Effects of Yulangsan polysaccharide on monoamine neurotransmitters, adenylate cyclase activity and brain-derived neurotrophic factor expression in a mouse model of depression induced by unpredictable chronic mild stress☆

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Abstract
The present study established a mouse model of depression induced by unpredictable chronic mild stress. The model mice were treated with Yulangsan polysaccharide (YLSPS; 150, 300 and 600 mg/kg) for 21 days, and compared with fluoxetine-treated and normal control groups. Enzyme-linked immunosorbent assay, radioimmunity and immunohistochemical staining showed that following treatment with YLSPS (300 and 600 mg/kg), monoamine neurotransmitter levels, prefrontal cortex adenylate cyclase activity and hippocampal brain-derived neurotrophic factor expression were significantly elevated, and depression-like behaviors were improved. Open-field and novelty-suppressed feeding tests showed that mouse activity levels were increased and feeding latency was shortened following treatment. Our results indicate that YLSPS inhibits depression by upregulating monoamine neurotransmitters, prefrontal cortex adenylate cyclase activity and hippocampal brain-derived neurotrophic factor expression.

Key Words: Yulangsan polysaccharide; anti-depressant; chronic stress; monoamine neurotransmitter; adenylate cyclase; brain-derived neurotrophic factor; Chinese medicine; neural regeneration

INTRODUCTION
Yulangsan (YLS) can increase resistance to fatigue, inhibit tumors and inflammation, improve learning and memory, and remove free radicals[1-5]. Polysaccharides can be extracted from YLS. Preliminary screening experiments of anti-depressant drugs show that YLS polysaccharide (YLSPS) can significantly shorten duration of mouse immobility[6-7]. Depression is associated with changes in intracerebral neurotransmitters and receptors[8-11], receptor signal transduction systems[12-14], genetic transcription, and neurotrophic function[15-16]. Stress is a precipitating factor for depression[17-18]. We hypothesized that YLSPS is a natural potential anti-depressant drug, and used a mouse model of depression induced by chronic mild unpredictable stress[19] to investigate the effects of YLSPS on monoamine neurotransmitter levels, prefrontal cortex adenylate cyclase activity and hippocampal brain-derived neurotrophic factor (BDNF) expression.

RESULTS
Quantitative analysis of experimental animals
A total of 180 Kunming mice were randomly assigned to six groups: normal control (routine feeding), model (depression model), fluoxetine (depression model + fluoxetine), and low-, moderate-, and high-dose YLSPS (depression model + 150, 300, or 600 mg/kg, respectively; L-YLSPS, M-YLSPS, H-YLSPS) groups, with 30 animals per group. Ten mice from each group were used for both behavioral and monoamine studies, and 10 each for adenylate cyclase and BDNF detection. In all mice except the normal control group, a mouse model of depression was established using chronic mild unpredictable stress[19-23]. Fluoxetine is an antidepressant selective serotonin reuptake inhibitor that served as positive control. All animals were tested after 21 days of treatment. A total of 180 mice were included in the final analysis, with no death or infection.
Effects of YLSPS on intracerebral monoamine neurotransmitter levels

The monoamine neurotransmitter levels in the brain were quantified using enzyme-linked immunosorbent assay (ELISA)\(^\text{[24]}\). Results showed that 5-hydroxytryptamine (5-HT) and norepinephrine (NE) content in the brain of the model group was significantly lower than in the normal control group \((P < 0.01)\). 5-HT and NE content was significantly increased in the M- and H-YLSPS groups compared with the model group \((P < 0.01, P < 0.05); \text{Figure 1})\.

![Figure 1](image)

**Figure 1** Concentration of 5-hydroxytryptamine (5-HT; \(A\)) and norepinephrine (NE; \(B\)) in the brain of chronically stressed mice. \(^aP < 0.05, ^bP < 0.01\), vs. normal control group; \(^cP < 0.05, ^dP < 0.01\), vs. model group. Data were expressed as the mean ± SD of ten mice in each group. One-way analysis of variance was used for comparison. NC: normal control; MC: model; FLU: fluoxetine; L-YLSPS, M-YLSPS, H-YLSPS: low-, moderate-, and high-dose Yulangsan polysaccharide (YLSPS).

Effects of YLSPS on prefrontal cortex adenylate cyclase activity

The activity of adenylate cyclase, which converts adenosine triphosphate into cyclic adenosine monophosphate (cAMP), was determined using radioimmunity methods, as previously described\(^\text{[25]}\). Following chronic stress, cAMP expression in the prefrontal cortex was significantly decreased in the model group compared with the normal control group \((P < 0.01)\). Compared with the model group, YLSPS at all three doses significantly increased cAMP expression in the prefrontal cortex of chronically stressed mice \((P < 0.01)\). Results indicate that YLSPS at 150–600 mg/kg can activate adenylate cyclase activity in the prefrontal cortex of chronically stressed mice (Figure 2).

![Figure 2](image)

**Figure 2** Changes in cyclic adenosine monophosphate (cAMP) activity in the prefrontal cortex of chronically stressed mice. \(^aP < 0.05, ^bP < 0.01\), vs. normal control group; \(^P < 0.01\), vs. model group. Data were expressed as mean ± SD of ten mice in each group. One-way analysis of variance was used for comparison. NC: normal control; MC: model; FLU: fluoxetine; L-YLSPS, M-YLSPS, H-YLSPS: low-, moderate-, and high-dose Yulangsan polysaccharide (YLSPS).

Effects of YLSPS on hippocampal BDNF-positive neurons

Hippocampal BDNF was determined using immunohistochemical staining. Under the light microscope, BDNF was strongly expressed in the hippocampus of the normal control group, showing dark brown cytoplasmic staining indicating high expression levels of BDNF. In the model group, BDNF immunoreactivity was weak, and the color of cytoplasmic staining was light. The mean gray-scale value of BDNF-positive neurons was significantly greater in the model group compared with the normal control group \((P < 0.01)\), but it was significantly lower in the M- and H-YLSPS groups compared with the model group \((P < 0.05); \text{Figure 3, Table 1})\.

**DISCUSSION**

Monamine neurotransmitters are distributed throughout the brain and control sleep, appetite, emotion and sexual desire. Neurotransmitter changes can influence these functions, leading to depressive symptoms, such as low emotion, insomnia, anorexia, sexual dysfunction and decreased activity levels.
Antidepressant drugs increase central monoamine levels by inhibiting monoamine synthesis or reuptake, thereby

Table 1  Effects of Yulangsan polysaccharide (YLSPS) on mean gray-scale value of brain-derived neurotrophic factor (BDNF)-positive cells in the hippocampus of chronically stressed mice

| Group           | Dose (mg/kg) | Mean gray scale value |
|-----------------|--------------|-----------------------|
| Normal control  | –            | 101.00±1.87           |
| Model           | –            | 104.00±2.12a          |
| Fluoxetine      | 20           | 102.80±0.45           |
| L-YLSPS         | 150          | 102.00±1.22b          |
| M-YLSPS         | 300          | 101.80±0.45           |
| H-YLSPS         | 600          |                       |

The mean gray scale value of BDNF-positive cells in every 400-fold field of view was determined and analyzed using a Q550 pathological imaging analyzer (Germany). *P < 0.01, vs. normal control group; aP < 0.05, bP < 0.01, vs. model group. Data were expressed as the mean ± SD of ten mice in each group. One-way analysis of variance was used for comparisons. L-YLSPS, M-YLSPS, H-YLSPS: Low-, moderate-, and high-dose YLSPS.

Table 2  Effects of Yulangsan polysaccharide (YLSPS) on behaviors of chronically stressed mice

| Group           | Dose (mg/kg) | Number of crossing grid | Feeding latency (s) |
|-----------------|--------------|--------------------------|---------------------|
| Normal control  | –            | 97.6±4.1                 | 40.6±2.8            |
| Model           | –            | 65.7±6.7b               | 69.9±4.2b           |
| Fluoxetine      | 20           | 93.2±4.0d               | 43.8±2.5d           |
| L-YLSPS         | 150          | 71.5±6.5bc              | 66.1±5.9bc          |
| M-YLSPS         | 300          | 90.8±4.0mm              | 49.2±3.8mm          |
| H-YLSPS         | 600          | 93.1±4.3g               | 44.2±2.2g           |

*aP < 0.05, bP < 0.01, vs. normal control group; *P < 0.05, **P < 0.01, vs. model group. Data were expressed as the mean ± SD of ten mice in each group. One-way analysis of variance was used for comparisons. L-YLSPS, M-YLSPS, H-YLSPS: Low-, moderate-, and high-dose YLSPS.

Antidepressant drugs increase central monoamine levels by inhibiting monoamine synthesis or reuptake, thereby...
reversing depression-like behaviors\textsuperscript{28}. Recent evidence indicates that adenylate cyclase activity is decreased in the cerebral cortex of depressed patients, resulting in dysfunction of the cAMP signaling pathway in neurons of the brain. Antidepressant drugs can restore decreased cAMP activity\textsuperscript{29}. The hippocampus is an important part of the limbic system. It is influenced by stress and is involved in stress regulation\textsuperscript{30}. According to the neurotrophic hypothesis, human depression is associated with reduced BDNF expression and function. BDNF mediates survival, growth, differentiation and apoptosis of nerve cells and possibly protects neurons against stress-induced injury through the regulation of neuronal regeneration and synaptic plasticity\textsuperscript{31}. BDNF is the growth factor of 5-HTergic neurons, and slow injection of BDNF in rat midbrain can increase 5-HT renewal and NE levels. Cortical injection of BDNF even stimulates a novel 5-HT nerve terminal\textsuperscript{32}. Therefore, there is an association between the neurotrophic hypothesis and the monoamine neurotransmitter hypothesis.

Our results showed that following YLSPS treatment for 21 days, spontaneous activity levels of mice were significantly increased, while the feeding latency in a novel environment was reduced. These results demonstrate that YLSPS exhibits anti-depressant activity in chronically stressed mice and may serve as a potential anti-depressant drug.

In summary, the effects of treatment with YLSPS in chronically stressed mice on NE and 5-HT levels, adenylate cyclase activity and BDNF expression were investigated in this study. NE and 5-HT content was significantly increased after YLSPS treatment compared with the model group, and YLSPS at 150-600 mg/kg activated prefrontal cortex adenylate cyclase activity. While there were no differences in the number of BDNF-positive cells, the intensity of staining was significantly lower in fluoxetine- and YLSPS-treated (300-600 mg/kg) animals compared to the model group, which was not fully consistent with previous findings\textsuperscript{33}. In conclusion, YLSPS enhances NE and 5-HT levels in the brain, increases prefrontal cortex adenylate cyclase activity, and improves stress-induced BDNF changes in chronically stressed mice, indicating that it is a potential anti-depressant drug.

MATERIALS AND METHODS

Design
A randomized, controlled, animal experiment.

Time and setting
This experiment was performed at the Laboratory of Pharmacology and Experimental Center of Medical Sciences, Guangxi Medical University, China from September 2007 to March 2009.

Materials
Animals
A total of 180 healthy, male Kunming mice, of specific-pathogen free grade, weighing 18 ± 22 g, were provided by the Laboratory Animal Center of Guangxi Medical University (license No. SYXK (Gui) 2003-0005). The experimental procedures were approved by the Animal Ethics Committee of Guangxi Medical University.

Drugs
YLSPS was extracted by our laboratory. Briefly, a dry sheet of YLS was soaked, decocted, centrifuged, filtrated, mixed with alcohol, washed, deproteinized using the Savage method\textsuperscript{24-30}, and filtrated. Sediments were collected and vacuum-dried, and white YLSPS was obtained. The collection rate was about 0.30%. YLSPS was dissolved in distilled water before intraperitoneal administration. The low dose was equal to six times the clinical adult dose (mg/kg), the moderate dose was equal to two times the low dose, and the high dose was equal to two times the moderate dose. Fluoxetine (No. 071201; Shanghai Zhongxi Pharmaceutical (Group) Co., Ltd., China) was diluted using distilled water and stored at 4°C.

Methods

Chronic stress depression model and intervention
The model of chronic stress depression was established as previously described\textsuperscript{19-33} with some modifications. The normal control group was fed routinely and the other groups were subjected to unpredictable chronic mild stress as follows:

Drugs were intraperitoneally administrated 30 minutes prior to stress stimulation, once daily, and the normal control group received distilled water.

Observation of behaviors in chronically stressed mice following YLSPS treatment
For the open field test\textsuperscript{26}, mice were separately placed in a self-made open field in a quiet and dark environment 30 minutes after the final drug administration, to record the number of times the mouse crossed the grid in 5 minutes.

For the novelty-suppressed feeding test\textsuperscript{27}, mice were deprived of food but not water for 48 hours and placed in a 30 cm × 30 cm × 30 cm organic glass case in a quiet and dark environment. A small piece of mouse chow was placed at the center to observe feeding latency in 5 minutes.

Determination of brain monoamines
The mice were sacrificed immediately following the behavioral tests. The entire brain was harvested, homogenized with pre-cooled normal saline at a volume: weight ratio of 5:1, and centrifuged at 14 000 r/min for 10 minutes.
15 minutes. The supernatant was harvested and stored at -86°C. NE and 5-HT content was determined using an MK3 microplate reader (Thermo Electron Corporation, Waltham, MA, USA) according to the manufacturer’s instruction for the NE and 5-HT kit (ADL, San Diego, CA, USA).

**Determination of adenylate cyclase activity**

A total of 50 mg of brain tissue from each group was harvested, mixed with 2 mL cold acetic acid buffer solution (50 mM, pH 4.75), homogenized, mixed with 2 mL absolute alcohol, left to stand for 5 minutes, and centrifuged at 3 500 rpm for 15 minutes. The supernatant was collected, and the sediment was washed with 75% alcohol (2 mL), homogenized, centrifuged, mixed with supernatant, and dried in oven at 60°C. The residue was stored at 4°C and dissolved in 1 mL acetic acid buffer solution, and 0.1 mL solution was used for activity determination. The cAMP amount in each reaction tube was quantified with a 125I-cAMP radioimmunoassay kit (Beijing Puerweiyi Biotechnology, Beijing, China) using a GC-1200y radioimmunity counter (Zhongjia Optical and Electrical Instrument Branch Company, University of Science and Technology of China), Gama software (GC-1200 for dos-GAMA.EXE) was used for calculations, and the unit was M.

**Determination of hippocampal BDNF**

The mice were anesthetized, and the ascending aorta was cannulated. The animals were perfused with normal saline and 4% paraformaldehyde, and the brains were harvested and post-fixed. The hippocampi were collected, dehydrated using gradient alcohol, cleared using xylene, dehydrated using gradient alcohol, and stained. The sections were stained with diaminobenzidine using MaxVision™ rapid immunohistochemistry (two-step method). Briefly, the sections were dewaxed with xylene, dehydrated using gradient alcohol, and washed with phosphate buffered saline (PBS), followed by antigen retrieval under high pressure using one drop or 50 μL of 3% H2O2. The sections were incubated at room temperature for 10 minutes, washed with PBS, incubated with one drop or 50 μL primary antibody (1: 100; anti-mouse BDNF antibody, Beijing Biosynthesis Biotechnology, China) at room temperature for 60 minutes or overnight at 4°C, washed with PBS, incubated with one drop or 50 μL ready-to-use MaxVision™ rapid immunohistochemistry reagent (Fuzhou Maxim, Fuzhou, China) at room temperature for 10–15 minutes, washed with PBS, mixed with two drops or 100 μL of freshly prepared diaminobenzidine solution (Fuzhou Maxim), and observed under a microscope for 3–5 minutes. The sections were washed with tap water, counterstained with hematoxylin, washed with PBS or tap water, dried using gradient alcohol, cleared with xylene, mounted with neutral gum and observed by light microscopy (Olympus, Tokyo, Japan). Three fields of view (400×) from the hippocampus were selected, and the mean gray scale value of BDNF-positive cells in each field of view was determined and analyzed using the Q550 pathological imaging analysis system (Leica, Wetzlar, Germany).

**Statistical analysis**

Data were expressed as mean ± SD and analyzed using SPSS version 13.0 software (SPSS, Chicago, IL, USA). Data were analyzed with one-way analysis of variance for multiple groups, and intergroup mean differences were compared using paired t-test. A value of P < 0.05 was considered statistically significant.

**Author contributions:** Shuang Liang conceived and designed this study, analyzed data and wrote the manuscript. Renbin Huang was in charge of funds, revised the manuscript, provided technical support and guided the experiments. All authors conducted the experiments and evaluated the study with the blind method.

**Conflicts of interest:** None declared.

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