Antidepressant effect of Yueju-Wan ethanol extract and its fractions in mice models of despair

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Abstract

\textbf{Aim of the study:} Yueju-Wan (YJ), a traditional Chinese medicinal formula, is commonly used for the treatment of depression-related syndromes in China. This study was conducted to evaluate the antidepressant activity of YJ ethanol extract (YJ-E) and its four different fractions, the petroleum ether fraction (YJ-EA), ethyl acetate fraction (YJ-EB), n-butanol fraction (YJ-EC) and final aqueous fraction (YJ-ED).

\textbf{Materials and methods:} Two experimental despair animal models: the mice tail suspension test (TST) and the mice forced swimming test (FST) were used to evaluate the antidepressant activity of YJ-E and its fractions. These extracts or fractions were administered orally for 7 days, while the parallel positive control was given at the same time using fluoxetine hydrochloride (FLU) in TST and imipramine hydrochloride (IMI) in FST respectively.

\textbf{Results:} YJ-E high dose (YJ-E2), YJ-EA, YJ-EC and the positive control groups could decrease the duration of immobility in the TST and FST and have no significant changes in locomotor activity. YJ-E low dose (YJ-E1), YJ-EB, YJ-ED and the vehicle solvent (VEH) control group have no obvious effect on these same tests.

\textbf{Conclusions:} In these despair animal models, YJ ethanol extract, the petroleum ether fraction and n-butanol fraction show potent antidepressant effects. The petroleum ether fraction and n-butanol fraction appear to be the active fractions of YJ-E.

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Keywords: Yueju-Wan; Antidepressant activity; Tail suspension test; Forced swimming test; \textit{Cyperus rotundus} L.; \textit{Ligusticum chuanxiong} Hort.; \textit{Gardenia jasminoides} Ellis.; \textit{Atractylodes lancea} (Thunb.) DC.; Massa Fermentata

1. Introduction

Depression is one of the major mental disorders associated with symptoms such as regular negative moods, decreased physical activity, feelings of helplessness, and sluggish thought and cognitive function. It was estimated by National Institute of Mental Health (NIMH) that more than 12 million women and 6 million men in the USA are affected by depressive illnesses in any given year (Rudy, 2004). Yueju-Wan (YJ), an oriental herbal formula popular within folk medicine for relieving a wide variety of symptoms caused by qi stagnation, was originally prescribed by the famous Chinese doctor Zhu Dan-xi, and is nowadays still used to efficiently treat depression, anxiety and irritability.

YJ is comprised of five indigenous medicines: Xiang Fu, Chuan Xiong, Zhi Zi, Cang Zhu and Shen Qu. Xiang Fu is the rhizoma of \textit{Cyperus rotundus} L. (Cyperaceae) and its active components reported mostly are volatile oil such as \(\alpha\)-cyperone, \(\beta\)-cyperone, \(\alpha\)-rotunol, \(\beta\)-rotunol, cyperene, patchoulenone, etc. (Hiking et al., 1971; Thebtaranonth et al., 1995; Mesmin and Wilfried, 2001). Chuan Xiong is the rhizoma of \textit{Ligusticum...
chuanxiong Hort. (Umbelliferae) and its active components reported include ligustilide, butyldene phthalide, cniidium lactone A, teramethylpyrazine and ferulic acid (Liang et al., 2005; Chan et al., 2006; Zhang et al., 2007). Zhi Zi is the fruit of Gardenia jasminoides Ellis. (Rubiaceae) and its active components reported include geniposide, gerdenoside, crocin-1, crocetin, and gardenin (Oshima et al., 1988; Choi et al., 2001). Cang Zhu is the rhizoma of Atractylodes lancea (Thunb.) DC. (Compositae) and its active components reported mainly are volatile oil including atracytlin, hinesol, β-eudesmol, etc. (Nakai et al., 2003; Suto et al., 1998; Tsumeki et al., 2005). Shen Qu (Massa Fermentata) is a mixture of six kinds of substances produced by fermentation including Prunus armeniaca L. (Rosaceae), Phaseolus angularis Wight. (Leguminosae), Artemisia annua L. (Compositae), Xanthium sibiricum Patrin (Compositae) and Polygonum hydropiper Linn. (Polygonaceae) and the active components of Shen Qu are also volatile oil.

Based on the theory of traditional Chinese medicine (TCM), the effect of YJ on depression can be attributed to its regulating function of qi and its eradication of six depression syndromes: qi depression, blood depression, fire depression, damp depression, phlegm depression and food depression. Therefore, we postulated that YJ might have antidepressant activity and sought evidence of this experimentally.

In this study, the antidepressant effect of YJ ethanol extract (YJ-E) and its four different fractions, the petroleum ether fraction (YJ-EA), ethyl acetate fraction (YJ-EB), n-butanol fraction (YJ-EC) and final aqueous fraction (YJ-ED), on two despair animal models: the mice tail suspension test (TST) and the mice forced swimming test (FST) were evaluated, accompanied with the phytochemical analysis by HPLC fingerprint to identify the active fractions.

2. Materials and methods

2.1. Plant material

The medicinal plants used to prepare YJ-E are Cyperus rotundus L., Ligusticum chuanxiong Hort., Gardenia jasminoides Ellis., Atractylodes lancea (Thunb.) DC. and Massa Fermentata. All the medicinal plants were purchased from Shanghai Yanghetang Medicinal Material Company (Shanghai, China) and authenticated by Dr. Wu Lihong, Shanghai R&D Center for Standardization of Traditional Chinese Medicine, based on their micro- and macroscopic characteristics. The voucher specimen of each plant was prepared and deposited in the herbarium of Shanghai R&D Center for Standardization of Traditional Chinese Medicine and the numbers of the voucher specimen are no. xf-060320, no. cx-051129, no. zz-050916, no. cz-050913 and no. sq-050817 respectively. The quality of these crude drugs is controlled and processed according to the Chinese Pharmacopoeia (2005).

2.2. Preparation of the extracts

According to the preparation of YJ formula, five plant materials weighted about 3000 g were powdered into 12 mesh. YJ-E was obtained (458 g) by maceration of the plant material with 1.5 L ethanol 90% for 2 days at room temperature, and this procedure was repeated twice. The extract was filtered and dried under reduced pressure at a temperature below 60°C. Then part of YJ-E (229.5 g) suspended in water was partitioned between petroleum ether and water and the petroleum ether fraction (YJ-EA) was obtained (80.5 g). Afterwards with the aqeous fraction, a distribution between ethyl acetate/water (EtOAC/H2O) was made and the ethyl acetate fraction (YJ-EB) obtained (13.5 g). Finally, the aqeous was subjected to a separation between n-butanol/water (n-BuOH/H2O). The n-butanol fraction (YJ-EC) and the final aqueous fraction (YJ-ED) were obtained for 25.9 and 109.0 g, respectively. The concentrated fractions of YJ-E (15.3%, w/w), YJ-EA (5.37%, w/w), YJ-EB (0.9%, w/w) and YJ-ED (7.27%, w/w) were all stored at −20°C and the YJ-EC (1.73%, w/w) powder was stored in a desicator until use.

2.3. Phytochemical analysis

YJ-E and its two different fractions were analyzed using HPLC fingerprinting analysis. All the samples were dissolved in methanol and then filtrated through a 0.45-µm membrane filter. HPLC analysis was performed on a Shimadzu LC-20AB Series System (Shimadzu corporation, Kyoto, Japan). Chromatographic separation was carried out on a ZORBAX Extend-C18 column (4.6 mm × 250 mm, 5 μm) heated to 25°C with a injection volume of 10 μL using a gradient elution of solvent (A) H2O and solvent (B) acetonitrile at a flow rate of 1 mL/min as follows: 9–20% B (15 min), 20–45% B (10 min), 45–55% B (30 min), 55–65% B (25 min) and 65–100% B (5 min) for YJ-EA, while 46–55% B (30 min), 55–57% B (1 min), 57–62% B (24 min), 62–100% B (1 min) and 100% B (5 min) for YJ-EB, and 46–55% B (30 min), 55–57% B (1 min), 57–62% B (24 min), 62–100% B (1 min) and 100% B (5 min) for YJ-EC. Peaks were detected at 254 nm of UV detection.

2.4. Animals

Male ICR mice (18–22 g) were used in the TST and FST. 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 Shangh hai Institute of Materia Medica’. The animals were housed for 1 week under controlled conditions before the experiments took place. These conditions were as follows: light (12 h light/dark cycle, lights on at 7:00 am), temperature (25 ± 1°C), free access to food and water. The animals were randomly assigned to 16 equal groups for the experiments: two vehicle solvent control groups (VEH), a group for fluoxetine hydrochloride (FLU), a group for imipramine hydrochloride (IMI), two groups for YJ-E low dose (YJ-E1), two groups for YJ-E high dose (YJ-E2), two groups for YJ-EA, two groups for YJ-EB, two groups for YJ-EC and two groups for YJ-ED. Each experimental group consisted of sixteen mice. All the animals were purchased from Shanghai Institute of Materia Medica, Shanghai Institutes for Biologi-
Fig. 1. Fingerprints of YJ-E, YJ-EA and YJ-EC.
2.5. Drug administration

YJ-E, YJ-EA and YJ-EB were dispersed in 1% CMC-Na added Tween 80 (0.5%, w/v). The other tested substances were dissolved in 1% CMC-Na. Vehicle solvent (0.5%, w/v Tween 80 dissolved in 1% CMC-Na) served as negative control. FLU (administered at 20 mg/kg) served as positive control in TST and IMI (administered at 30 mg/kg) in FST, respectively (provided by Department of Medicinal Chemistry, SIMM, purity >99.8%).

2.6. Antidepressant activity evaluation

2.6.1. Measuring locomotor activity

In order to detect any link between immobility in the TST and FST and changes in motor activity, the activity level of animals treated with the extracts was analyzed in the mice locomotor activity recorder apparatus (model YLS-1A, Shandong Academy of Medical Sciences, China). The mice were placed in the apparatus for 8 min. During this period, measurements were taken only in the final 5 min. The behavioral tests on the mice were started 60 min after the final administration. The behavioral tests on the mice were started 60 min after the final administration.

2.6.2. Tail suspension test

The mouse was hung by the tail (clipped 2 cm from the end) for 6 min in a box of dimensions 50 cm × 25 cm × 50 cm, its head 15 cm above the bottom of the box. Data was recorded only in the final 4 min of the test (Steru et al., 1985). ‘Immobility’ was scored as a failure to make any struggling movements, attempts to catch the adhesive tape, or body torsions or jerks. During the test session, the immobility time was recorded using a video camera. Two observers who had no knowledge of the type of treatment each animal had received evaluated the tapes.

2.6.3. Forced swimming test

The mouse was dropped into a glass cylinder (20 cm in height and 10 cm in diameter) containing 10 cm-deep water at 25 ± 2°C, and left for 6 min. The duration of immobility during the final 4 min interval of the swimming test was measured (Porsolt et al., 1978). ‘Swimming’ was defined as the carrying out of ‘escaping behaviors’, i.e. diving, vigorous paddling with all four legs, circling the tank, and attempts to clamber up the walls. ‘Immobility’ was defined as floating and treading water just enough to keep the nose above water. The water was changed after each mouse was tested. During the test session, the immobility time was recorded using a video camera. Two observers who had no knowledge of the type of treatment each animal had received evaluated the tapes.

2.6.4. Statistical analysis

Data were reported as the means ± S.E.M. Overall differences according to the treatment were confirmed using the one-way analysis of variance (ANOVA). Analysis of variance and least significant difference tests (SPSS for Windows, Version rel. 11.5, SPSS Inc., Chicago, IL) were conducted to identify differences among means. Statistical significance was declared at p < 0.05.

3. Results

By referring to standards, the chromatographic fingerprinting analysis showed that the main components of YJ-E are geniposide, atratylodinol, liguistilide, α-cyperone, (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyldiacetate and aractylin, while the main components of YJ-EA are atratylodinol, liguistilide, α-cyperone, (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyldiacetate and aractylin, and the main components of YJ-EC are geniposide and crocin-1 (Fig. 1).

Fig. 2 shows the effects of drug administration on locomotor activity in the mice. Locomotion did not differ statistically across YJ-E1, YJ-E2, YJ-EA, YJ-EB, YJ-EC, YJ-ED and the control groups. There were no significant changes in locomotor activity.

The behavioral effects produced in the TST test owing to drug administration are presented in Fig. 3. A significant reduction in immobility time was observed in the mice treated with FLU, YJ-E2, YJ-EA and YJ-EC versus VEH. The other groups showed no significant reduction.

The behavioral effects produced in the FST test owing to drug administration are presented in Fig. 4. A significant reduction in immobility time was observed in the mice treated with IMI, YJ-
It is difficult to mimic depression in the laboratory, because depression-related syndromes are associated with multifaceted symptoms which manifest themselves at the psychological, behavioral and physiological levels. The TST and FST were the most widely accepted models for assessing antidepressant-like activity in mice. The immobility taken on in these two tests is understood to reflect a state of ‘behavioral despair and variants’ or ‘failure to adapt to stress’ (Wilner, 1997; Borsini and Meli, 1998). These two tests are based on the fact that animals will develop an immobile posture when subjected to the short-term, inescapable stress of being suspended by their tail or being dropped into water. These two models are widespread in the laboratory largely due to their ease-of-use, consistency across laboratories, and their ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the TST and FST, while neuroleptics and anxiolytics produce different effects. Antidepressants can also be distinguished from stimulants because stimulants cause marked motor simulation, in contrast to antidepressants, which do not (Borsini and Meli, 1998). However, inconsistent scoring techniques and poor reproducibility may result from their reliance on subjective ratings by observers to score behavioral changes. In this study, we used two fixed observers who had no knowledge of the treatment of each group to evaluate the video tapes, consolidated the standardization for scoring and enlarged the amount of animals (16 mice each group) to minimize the deviation and improve the reliability of the result.

The data presented has demonstrated that YJ-E high dose, YJ-EA and YJ-EC can significantly reduce the immobility time in the TST and FST, indicating significant antidepressant effects in the two animal model tests used. In addition, the antidepressant effects of YJ-E, YJ-EA and YJ-EC cannot be attributed to an increase in motor activity because they did not induce hyperlocomotion in the mice. Likewise, the positive control did not alter locomotion. In the TST test, the effect of YJ-E was superior to that of the two active fractions YJ-EA and YJ-EC. YJ-E, YJ-EA and YJ-EC also showed a similar relationship in the FST test. These results suggest that the combination of these herbal drugs is necessary to give the antidepressant effect of YJ-E, and the petroleum ether fraction and n-butanol fraction contribute most to the antidepressant effect of YJ-E.

Using HPLC analysis, we found that the main components of YJ-EA are atratylodinol, atractylin, (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diy diacetate (the three former components from Cang Zhu), liguistilide (from Chuan Xiong) and α-cyperone (from Xiang Fu). The main components of YJ-EC are geniposide and crocin-1, both of the active components in Zhi Zi. It has been reported that liguistilide could directly suppress the activity of CRF systems, or indirectly affect the GABA_A receptor systems (both of the two effects may be related to the mechanism of depression), resulting in a reversal of social isolation stress- and FG7142 (a GABA_A/benzodiazepine receptor inverse agonist) -induced decrease in pentobarbital sleep (Matsumoto et al., 1998). The extract of Zhi Zi and geniposide can increase the social interaction time of mice through an anxiolytic effect (Torrizuka et al., 2005). In addition, crocin-1 can reduce immobility time in the FST, indicating an antidepressant-
like effect (Hossein et al., 2004). All of the above information suggests that the antidepressant activity of YJ-E maybe related to some compounds from Xiang Fu, Chuan Xiong, Cang Zhu and Zhi Zi, such as ligustilide, geniposide and crocin-1.

In summary, our results suggest that Yueju-Wan and its petroleum ether fraction and n-butanol fraction exert antidepressant effects in experimental animal models. The petroleum ether fraction and n-butanol fraction appear to be the fractions that contain the active constituents of Yueju-Wan. Further research elucidating the action mechanism of these effects using purified ingredients will give an insight into the usefulness of this herbal remedy in the treatment of depression.

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References


