

Original Research Article

Investigation of the antidepressant effects of Shu-Gan-Jie-Yu granule and its mechanism of action

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Abstract

Purpose: To study the antidepressant effects of Shu-Gan-Jie-Yu granule (SJG) and its possible mechanisms in mice.

Methods: The anti-depressive effects of SJG were evaluated by three techniques, viz, forced swimming test (FST), tail suspension test (TST) and open field test (OFT). The levels of the neurotransmitters norepinephrine (NE), DA, and 5-HT in the brains of depressive mice were determined using commercially available kits. In addition, the effects of SJG on the BDNF expression in the mice brain were determined by western blot.

Results: Administration of SJG significantly reduced the duration time of immobility in the experiments of FST and TST. In addition, relative to the control mice, SJG (800 mg/kg) administration significantly affected the mobility performance ($p < 0.05$) of mice. The levels of the three neurotransmitters (DA, NE and 5-HT) and BDNF in the brains of depressive mice were increased by treatment with SJG at the doses of 200, 400 and 800 mg/kg ($p < 0.05$). The results suggested that SJG exerted a significant antidepressant effect, which could be attributed to increases in the levels of neurotransmitters, and the up-regulation of BDNF expression.

Conclusion: The results suggested that SJG exerted a significant antidepressant effect, most probably via regulation of related neurotransmitters (including DA, NE, and 5-HT) and BDNF in the brain.

Keywords: Shu-Gan-Jie-Yu granule, Antidepressant, dopamine, norepinephrine, 5-hydroxytryptamine, brain-derived neurotrophic factor

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INTRODUCTION

It's well known that depression is a very common chronic recurrent syndrome and the main reason for the increase of suicide and physical disorders [1]. Despite significant progress in the diagnosis and care of patients with depression, many patients who experience depression still have no

access to satisfactory treatment. Depression is a mental disorder characterized by persistent depression and self-denial, and its pathogenesis is highly complex. Its pathogenesis is very complex. Depression is generally considered to be caused by genetic and environmental stressors, although the specific pathological mechanism remains unclear [2]. Furthermore, it

has been reported that current medications used to treat depression have a series of side effects, such as constipation, dry mouth, and sexual dysfunction [3]. Therefore, it is urgent to find more safe and effective drugs to treat depression.

Shu-Gan-Jie-Yu granule (SJG) is the first traditional Chinese medicine approved for clinical prescription by the State Food and Drug Administration to treat depression [2]. SJG is a compound preparation purified using advanced technology [4]. Its primary components include extracts of *Hyperici perforati Herba* and *Acanthopanax Senticosi Radix et Rhizoma Seu Caulis* [5]. Numerous studies have reported that SJG exerts significant depressant effects in the clinic with fewer/less severe side effects than Western medicines [5]. However, the antidepressant mechanism of SJG is still unclear. Accordingly, the aim of this study was to systematically investigate the antidepressant effects of SJG and explore its mechanism of action. The results could provide more scientific support for its use in treating clinical depression.

EXPERIMENTAL

Animals

Male ICR mice (mean \pm SD] weight, 20 ± 2 g) were obtained from the Shanghai Laboratory Animal Center (Shanghai, China). The animals were maintained in a temperature and light controlled environment with *ad libitum* access to chow and water. The animal protocol adhered to the principles of the Declaration of Helsinki promulgated in 1964 as amended in 1996 [6], and was approved by the animal experiment management department of Affiliated Hangzhou First People's Hospital (Zhejiang, China; no. HZ2018-065).

Chemicals and reagents

Shu-Gan-Jie-Yu granule (SJG) was product of Beijing Tongrentang (Beijing, China). Imipramine was obtained from Sigma (Sigma-Aldrich Co., St Louis, MO, USA). Commercially available kits used to determine DA, NE, and 5-HT were products of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Primary antibody for BDNF and β -actin were purchased from Abcam (Cambridge, United Kingdom).

Forced swimming test (FST)

The FST experiment was carried out based on a method previously described in the literature [7]. The animals were grouped as follows: control

(saline); positive (imipramine, 15 mg/kg); and SJG-treated groups (200, 400, and 800 mg/kg). Imipramine (IMP) was treated by intraperitoneal injection (i.p.) at 30 min before the experiment. SJG was administered orally at 1 h before the FST. The mice were forced to swim for 6 min in a glass jar (14 × 14 × 25 cm) with 20 cm-deep water (mean temperature $25 \pm 2^\circ\text{C}$). In the last 4 min of the test, the duration time of immobility exhibited by the mice was recorded.

Tail suspension test (TST)

The TST experiment was also carried out as previously reported [8]. Fifty male mice were grouped as follows: control (saline); positive (IMP); and SJG -treated groups (200, 400 and 800 mg/kg). Before 30 min of the TST experiment, mice in the positive group were treated with IMP (15 mg/kg, i.p.), and SJG was orally administered 1h before the TST. Mouse was hung by the tail in a clear black acrylic box (30 × 30 × 45 cm), respectively. Immobility duration was recorded over a test period of 6 min.

Spontaneous behavior in the open-field test (OFT)

The OFT experiment was carried out as reported in a previous literature [1]. Transparent black acrylic box (41 × 41 × 41 cm) was used and monitored by a video-based camera system (Etho-vision, Noldus, Wageningen, The Netherlands). Fifty male mice were grouped as follows: control (saline); positive (IMP); SJG -treated groups (200, 400 and 800 mg/kg). Before 30 min of the OFT experiment, mice in the positive group were treated with IMP (15 mg/kg, i.p.). The SJG (200, 400 and 800 mg/kg) were orally administered 1 h before assessing spontaneous behavior in the OFT. To evaluate horizontal locomotor activity of the mice in different groups, mouse was placed in the center of device for 1 h and recorded by video for 5 min. The frequency of rearing and total ambulatory distance was used to evaluate the horizontal loco-motor activity of mice.

Determination of neurotransmitters in the depressive mice brain

Depressive mice were identified as described in the TST. These mice were decapitated and the entire brain was removed. The levels of neurotransmitters (DA, NE, and 5-HT) in brain tissue were determined using commercially available kits according to manufacturers' instructions.

Western blot analysis

Collected brain tissues were homogenized to extract total proteins. Subsequently, samples (40 µg of total protein in each sample) were separated on SDS-PAGE. Proteins were transferred to polyvinylidene fluoride membranes, and then probed using various primary BDNF antibody, followed by the appropriate secondary antibody. The protein bands were stained with chemiluminescence reagent. Protein content was normalized to a β-actin control.

Statistical analysis

Data are expressed as mean ± S.D. Statistical analyses were carried out using the two-tailed Student's *t* test (ANOVA) using SPSS 16.0 software (SPSS Inc., USA). $p < 0.05$ was regarded as a significance level.

RESULTS

Effect of SJG on depressive mice in FST

As shown in Figure 1, immobility time of mice treated with imipramine was obviously shorter ($p < 0.01$) than that of the mice in the control group. Interestingly, SJG treatments (200, 400 and 800 mg/kg) also markedly shortened the immobility duration time of depressive mice ($p < 0.05$, $p < 0.05$, $p < 0.01$, respectively).

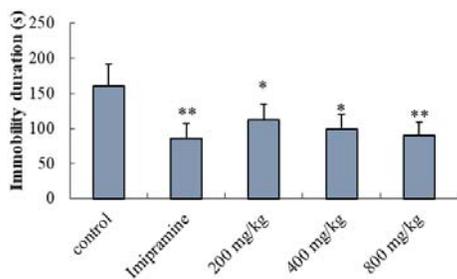


Figure 1: FST results for SJG. Each column represented the mean ± SD. (n = 10). Asterisks indicated significant difference from control; * $p < 0.05$, ** $p < 0.01$

Effect of SJG on mice with depression in TST

Results of effects of SJG on the mice with depression in TST experiment are presented in Figure 2. Relative to the control group, IMP (15 mg/kg) treatment markedly reduced the immobility time of depressive mice ($p < 0.01$). The immobility time of mice in SJG-treated groups (400 and 800 mg/kg) was apparently

shorter ($p < 0.05$) than that of the normal control mice.

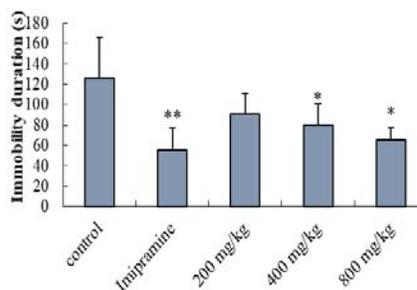


Figure 2: TST results for the SJG. Each column represents the mean ± SD. (n = 10) Asterisks indicated significant difference from control; * $p < 0.05$, ** $p < 0.01$

Effect of SJG on mice with depression in OFT

As shown in Figure 3, compared with mice in the control group, after SJG (800 mg/kg) treatment, the mobility of depressive mice was markedly increased ($p < 0.05$). In addition, IMP treatment also significantly affected the mobility of depressive mice when compared with the normal control mice ($p < 0.05$).

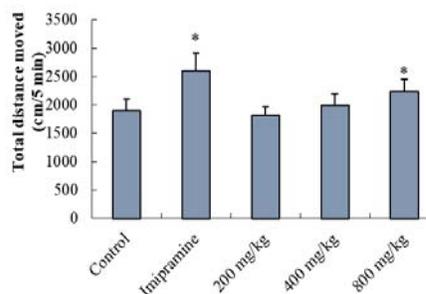


Figure 3: OFT results for SJG. Each column represented the mean ± SD. (n = 10). Asterisks indicate significant difference from control; * $p < 0.05$

Effect of SJG on neurotransmitters in depressive mouse brain

Effect of SJG on neurotransmitters in depressive mouse brain were shown in Figure 4. Compared with the control group, it can be seen that different doses of SJG remarkably increased the 5-HT level in the brains of depressive mice at 200, 400, and 800 mg/kg. The DA and NE levels in mice in the SJG-treated group were also significantly increased ($p < 0.05$) when compared to the normal control mice.

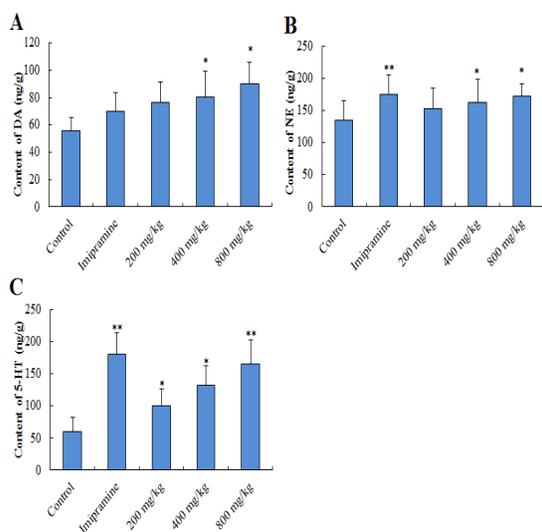


Figure 4: DA, NE, and 5-HT levels in depressive mouse brain. Each column represented the mean \pm SD ($n = 10$). Asterisks indicated significant difference from control; * $p < 0.05$, ** $p < 0.01$

Effect of SJG on BDNF expression in depressive mouse brain

As shown in Figure 5, different doses of SJG (200, 400 and 800 mg/kg) markedly enhanced the expression of BDNF in the brains of depressive mice ($p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively), relative to the normal control mice.

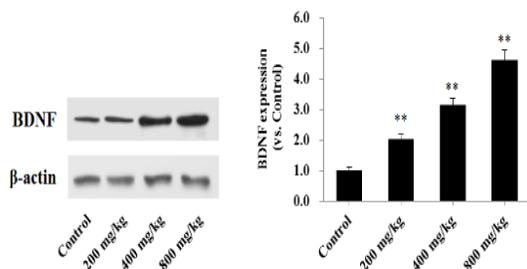


Figure 4: Expressions of BDNF in the depressive mice brain. Each column represented the mean \pm SD. ($n=10$). Asterisks indicate significant difference from control. * $p < 0.05$, ** $p < 0.01$

DISCUSSION

Behavioral despair models often include forced swim and tail suspension, both of which provide an unavoidable and oppressive environment. The duration of immobility of animals in the experiment after effort reflects a state known as “behavioral despair” [10,11]. The immobility time of “behavioral despair” can reflect the degree of depression in animals, while antidepressants

reduce immobility time; in other words, the lower the immobility time, the stronger the antidepressant effect.

These two models are fast, simple and sensitive to most antidepressants. Thus, they have been widely used in the preliminary screening of various antidepressants. In addition, the OFT can effectively reflect spontaneous behavior, activity, and emotional state of the animals, and its activity intensity can also indicate excitation or inhibition of the central nervous system (CNS) [12]. It is known that increases in animal motor activity elicited by a drug(s) reflects excitation of the CNS.

The behavior despair models were employed in in this study, and the results showed that SJG exhibited anti-depressive activity, which reflect its powerful anti-depressive effects. Moreover, the results of OFT demonstrated that SJG treatment clearly altered the locomotor activity of depressive mice.

The decrease of some brain neurotransmitters (DA, NE and 5-HT, etc.) can lead to depression. Therefore, upregulation of these neurotransmitters in the brain is of great significance for the improvement of depression [13]. The present results indicated that the levels of brain neurotransmitters (DA, NE, and 5-HT) were increased after SJG treatment. In addition, as a neurotrophic factor, BDNF is indispensable for the function of neurons, such as growth, survival and differentiation [14]. It is also involved in improving the function of learning and memory, and has antidepressant effect [1]. The present study found SJG exerted significantly antidepressant effect by increasing the expression of BDNF in the brains of depressive mice.

CONCLUSION

The findings of this study demonstrate that SJG possesses antidepressant effects that modified the CNS, leading to reduced immobility, and altered motor and exploratory activity. Thus, SJG possesses antidepressant activity, and can be potentially developed as an antidepressant medication.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. All authors approved the manuscript for publication. For preparation of the paper, Min Wu conceived the study; Li-shu Gao, Min Wu, Yue Gao, En-ping Xu, Jian Xie collected and analyzed the data; Li-shu Gao and Min Wu wrote the manuscript.

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