Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2016, **14**(8): 0590–0598
Chinese Journal of Natural Medicines

Traditional herbal formula Sini Powder extract produces antidepressant-like effects through stress-related mechanisms in rats

WEI Shan-Shan [1], YANG He-Jin [2], Huang Jia-Wen [2], LU Xue-Ping [2], PENG Ling-Fang [2], WANG Qing-Guo [1*]

[1] *School of Basic Medicine, Beijing University of Chinese Medicine, Beijing 100029, China;*
[2] *Department of Drug Screening, Yunnan Institute of Materia Medica, Kunming 650111, China*

Available online 20 Aug., 2016

[ABSTRACT] Sini Powder (SP), a traditional Chinese herbal formula, has long been used to treat depression in patients, although the underlying mechanisms remain to be elucidated. In the present study, we found that rats treated with SP extract for 7 days showed a significant increase in swimming time and reduction in immobility time in forced swimming test in a dose-dependent manner, without changes in locomotion. These effects could be attributed to SP's modulation of the hypothalamus-pituitary-adrenal axis, because a single pretreatment of SP extract could rescue increased serum corticosterone and plasma adrenocorticotropin levels induced by acute elevated platform stress. A single pretreatment of SP extract could also elevate the mRNA expression of hippocampal glucocorticoid receptors. In conclusion, our results suggest that SP extract may act as an anti-stress medication to produce antidepressant-like effects.

[KEY WORDS] Sini Powder extract; Antidepressant-like; Hypothalamic-pituitary-adrenal axis; Glucocorticoid receptor

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2016)08-0590-09

Introduction

Depression is a common mental disorder that causes an enormous burden on society worldwide. Clinically available antidepressants rescue many symptoms; however, the effect is far from satisfactory because only some patients respond to treatment [1-3], with a long delay of action [4] and side effects [5]. Traditional Chinese medicine (TCM) is a promising alternative approach to treating or preventing depression [6-8]. Herbal formulae in TCM have been used for a long time to relieve symptoms of mood disorders [9]. TCM uses multiple active components with a synergistic effect to enhance efficacy and reduce toxicity [10]. In recent years, psychiatrists worldwide have employed a similar idea using a combination of several drugs to enhance the efficacy and reduce the delay in action to treat depression [11].

Sini Powder (SP) is a well-known herbal formula that has been prescribed to treat symptoms related to depression. SP which was described in "*Treatise on Febrile Diseases*" 1800 years ago [12], is comprised of four herbs in a 2 : 3 : 2 : 2 ratio: Bupleuri Radix (Chaihu), Paeoniae Radix alba (Shaoyao), Aurantii Fructus immaturus (Zhishi), and Glycyrrhizae Radix et Rhizoma (Gancao). Today, many Chinese physicians still prefer to use SP to treat a wide range of diseases, such as dysthymia [13], depression [14-15], functional dyspepsia [16], and irritable bowel syndrome [17], which are all known to be associated with stress [18-20]. This classical idea of SP is supported by modern clinical studies For example, Xie *et al.* have reported that a modified SP treatment for 6 weeks significantly reduces Hamilton Depression Rating Scale scores in 38 depressed patients, along with the relief of anxiety and insomnia [13].

Nevertheless, batch-to-batch stability of TCM is always a deterring factor in achieving quality control and consistency of efficiency. Thus, our laboratory has demonstrated a novel and stable method to obtain the active extract of SP (Patent No. CN 1416880A) [21] and found that a dose of 1.5 g·kg^{-1} of the active extract can normalize the reduced sucrose intake and auto-activities as well as upregulate the hippocampus/cortex 5-hydroxytryptamine (5-HT) levels and cortex dopamine (DA)

[Received on] 17- Apr.-2016
[Research funding] This work was supported by the National Basic Research Program funded by Ministry of Science and Technology of People's Republic of China (No. 2011CB505100).
[*Corresponding author] Tel: +86-10-64286727, Fax: +86-10-64213817, E-mail: wangqg8558@sina.com
These authors have no conflict of interest to declare.

in rats subjected to chronic unpredicted mild stress [22-23]. Interestingly, after 28 days of oral administration, 1.5 g·kg^{-1} of SP extract seemed to produce greater antidepressant-like effects than 20 g·kg^{-1} of SP water decoction (the traditional method), which could deduct the clinical dosage.

Extensive epidemiological studies have revealed that stress contributes to the development of depression [24-26]. Stress is a complex process involving the disruption and re-establishment of homeostasis that occurs when living organisms are challenged by intrinsic or extrinsic disturbing forces (stressors) [27-28]. The most important stress response system is the HPA axis in addition to the sympathetic autonomic nervous system. Within seconds after exposure to a stressor, corticotropin-releasing hormone (CRH) synthesis is increased in the hypothalamus and then transported to the pituitary, where it stimulates adrenocorticotropin (ACTH) secretion. ACTH is transported by systemic circulation to the adrenal cortex, where it rapidly stimulates the synthesis and secretion of glucocorticoids (GCs; cortisol in primates and corticosterone in rodents) [29]. Repeated stress and abnormal HPA axis activity are associated with many depressed patients [30-31]. Nowadays, stressful life events can happen at any time due to the increasingly bitter competitions and complicated social relationships. How to ameliorate the damage of stress is a critical issue in prevention and treatment of depression.

In the present study, we used the forced swimming and open field tests to evaluate the antidepressant-like effects of SP extract in rats. We also explored the potential mechanism, such as alleviating changes in hormones and related gene expressions after exposure to acute stress.

Materials and Methods

Materials and reagents

Crude drug of *Bupleurum chinense* DC. *(*Batch #130106*)*, *Paeonia lactiflora* Pall. (Batch #130201), *Citrus aurantium* L. (Batch #120405), and *Glycyrrhiza uralensis* Fisch. (Batch #121213) were purchased from Kunming Drugstore LLC., which is affiliated with Beijing Tongrentang Group (Kunming, China). The herbs were authenticated by Professor LI Gao, Department of Natural Medicine Resource, Yunnan Institute of Materia Medica, according to Chinese Pharmacopoeia (The Pharmacopoeia Commission of PRC, 2010). Fluoxetine (FLU) hydrochloride capsules were purchased from Lilly Suzhou Pharmaceutical Co., Ltd. (Suzhou, China). Isoflurane was purchased from Hebei Jiupai Pharmaceutical Co., Ltd. (Shijiazhuang, China). Neohesperidin, naringin, and glycyrrhizic acid were purchased from Chengdu Must Bio-technology Co., Ltd. (Chengdu, China). All of other reagents used in the present study were of analytical grade.

Preparation of SP extract and quality analysis

The SP extract was obtained according to our patented method [20]. The four herbs were mixed as shown in Table 1. The 1st decoction was performed in 200 L of water for 2 h. The 2nd decoction was performed in 160 L of water for 1.5 h. The filtered decoctions were then mixed and concentrated in a volume of 70 L using a rotary evaporator. The solution was eluted on an AB-8 macroporous resin column (15 L, Tianjin Bohong Technology Co., China) at a rate of 2 L·h^{-1} with distilled water and 50% ethanol. Finally, SP extract (1 840.7 g) was obtained by evaporation under vacuum.

Table 1 Composition of Sini Powder (SP)

Herbs	Local name	Parts of the Herb	Amount (kg)
Bupleurum chinense DC.	Chaihu	Root	4.8
Paeonia lactiflora Pall.	Shaoyao	Root	7.2
Citrus aurantium L.	Zhishi	Fruit	4.8
Glycyrrhiza uralensis Fisch.	Gancao	Root and Rhizome	4.8

The quality of SP was compared with our previous patented SP and the results indicated that our SP's quality was reproducible. The fingerprint of SP (Fig. 1) was analyzed by the same method [32] with minor modifications. It was performed on an Agilent Technologies 1260 Infinity system with a 1260 DAD VL detector at 230 nm. Thermo C_{18} (250 mm × 4.6 mm, 5 μm) column（Thermo, USA）was used and the temperature was set at 30 °C. The mobile phase was consisted of acetonitrile (A) and water containing 0.1% phosphoric acid (B). A linear gradient elution program was used as follows: 0–35 min, 15%–25% A; 35–65 min, 25%–50% A; and 67–72 min, 50%–15% A. The flow rate was set at 1.0 mL·min^{-1}.

The patented SP extract contains two main groups of active compounds: flavonoid glycosides and saponins. Using our previous method [32], the contents of flavonoid glycosides (56.36%) and saponins (12.067%) were detected by UV. The contents of three major components, neohesperidin (24.547%), naringin (20.20%) and glycyrrhizic acid (2.654%) were measured by HPLC-UV. They were quite similar with that in the previous batch [21].

Animals

Male Sprague-Dawley (SD) rats (weighing 180–220 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China). The animals (five per cage) were housed under controlled lighting (12 : 12 h light-dark cycle, lights on at 8 : 00 a.m.), temperature (22 ± 2 °C), and humidity (40%–70%) conditions, with free access to food and water. The animals were acclimatized to the laboratory environment for at least 3 days prior to the experiments. All of the experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by Yunnan Institute of Materia Medica. To minimize suffering, the animals were anesthetized with 5% inhaled isoflurane before the collection of blood samples and before they were sacrificed.

Forced swimming test (FST)

A total of 38 male SD rats were randomly assigned to 5 groups, which were treated with vehicle (distilled water, intragastrically (i.g.)), SP extract (1, 2, and 4 g·kg^{-1}, i.g.) and FLU