (Butterweck et al., 2001; Nolder and Schotz, 2002), the number of flavone compounds found to have antidepressant effects has increased (Butterweck et al., 2000; Zhu et al., 2006). In the present study, liquiritin and another flavonoid from *G. uralensis*, isoliquiritin, were investigated to determine their antidepressant activities. In addition, the probable mechanism of antidepressant-like activity was explored by analyzing monoamine neurotransmitters in the mouse brain.

2. Materials and methods

2.1. Plant material and agents

Roots of *G. uralensis* were purchased from Tongrentang Medicinal Material Company in Beijing. Specimens of the plant materials were identified by Dr. Hongzhu Guo and were preserved at the Division of Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Peking University, Beijing, China.

A positive control: Fluoxetine-HCl (Lilly, USA, purity > 98%) was included. Reference standards for simultaneous determination of: 5-HT, 5-HIAA, DA, NE and DHBA were all purchased from Sigma, USA, purity > 98%.

2.2. Apparatus

^{1}H and ^{13}C-NMR spectra were obtained on a Bruker ARX 400 spectrometer (Bruker Co. Germany) in DMSO-d6 solution, using the corresponding solvent signal as an internal standard. ESI-MS was measured on a Finnigan LCQ Advantage mass spectrometer (Thermo Co. USA). Analysis and semi-preparative HPLC was carried out on an Alltech HPLC system equipped with a 426 HPLC pump and a Linear detector (Alltech Co., USA) by using Senshu Pak ODS C18 columns (4.6 × 250 mm 5 μm, 20 × 250 mm 5 μm, SSC Co., Japan).

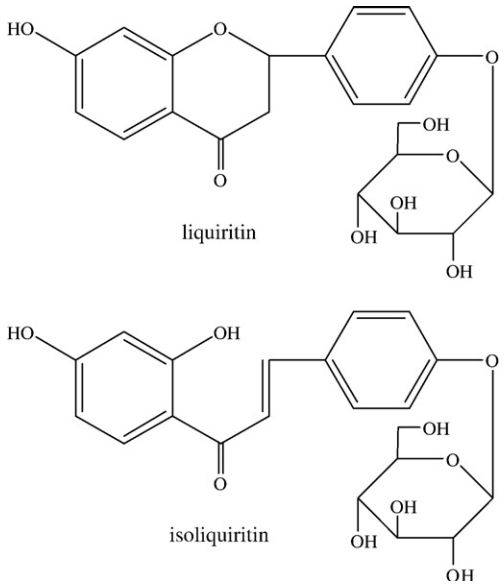

Fig. 1. Chemical structures of liquiritin and isoliquiritin.

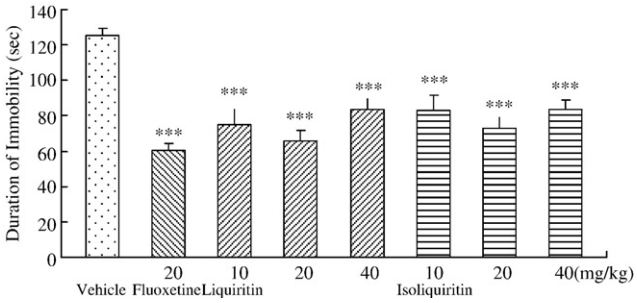

Fig. 2. Immobility time of liquiritin and isoliquiritin in the mouse FST. Data expressed as mean ± S.E.M. ($n = 10$). Statistical analysis of data was carried out by one-way analysis of variance followed by the LSD test. $F(7, 72) = 63.94$; $p < 0.0001$.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle.

2.3. Extraction and isolation

1000 g of *G. uralensis* roots, were extracted with deionized water (3 × 3 L) under reflux and repeated 3 times (1 h each time). The extracts were combined and concentrated under vacuum to afford 340 g aqueous extracts (AE). AE were chromatographed on a macroporous resin D101 column (10 × 80 cm, Naikai Chemical Co., China) and eluted with deionized water (1 L), 50% ethanol (3 L), 95% ethanol (3 L) to afford three fractions: water fraction (AE1, 122 g), 50% ethanol fraction (AE2, 26.8 g) and 95% ethanol fraction (AE3, 8.83 g). AE2 was subjected to a silica gel column (500 g, 5 × 50 cm, 200 mesh, Qingdao Haiyang Chemical Co., China) eluted with $CHCl_3$–MeOH (4:1, 3:1, 2:1, 1:1, each 300 ml) affording four subfractions (sfr): sfr.1 to sfr.4. Subfraction 3 was subjected to an ODS gel column (1 × 30 cm, 100 mesh, Fuji Silysia Chemical Ltd. Japan), eluted with MeOH–H_2O solution from 10% to 70% with 10% increments, each 100 ml. The 30%MeOH eluant afforded liquiritin (289 mg) and the 50% MeOH eluant afforded isoliquiritin (137 mg). The two compounds were further purified using isocratic semi-preparation HPLC with MeOH–H_2O (42:58). Their structures were identified by analysis of MS, ^{1}H-NMR, ^{13}C-NMR data (Zhang et al., 1994; Yang and Liu, 1998), purity > 98% (by HPLC) (Fig. 1).

2.4. Drug treatment

All the test materials were dissolved in deionized water. Vehicle solvent served as a negative control while fluoxetine served as a positive control. Fluoxetine, was administered via gastric intubation at a dose of 20 mg/kg. Liquiritin and isoliquiritin were also administered via gastric intubation at doses of 10, 20, 40 mg/kg, which was designed after the pretest. The volume of administration for vehicle and drug solutions was 0.1 ml /10 g of mouse. The vehicle or test drugs were administered 30 min before the test session.

2.5. Forced Swimming Test (FST)

Experiments were carried out on mice according to the method of Porsolt (Porsolt et al., 1977). Briefly, mice were forced to swim individually for 6 min, in a glass cylinder (20 cm × 14 cm)