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Antidepressant-like effect of ethanol extract from Zuojin Pill, containing two herbal drugs of Rhizoma Coptidis and Fructus Evodiae, is explained by modulating the monoaminergic neurotransmitter system in mice

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ABSTRACT

Ethnopharmacological relevance: Zuojin Pill (ZJP), a traditional Chinese medicinal decoction, contains two herbal drugs: *Coptis chinensis* Franch. and *Evodia rutaecarpa* (Juss.) Benth. in the ratio of 6:1 (w/w). Previous pharmacological studies have shown that two herbs in ZJP have the antagonistic effects on catecholamine secretion in bovine adrenal medullary cells. Furthermore, the alkaloids from the two herbs in ZJP may provide a protective effect for depression in individuals with a low expressing 5-HTT allele by increasing receptor concentration in serotonergic neurons. However, antidepressant effect has not been reported before and has not been fully clarified.

Aim of the study: The present study aimed to investigate the antidepressant potential of ethanol extract from ZJP and its monoaminergic mechanism in mice.

Materials and methods: Seven alkaloids were determined from the ethanol extract of ZJP using High Performance Liquid Chromatography (HPLC) with the gradient mobile phase. The ethanol extract from ZJP was used to evaluate the antidepressant potential in mice. Mouse models of depression including the tail suspension test (TST) and the forced swim test (FST) were used to evaluate the effects of the ethanol extract from ZJP. A possible mechanism was explored in the tests of antagonism of reserpine-induced ptosis and hypothermia, and 5-HTP induced head twitch response in mice. The contents of monoamine neurotransmitters including norepinephrine (NE), serotonin (5-hydroxytryptamine or 5-HT) in hippocampus of mice and NE, 5-HT, dopamine (DA) in striatum of mice were determined by HPLC system with Electrochemical Detector (ECD).

Results: The results showed that intragastric administration of the ethanol extract from ZJP (5, 10, 20 mg/kg) or fluoxetine (7.5 mg/kg) significantly reduced the duration of immobility in TST and FST. However, the effect was not dose-dependent. Ethanol extract from ZJP (5, 10, 20 mg/kg) also increased the accumulative number of the 5-HTP-induced head twitch response in mice. The mice were treated with the ethanol extract from ZJP (5, 10, 20 mg/kg) or fluoxetine (7.5 mg/kg), which could antagonize reserpine-induced ptosis and hypothermia, moreover, both of them could elevate the contents of NE, 5-HT in hippocampus as well as NE, 5-HT, DA in striatum significantly.

Conclusion: These results indicate that the ethanol extract from ZJP produced antidepressant-like effect and the possible mechanism, at least in part, is via the central monoaminergic neurotransmitter system and 5-HT plays a major role.

1. Introduction

Depression is a chronic, recurring and potentially life-threatening disease which could affect the performance and learning, social interactions and development of normal peer relationships, and even lead to suicidal ideations later in life. According to the report of World Health Organization, it may become the second cause of illness-induced disability by the year 2020 (WHO, 2001).

It is well known that monoamine neurotransmitters such as 5-HT, NE and DA in the central nervous system play a key role in the pathophysiology of depression (Elhwuegi, 2004). The increase in extracellular 5-HT and NE levels in the brain are thought to be

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closely related to the antidepressant effect (Kapur and Mann, 1992; Papakostas, 2006). At present, several types of classical antidepressants are primary modulators of monoamine neurotransmitters. Although many antidepressant drugs could improve depressive symptoms, 30% of depressive patients failed to respond satisfactorily to commercially available antidepressants as the undesirable side-effects of antidepressant drugs, such as bupropion, mirtazapine, venlafaxine. (Papakostas et al., 2008). Therefore, there is an urgent need for research and development of more effective antidepressant therapies without any or with less adverse effects. Nowadays, the use of traditional herbs such as traditional Chinese medicine, traditional Ayurvedic medicine and other folk medicines has provided us a prospective alternative in the treatment of depression (Singh et al., 2009; Yi et al., 2009).

Zoujin Pill (ZJP), a typical Traditional Chinese Medicine (TCM) formula, consists of two herbs: the rhizomes of *Coptis chinensis* Franch. (Ranunculaceae, officially recognized in the Chinese Pharmacopoeia as Rhizoma Coptidis) and the dried, unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae, officially recognized in the Chinese Pharmacopoeia as Fructus Evodiae) in the ratio of 6:1 (w/w). It was first recorded in Danxi's experiential therapy, a famous ancient medical manuscript, and is listed in the Chinese Pharmacopoeia as a prescription employed in patients suffering from gastric ulcer, gastroesophageal reflux disease, gastritis, and pyloric obstruction, among other disorders (The State Pharmacopoeia Commission of PR China, 2010).

Depression with psychotic and somatic symptoms has been observed in association with stress gastric ulcers. It seems that there is a marked overlap among the neuronal pathogenetic pathways involved in ulcer genesis and depression. The report showed that the antidepressants duloxetine, amitriptyline and mirtazapine were capable of exerting more potent and efficacious protective effects in various experimental gastric ulcer rat models (Ji et al., 2012). Clinical studies have shown that some peptic ulcer patients benefit from combination therapy with antidepressants, even in the absence of any type of depression (Suleyman et al., 2009). Tricyclic antidepressants are particularly useful in the treatment of endogenous depression. Some tricyclic antidepressants possess definite antimuscarinic actions, thus reducing gastric secretion (Sen et al., 2002). These laboratory data and clinical reports highlight the importance of studying the action of antidepressants, not only in depressive behavior, but also in other responses to stress (i.e., stress ulcer incidence).

Our previous pharmacological studies have shown that seven alkaloids, epiberberine, jatrorrhizine, coptisine, palmatine, berberine, evodiamine, rutaecarpine in the ethanol extract of ZJP, and the quantification of seven alkaloids in the ethanol extracts of ZJP was 32.65, 13.59, 110.64, 61.20, 153.0, 1.89, 1.47 mg/g, respectively, furthermore, the results showed that the alkaloids from the ethanol extract of ZJP have the anti-inflammatory (Wang et al., 2012) and two herbs in ZJP have the antagonistic effects on catecholamine in secretion in bovine adrenal medullary cells (Zhao et al., 2010). Furthermore, the evidence showed that the combination of berberine and evodiamine (the main bioactive ingredients in the herb of Rhizoma Coptidis and Fructus Evodiae respectively) might provide a protective effect for depression in individuals with a low expressing 5-HT transporter (5-HTT) allele by increasing receptor concentration in serotonergic neurons which could have more potential as an antidepressant or foodintake inhibitor than berberine or evodiamine alone (Hu et al., 2011). The 5-HTT regulates 5-HT transmission, which in turn modulates mood, emotion and appetite (Camarena et al., 2002). The 5-HTT is also well documented as the pharmacological target of selective 5-HT reuptake inhibitors, and its expression has been reported to be down-regulated by the antidepressants fluoxetine and sertraline (Johnson et al., 2009; Zhao et al., 2009).

However, the antidepressant potential and pharmacological mechanism of the ethanol extract from ZJP are not clear. The present study aimed to explore the antidepressant-like effects of the ethanol extract from ZJP (at doses of 5, 10, 20 mg/kg) using the tail suspension and forced swim tests. We investigated the probable mechanism of antidepressant-like activity by analyzing monoamine neurotransmitters, such as 5-HT, NE and DA levels in specific brain regions of striatum and hippocampus following the ethanol extracts from ZJP treatment and performing the reserpine test in depressive mice.

2. Materials and methods

2.1. Reagents

The rhizomes of *Coptis chinensis* Franch. and the dried, unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. were purchased from Baokang Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China) and authenticated by Prof. Tian-Xiang Li in Tianjin University of Traditional Chinese Medicine according to the Chinese Pharmacopoeia (2010 edition). The voucher specimen (No. 018723) was deposited at herbarium of Tianjin University of Traditional Chinese Medicine. The standards of berberine (98%), palmatine (98%), evodiamine (98%), rutaecarpine (99.9%) were purchased from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Coptisine (98%) and epiberberine (98%) were obtained from Chengdu Must-technology Co., Ltd. (Chengdu, China). Jatrorrhizine was purchased from Phytomarker Company (Tianjin, China). Methanol and acetonitrile were of HPLC grade and obtained from Tianjin Concord Technology Co., Ltd. (Tianjin, China). Triethylamine and phosphoric acid were of analytical grade obtained from Tianjin Chemical Reagent Company (Tianjin, China). Fluoxetine hydrochloride capsules were obtained from Eli Lilly and Company (Indianapolis, IN, USA). 5-HTP was purchased from Alfa Aesar (Ward Hill, MA, USA).

2.2. Preparation of ethanol extract from ZJP and quantitative analysis of various active compounds in the ethanol extract

Preparation of ethanol extract from ZJP and quantitative analysis of various active compounds in the ethanol extract have been published in our previous paper (Wang et al., 2012).

2.3. Animals

Male ICR mice (weighing 18–22 g), purchased from Beijing HuaFuKang Bio-technology Co. Ltd.(SCXK 2009-0004, Beijing, China) were housed under controlled light (12 h light/12 h dark, lights on at 07:00 a.m.). Ambient temperature and relative humidity were maintained at 24 ± 1 °C and 55 ± 5%, respectively. The animals were allowed to acclimate to the housing facilities for 7 days before the experiments began with access to water and food *ad libitum*. All the experiments were carried out in a quiet room between 9:00 a.m. and 3:00 p.m. Each animal was used only once. All the animals used in this study were in accordance with NIH Guide for the Care and Use of Laboratory Animals and protocol was approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (TCM-2009-037-E07).

2.4. Drug administration and treatment

The ethanol extract of ZJP was dissolved in 0.9% saline and sonicated for 45 min at room temperature. Animals were randomly divided into six groups ($n=10$ per group) for the tail suspension test (TST), forced swim test (FST) and 5-HTP-induced head-twitch test. The ethanol extract of ZJP (5, 10 and 20 mg/kg), fluoxetine (7.5 mg/kg) or 0.9% saline were given once by

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