Antidepressant-Like Effect of Bupleurum Paeonia Liujunzi Decoction on Depression Model Mice and its mechanism

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Abstract. To investigate the behavioral effects and mechanisms of Bupleurum Paeonia Liujunzi Decoction (BPLD) in depression model mice. Twenty male Kunming mice were randomly divided into four groups: blank group, model group, fluoxetine group and Bupleurum Paeonia Liujunzi Decoctiction group. Fluoxetine group were given fluoxetine treatment at 2mg/kg/d and Bupleurum Paeonia Liujunzi Decoctiction group. Depression model mice were induced by the chronic unpredictable mild stress (Con group were given BPLD treatment at 8g/kg/d. The model group and the blank group were given normal saline once a day. The experiment lasted for 7 days, during which the general state (body weight, hair color, etc.) was recorded and the changes were observed. The serum levels of norepinephrine (NE) and dopamine (DA) were detected by ELISA kit. Compared with the model group, the body weights of the diazepam group and the Chinese medicine group were significantly increased (P<0.01). In addition, it produced the concentrations of NE and DA in the serum were significantly increased (P<0.05) in the two groups. Bupleurum Paeonia Liujunzi Decoction has obvious therapeutic effect on depressed mice, and its mechanism may be related to the increase of NE and DA concentrations.

Keywords: Bupleurum Paeonia Liujunzi Decoction, Antidepressant, Depressive-like behavior, Norepinephrine, Dopamine.

1. Introduction
Depression disorder (depression) is a form of central disorder in psychiatry, which is called depression clinically and in folk practices [1].

Depression is caused by a variety of causes, with low emotional continuity as the main manifestation of a group of mental disorders and emotional disorders of the clinical syndrome. According to the World Health Organization (WHO) survey data, depression takes responsibility for 40.5% of all disability-adjusted life-like conditions due to mental and substance use disorders [2].

Modern depression is mainly the drug treatment of Western medicine, but the drugs have toxic side effects, potential cancer risk, slow clinical results, high drug price, and easy to relapse, ect [3].

Bupleurum Paeonia Liujunzi Decoction is derived from synopsis of golden chamber, and it’s consist is Renshen, Baizhu, Fuling, Chenpi, Banxia, Gancao, Bupleurum, Paeonia, and Bupleurum and...
Paeonia are two drugs considered to contribute to depression [4]. Bupleurum Paeonia Liu junzi Decoction has a therapeutic effect on depression clinically [5, 6] and the basic medical research on BPLD is lacking. In this study, we used the chronic unpredictable mild stress combined with reserpine gavage to induced depression model mice and observe the effect on behavior and the neurotransmitter of BPLD in depression model mice.

2. Materials and methods

2.1. Animals
The Kunming species male mice, SPF grade and body weight (18-24)g, were housed in a constant temperature and humidity environment (20°C-24°C, 50%-60%) on a day and night natural light and dark alternate lighting cycle, feeding mice the same standard diet and water.

2.2. Experimental Materials

2.2.1. Medicine and reagents. Experimental reagent: mice Norepinephrine (NE) ELISA kit and mice Dopamine (DA) ELISA kit were from Shanghai Fanke Biotechnology Co., Ltd., 96T; a combination pill of fluoxetine, Single Concentrated Chinese Herbal Tea: Chaihu 2.8g; Baishao 0.9g; Banxia 2.0g; Chenpi 2.0g; Fuling 1.5g; Gancao 1.0g; Dangshen 9.0g; Baizhu 12.0g (Jiangsu Jiangyin Tianjiang granule).

2.2.2. Experimental equipment. Electronic needle treatment instrument, microplate reader were provided.

2.3. Methods

2.3.1. Grouping and management. Divide 20 male Kunming mice into 4 groups: model group, fluoxetine group (2mg·kg⁻¹), Bupleurum Paeonia Liu junzi Decoction group (8g·kg⁻¹) and blank group. Except for the blank group were administered with distilled water at 0.4 ml/kg/d, model group, fluoxetine group and Bupleurum Paeonia Liu junzi Decoction group were given corresponding drugs by gavage.

2.3.2. Model preparation. The depression model mice were induced by the the chronic unpredictable mild stress (CUMS) combined with reserpine gavage. Except for the blank group, Give each model mice of the other three groups for 2-3 kinds unpredictable stress every day including 14 kinds of stimulation factors, including fasting for 24 hours, water prohibition for 24 hours, ice water swimming for 5 minutes at 4°C, hot water swimming for 5 minutes at 40°C, tail clamping for 5 minutes, wet bedding for 12 hours, tilting for 45º 12 hours, noise stimulation for 10 minutes, horizontal shaking for 30 minutes, day night reversal for 24 hours for 7 days except the blank group. These methods were randomly assigned to seven days, each stimulus was used 2-3 times for 7 days.

2.4. Observations

2.4.1. Mice’s weight observation. The mice’s weight was weighed 2 hours after the drug was administered every day. The data of each group were recorded.

2.4.2. NE and DA. The concentrations of NE and DA in mice’s serum were determined strictly follow the instructions in the ELISA kit.
2.5. **Statistical analysis**

The experimental data are mean ± standard deviation and all data are statistically processed by SPSS 21.0 software. First of all, the data in each group is tested for normal distribution, and a One-way ANOVA is used for normal distribution, and non-parameter test is used for that does not conform to normal distribution. P<0.05 is considered as be statistically significant.

3. **Results**

3.1. **Effect of BPLD to body weight of depression model mice**

On the first day in experiment, differences in weights among the groups have no statistical significance (Figure 1).

On the seventh day of the experiment, compared to model group, body weight of the FLU and CLT groups was obviously higher, which was statistically significant (P<0.01) (Figure 2).

![Figure 1](image1.png)

**Figure 1.** Body weight of each group on day 1.

Note: Data in Figure 1 were expressed as average ± S.E.M. Relative to control group, * indicates p<0.05, ** indicates p<0.01. Relative to model group, * indicates p<0.05, ## indicates p<0.01.

![Figure 2](image2.png)

**Figure 2.** Effect of Bupleurum Paeonia Liujunzi Decoction (BPLD) on weight at day.

Note: Data in Figure 2 were expressed as average ± S.E.M. Relative to control group, * indicates p<0.05, ** indicates p<0.01. Relative to model group, * indicates p<0.05, ## indicates p<0.01.
3.2. Effect of BPLD on the serum NE concentration of depression model mice
Relative to the model group, the NE concentration of FLU and CLT group was significantly shortened, which was statistically significant (P < 0.05) (Figure 3).

![Figure 3](image_url)

**Figure 3.** Effect of Bupleurum Paeonia Liujunzi Decoction (BPLD) on NE levels in the serum.

Note: Data in Figure 3 were expressed as average ± S.E.M. Relative to control group, * indicates p<0.05, ** indicates p<0.01. Relative to model group, # indicates p<0.05, ## indicates p<0.01.

3.3. Effect of BPLD on the serum DA concentration of depression model mice
Relative to the model group, the DA concentration of the FLU and the CLT group was significantly shortened, which was statistically significant (P<0.05) (Figure 4).

![Figure 4](image_url)

**Figure 4.** Effect of Bupleurum Paeonia Liujunzi Decoction (BPLD) on NE levels in the serum.

Note: Data in Figure 4 were expressed as average ± S.E.M. Relative to control group, * indicates p<0.05, ** indicates p<0.01. Relative to model group, # indicates p<0.05, ## indicates p<0.01.

4. Discussion
CUMS and reserpine modeling are internationally recognized methods for modeling rodent depression. The animal models of depression prepared by the two methods have been widely used in the study of pathological mechanisms of depression and screening of antidepressants [7, 8].
Depression model mice often suffer from weight loss due to slow thinking, movement reduction, and loss of appetite [9, 10]. Our experimental results show that after the intervention of Bupleurum Paeonia Liujunzi Decoction, the weight of the depression modeling mice were significantly higher, suggest that Bupleurum Paeonia Liujunzi Decoction have an improved effect on depression.

NE and DA are central monoamine neurotransmitters that can transmit excitatory signals and participate in the regulation of cardiovascular, body temperature, mood and other physiological functions. Reduced levels or functional defects often lead to depression [11, 12]. In the study, the NE and DA levels in the CLT group’s serum increased significantly relative to the model group.

The results of this experiment indicate that the therapeutic mechanism of Bupleurum Paeonia Liujunzi Decoction in depressed mice may be related to elevated serum NE and DA concentrations.

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