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Fructus Aurantii induced antidepressant effect via its monoaminergic mechanism and prokinetic action in rat

Ying-jin Zhang[a,b], Wei Huang[a,c], Xi Huang[a,c,d,*], Yang Wang[a,c], Zhe Wang[b], Cheng Wang[e], Bing-wu Zhong[b], Chen-xia Sheng[b], Bing Wang[b], Si-fang Zhang[b], Nan-xiang Su[b], Zhao-qian Liu[d], Hong-hao Zhou[d], Ping Ren[a,c]

[a] *Laboratory of Ethnopharmacology, Institute of Integrated Traditional Medicine and Western Medicine, Xiangya Hospital, Central South University, Changsha 410008, China*
[b] *Department of traditional chinese medicine, The Second Xiangya Hospital, Central South University, Changsha 410011, China*
[c] *National Key Clinical Specialist Vocational School of TCM Encephalopathy, Xiangya Hospital, TCM Pharmacogenetics Laboratory, Central South University, Changsha 410008, China*
[d] *Instutite of Clinical Pharmacolgy, Central South University, Changsha 410008, China*
[e] *Institute of Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

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ABSTRACT

Depression could hardly get a satisfactory effect from the currently available antidepressants. To get a more effective treatment, antidepressant effect and monoaminergic mechanism of Fructus Aurantii (FRA) in the rat forced swimming test (FST) and open field test (OFT), and its prokinetics were examined. FST and OFT were respectively used to evaluate the antidepressant effect and locomotor activity of FRA. We observed the effects of monoamine receptor antagonists on FRA-induced antidepressant effect in rat. The effects of FRA on intestinal transit, gastric emptying and *in vitro* jejunum contractile activity were assessed. FRA decreased significantly the immobility time (32.6 ± 8.5, 30.3 ± 5.2 vs 56.4 ± 9.4, all $p < 0.01$) in FST, dose-dependent increased the locomotor activity (102 ± 17.5, 120 ± 18.5 vs 89 ± 9.8, $p < 0.05$ or 0.01), significantly accelerated gastric emptying (GE: 48.1 ± 6.3, 39.5 ± 5.7 vs 19.5 ± 3.8, $p < 0.01$) and intestinal transit (IT: 67.3 ± 9.1, 64.2 ± 6.3 vs 49.1 ± 8.2, $p < 0.01$) of the semi-liquid meal, compared with vehicle. And FRA (1 μM, 10 μM) significantly increased the mean amplitude (0.24 ± 0.021 and 0.281 ± 0.015) of contraction in jejunum of rat compared with vehicle (0.149 ± 0.011) *in vitro*. FRA (10 μM) could induce a largest amplitude (0.281 ± 0.015) of contraction in jejunum. The anti-immobility effect of FRA in FST was prevented by pre-treatment of rat with p-chlorophenylalanine methyl ester, WAY100635, ketanserin, haloperidol, SCH233390, sulpiride, yohimbine, but not prazosin. FRA could simultaneously induce prokinetics and antidepressant effect, deserves further to investigate.

Introduction

Depression, a common mental disorder, will become the second most common cause of disability worldwide by 2020 (Peveler et al. 2002). A large percentage of depressive patients could not get a satisfactory effect from currently available antidepressants with low rates of response and remission. And these drugs are often attended by many side effects (Mai et al. 1993; Kim et al. 2005). For example, selective serotonin reuptake inhibitors (SSRIs), as most commonly prescribed antidepressants since mid-1980s, were forced to discontinue because of digestive-tract side effects such as nausea and vomiting (Poyurovsky et al. 1999; Wagstaff et al. 2002; Pigset 1999). These resulted in about 40% of antidepressant invalid, thus a great gap between mechanism and treatment of depression (Kiss 2008) formed.

Noteworthy in this regard is that psychological stimulation elicits delayed gastric emptying, circulating gastrointestinal hormone abnormalities (Zhang et al. 2008; Guo et al. 2001; Liu et al. 2004). Also, depression is often associated with gastrointestinal dysfunction such as abdominal discomfort, nausea, heartburn, bloating, diarrhea and constipation (Zou et al. 2004). Therefore, it is very necessary to develop an antidepressant with prokinetic activity.

Fructus Aurantii (FRA) is the dried immature fruit of Citrus aurantium L. (Rutaceae) and its cultivated variety. Previous study showed that FRA possesses prokinetic, anti-dyspepsia, antioxidative and anti-inflammatory effects (Huang et al. 2011). FRA as a representative for dispersing stagnated liver qi stagnation in traditional Chinese medicine, has been popularly used for relieving depression-like symptoms such as pain, insomnia, sad

Abbreviations: FRA, Fructus Aurantii; FST, forced swimming test; OFT, open field test; FD, functional dyspepsia; IBS, irritable bowel syndrome.

* Corresponding author at: Institute of Integrated Traditional Medicine and Western Medicine, Xiangya Hospital, Central South University, Changsha 410008, China.
E-mail address: tcmhuangx59@163.com (X. Huang).

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and depressed for more than 2000 years. According to these, we proposed a hypothesis "Chinese medicine or herbs for regulating qi-flowing or relieving qi stagnation often has antidepressant and gastroprokinetic effects". Therefore, we evaluated FRA's antidepression by the OFT and FST, and gastroprokinetic by the measurements of the gastric emptying and gastrointestinal transit and *in vitro* jejunum contractile activity in rats. To verify the effect of FRA's antidepression over serotonergic, noradrenergic and dopaminergic system, the anti-immobility effect of FRA in FST was prevented by pre-treatment of rat with their receptor antagonists.

Materials and methods

Animals

Male Sprague Dawley rats (Changsha, China), weighing 160–200 g, were housed under standardized environmental conditions (20–22 °C, 12 h light/dark cycle with light on at 6.30 a.m., 50 ± 10% relative humidity), with free access to food and water. All experimental procedures were conducted in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (1988) and approved by the Animal Experimental Center for Central South University.

Preparation of FRA and quantitative analysis of main components

Preparation of FRA

Fructus Aurantii (FRA) granula was purchased from China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. It was extracted by 70% ethanol. In short, the materials (100 g) are extracted twice with 5-fold volumes of 70% ethanol (500 ml) at 70 °C for 1 h each time. Then the supernatant, after centrifuging at 2000 g, is pooled and lyophilized to make a powder with 22.8% yields for FRA.

Sample preparation

For UPLC analysis the lyophilized powder was dissolved in distilled water, the amount dissolved being equivalent to 15 mg FRA ml^{-1} water. All solutions were filtered through a 0.45 μm pore size filter before analysis. The injection volume was 3 μl, FRA's concentration in each decoction before injection is 0.31 mg/ml.

Apparatus

A Waters Acquity UPLC BEH 2.1 mm × 100 mm, 1.7 μm C18 column system (Waters Corporation, USA) was used to analyze. The system consisted of a quaternary pump solvent management system, an on-line degasser and an autosampler. The raw data were detected, acquired and processed with Empower Software.

Chromatographic conditions

The mobile phase was acetonitrile –0.5% aqueous acetic acid with gradient elution (0 min, 13:100; 10 min, 18:100; 20 min, 25:100; 25 min, 60:100). The components were quantified based on peak areas at the maximum wavelength in their UV spectrum.

Preparation of standard solutions

A standard stock solution of each of the 5 components and their standard solutions were directly prepared and diluted with methanol to establish calibration curves. All were prepared in dark brown calibrated flasks and stored at 4 °C. The linearity of the responses was determined for seven concentrations. The standard curves from the peak area of each compound were prepared by Empower software. Their contents in the test samples were calculated by the regression parameters obtained from the standard curves respectively.

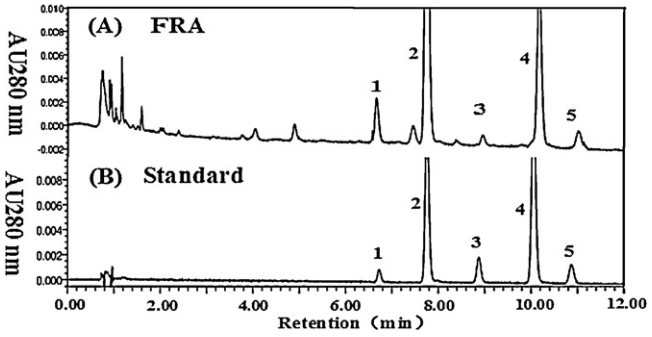

Fig. 1. Typical chromatograms of 70% ethanol extracts of Fructus Aurantii (FRA) granula (A) and the standard mixture (B) at 280 nm. (1) Narirutin, (2) hesperidin, (3) naringin, (4) neohesperidin, and (5) meranzin hydrate.

Quantitative analysis results

The contents of main components in the 70% ethanol extracts of FRA are determined by Ultra Performance Liquid Chromatography (UPLC) as 2.8% of Naringin, 6.3% of Hesperidin, 8.6% of Narirutin, 6.7% of Neohesperidin, 4.5% of Merazin hydrate (Fig. 1).

Drug administration

The following drugs were used: prazosin Hydrochloride, yohimbine hydrochloride, ketanserin, p-chlorophenylalanine (PCPA), haloperidol, sulpiride, WAY100635, SCH23390, Fluoxetine, Mosapride, Evans blue, methylcellulose (all from Sigma Chemical Co., St. Louis, U.S.A.). Sulpiride and prazosin were diluted in saline with 5% dimethyl-sulfoxide (DMSO). Ketanserin, prazosin, yohimbine, propranolol and sulpiride were administered by intraperitoneal (i.p.) route. SCH23390 and WAY100635 were administered by subcutaneous (s.c.) route.

Fructus Aurantii (FRA) granula was purchased from China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. All drugs were dissolved in distilled water and administered in a constant volume of 10 ml/kg body weight (Kim et al. 2005; Aynara et al. 2010). FRA (3, 9 or 18 mg/kg, p.o.), Fluoxetine (20 mg/kg, p.o.) and their Vehicle (1.5 ml saline, p.o.) were administered half an hour before the behavior test. The cut crude drug of FRA of 3, 9 or 18 mg is equivalent to 0.68 g, 2 g and 4 g of FRA granula respectively. Appropriate vehicle-treated groups were also assessed simultaneously.

In the experiments designed to study the time-course effect of FRA (18 g/kg), the immobility time in FST was assessed in an independent group of rat, 0.5 h, 1 h, 2 h and 4 h after the administration of FRA (Posser et al. 2009). To investigate the influence of the serotonergic system in the antidepressant effect of FRA, rats were pretreated with PCPA (100 mg/kg, i.p., an inhibitor of serotonin synthesis) or vehicle once a day for 3 days. Then, 24 h after the last PCPA injection, FRA was given orally to rats. 30 min later, rats were tested in FST for 5 min. And the involvement of the 5-HT receptor subtypes, the noradrenergic and the dopaminergic systems in the effect of FRA in FST were also studied. Rats were pretreated with WAY100635 (0.1 mg/kg, s.c., a selective 5-HT1A receptor antagonist), ketanserin (5 mg/kg, i.p., a preferential 5-HT2A receptors antagonist), prazosin (1 mg/kg, i.p., an α1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α2-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, s.c., a dopamine D1 receptor ant agonist) or sulpiride (50 mg/kg, i.p., a dopamine D2 receptor antagonist), or vehicle and after 30 min, received FRA (18 g/kg, p.o.) or vehicle injection before being tested 30 min later.