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Potential antidepressant properties of Radix Polygalae (Yuan Zhi)

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ABSTRACT

Radix Polygalae (“Yuan Zhi”, the roots of *Polygala tenuifolia* Willd., YZ) is an important herb used in traditional Chinese medicine to mediate depression. The present study was designed to verify the antidepressant effects of the standardized YZ ethanol extract (YZE) and its four fractions YZ-30, YZ-50, YZ-70 and YZ-90 on the tail suspension (TST) and forced swimming test (FST). Furthermore, the standardization of the fractions obtained from the separation procedures was carried out by high-performance liquid chromatography (HPLC)-fingerprint. The YZ-50 fraction (Oligosaccharide esters – enriched, oral (200 mg/kg) showed a significant anti-immobility like effects. The data of YZ-50 on the corticosterone-induced injure of SH-SY5Y human neuroblastoma cell indicated that YZ-50 may have biological effects on neuroprotection. Proliferation of cell lines was assessed by dimethylthiazoldiphenyltetrazoliumbromide (MTT) and 5-bromo-2′-deoxyuridine (BrdU) incorporation assays. It was found that YZ-50 and its two bioactive compounds, 3,6′-di-o-sinapoyl-sucrose (DISS) and tenuifoliside A(TEA) showed protection activities in SY5Y cells from the lesion. By using bioassay-screening methods, our results indicate that the presence of oligosaccharide esters such as DISS and TEA in this herb may be responsible for the cytoprotective activity effects.

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Introduction

Depression is one of the major mental disorders associated with symptoms such as regular negative moods, decreased physical activities, feelings of helplessness, and sluggish thought and cognitive function (Rudy, 2004). Selective and reversible monoamine oxidase inhibitor (e.g., moclobemide), selective serotonin reuptake inhibitors (e.g., citalopram) or serotonin and noradrenaline reuptake inhibitors (e.g., venlafaxine) are the typical antidepressants usually used. However, most of the drugs are synthetic nitrogen-bearing compounds which have inevitably some serious adverse-effects such as causing cardiovascular disease, narrowing the scope of remedial spectrum and shortening of $t_{1/2}$. Hence, there is an urgent need for the research and development of more effective antidepressants without any (or with minor) adverse-effect (Chen et al., 2004).

According to the theory of the traditional Chinese medicine (TCM), the clinical condition of depression could be mainly classified into sentiment and mental inquietude (asthenia syndrome). The symptom of asthenia syndrome can be described as mental stress, loss of memory, sorrowful, emotion unrest, or discomfort. Based on this view point, many Chinese medicinal plants have been successfully used to treat sentimental diseases, which is similar to depression in the Western medicine. Moreover, active principles from them were extracted and isolated (Yu et al., 2002; Xia et al., 2007; Rocha et al., 2007).

Radix Polygalae is the dried root of *Polygala tenuifolia* Willd, which has been used as a traditional medicine for expectorant, tonic, tranquillizer and antipsychotic agent etc. (Chung et al., 1992; Huang, 1993). Radix Polygalae is classified as a high grade herb in the “Shennong’s Herbal”, and was widely used in the vast majority of Chinese medicine prescription to treat mental diseases, for example, amnesia, neurasthenia, palpitation, and insomnia (Chang and But, 1986). Chemically, the dried root of this plant contains polygalitol, tenuigenin, polygalasaponin, oligosaccharides (Ikeya et al., 2004) and xanthone derivatives.

In this study, the mice despair models were used for screening of the antidepressant fractions and substances obtained from *Polygalae Radix* (YZ), accompanied with the phytochemical analysis by HPLC. The present study was designed to verify the promote effect of the YZ-50 on hippocampal neurogenesis. Considering major antidepressant drug have the ability for neuroprotection pain, we have used corticosterone-induced injure cell model to screen cytoprotective and anti-depressant effects of fractions and substances which obtained from Radix Polygalae.

Materials and methods

Plant material

Radix Polygalae were purchased from traditional Chinese medicinal (TCM) pharmacy, Chinese People’s Liberation Army (PLA) General Hospital (Beijing, China) and authenticated by

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Professor LIU Ping, TCM pharmacy, PLA General Hospital. A voucher specimen (NU-80617) has been deposited in the Herbarium of PLA General Hospital. The quality of these crude drugs was controlled and processed according to the Chinese Pharmacopoeia (Zheng, 2005).

Preparation of the extracts

The air-dried Radix Polygalae (965.27 g) was powered and extracted with 60 % EtOH (10 l) under reflux. After removal of EtOH under reduced pressure, the aqueous brownish syrup (1 l) was lyophilized into powder to get YZE. Then part of YZE (201.5 g) was suspended by water and subjected to a macro porous resin column (1300 Version), eluting with H_2O, 30% EtOH, 50% EtOH, 70% EtOH and 95% EtOH successively, to yield 5 fractions (YZ-H_2O, YZ-30, YZ-50, YZ-70, YZ-95 (98.6 g, 16.8 g, 48.4 g, 26.0 g, 4 g)). YZ-50 was repeatedly chromatographed on a silica column eluting with $CHCl_3$-MeOH-H_2O (9: 1: 0.1; 8: 2: 0.2; 6: 4: 0.4) to afford four compounds (sibiricose A6(E-10-1), 3′,6-di-o-sinapoyl-sucrose (DISS), tenuifoliside A (TEA) and tenuifoliose H (tb-C19)) as described previously by Tu (Tu et al 2008). The structures were identified by a combination of spectral methods (UV, IR, MS and NMR), see Fig. 1.

Phytochemical analysis: HPLC fingerprint of the fractions

Chemical fingerprinting of the extracts and fractions were analyzed. Samples were dissolved with solvent (methanol: H_2O=1:1) and filtered through a membrane filter (0.45 μm, Alltech, Germany).

The HPLC system (Hitachi-2000, Japan) consisted of a quaternary pump, an autosampler, a degasser, an automatic thermostatic column compartment and a computer with a Chemstation software (Analyst 1.4, Applied Biosystems Inc, USA). The analytical column used was a Kromasil SB-C18 (3.5 μm, 250 mm × 4.6 mm, Agilent, USA) and an Agilent Zorbax Extand -C18 guard column (5 μm, 12.5 × 4.6 mm) with column temperature set at 30 °C.

The mobile phase consisted of A, H_2O (0.05% phosphoric acid), B, CH_3CN; gradient: 11% B linear in 7 min; 11-15% B linear in 1 min; 15% B linear in 16 min; 15-20% B linear in 23 min; 20-25% B linear in 5 min; 25% B linear in 12 min; 25-29% B linear in 8 min; 29-35% B linear in 8 min; 35-40% B linear in 1 min; 40-43% B linear in 3 min; isocratic on 43-11% B for 10 min. The mobile phase was degassed automatically using the electronic degasser system. The flow rate was 1.0 ml/min. The detector wavelength was set at 318 nm.

Animals and Drugs Administration

The experiments were carried out on ICR mice (18–26 g) for the tests. All the animals used in this study were cared for and treated humanely according to the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised in 1985) and the 'Guide for the Care and Use of Laboratory Animals of Shanghai Institute of Material Medica. In this experiment, the mice were divided into six groups in six cages, including control group, desipramine group, YZ-30 group, YZ-50 group, YZ-70 group and YZ-95 group. 10～12 mice were kept in each cage under standard laboratory conditions (at a temperature of 20 ± 1° C and a 12 h light/dark cycle) with free access to food and water. All experiments were performed from 9:00 am to 4:00 pm. Desipramine (Sigma, USA), administered at 20 mg/kg served as positive control in tests. The solutions of the tested samples were administered to the mice via gastric intubation at different dosages once a day at 9:00 am. The control group received the same volume of the dosing vehicle.

Evaluation of antidepressant activity

Tail suspension test

The tail suspension test was based on Steru's method (Steru et al., 1985). Briefly, the mouse was individually suspended by the tail with a clamp (2 cm from the end) for 6 min in a box (25 × 25 × 30 cm) with the head 5 cm above the bottom. 8-12 animals were used for the preliminary activity screening test, carried out in a darkened room with minimal background noise. Half an hour after administrating the test sample, the duration of immobility (counting by seconds) were evaluated at 2 different doses and recorded during the final 4 min interval of the test.

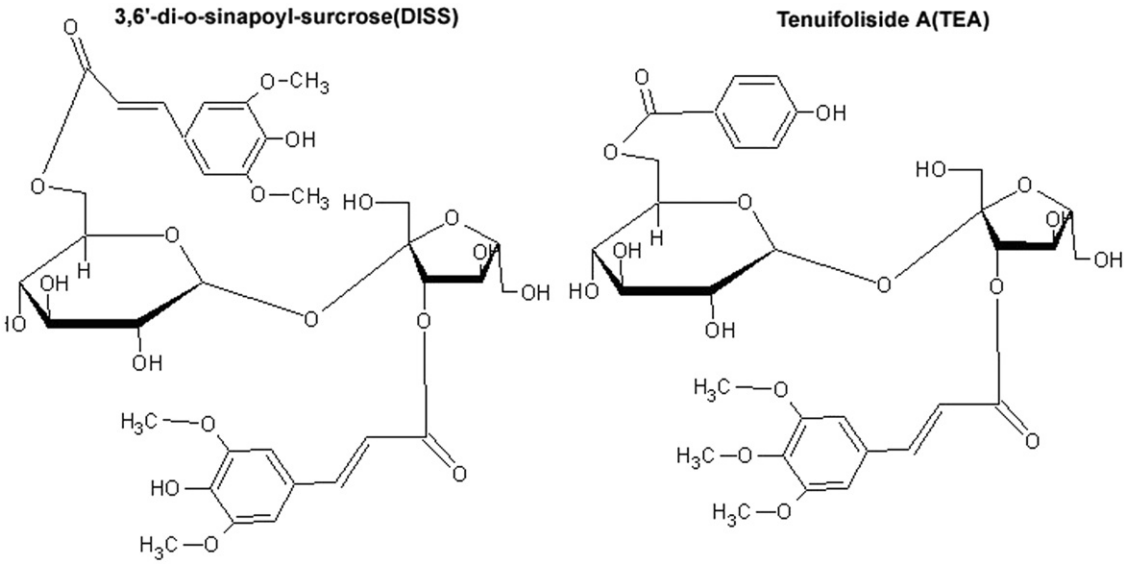

Fig. 1. Chemical structures of compounds.

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