Gallbladder-warming Decoction with Bupleurum and Scutellaria inhibits Anxiety and Depression of Rats

Ning Sun\textsuperscript{1}, Yumei Ren\textsuperscript{2}, Mingyu Wang\textsuperscript{2}, Xinyan Miao\textsuperscript{2}, Zhiyong Meng\textsuperscript{3}, Chaobo Wang\textsuperscript{1}, Xiaolong Yuan\textsuperscript{3}, Liang Wang\textsuperscript{2}, Chen Fu\textsuperscript{2}, Weiguang Zhang\textsuperscript{3}, Donghan Zhang\textsuperscript{2}, Ran Cheng\textsuperscript{2}, Pengyu Wang\textsuperscript{2}, Hao Yang\textsuperscript{2}, Binbin Qi\textsuperscript{4}, Yuzhu Li\textsuperscript{4}, Ruijuan Cai\textsuperscript{5,*}

\textsuperscript{1}Experimental Research Center, Henan University of Traditional Chinese Medicine, Zhengzhou, China
\textsuperscript{2}The Second school of clinical medicine of Henan University of Traditional Chinese Medicine, Zhengzhou, China
\textsuperscript{3}College of Acupuncture-Moxibustion and Tuina, Henan University of Traditional Chinese Medicine, Zhengzhou, China
\textsuperscript{4}The first Clinical Medical College of Henan University of Chinese Medicine, Zhengzhou, China
\textsuperscript{5}Henan University of Chinese Medicine, Zhengzhou, China

*Corresponding author e-mail: baishaoyao@163.com

Abstract. Objectives: To search the behavioral effect of Gallbladder-warming Decoction with Bupleurum and Scutellaria (GWBS) on anxiety and depression model rats and explore its mechanism. Methods: Twenty SD rats were randomly divided into four groups with 5 in each group: blank control group, model group, weastern medicine group and GWBS group. The unpredictable chronic emotional stress method was used to induce anxiety and depression of model rats. Rats in weastern medicine group were given diazepam at 0.26 mg/kg/d and the 5 rats in GWBS group were given GWBS at 11.5g/kg/d, otherwise, the other two groups were given the same volume of normal saline once a day. The experiment lasted for 10 days, during which the general state including body weight, hair color, activity level and so on were observed and the data were recorded. The results of sucrose consumption test and the light-dark box test were also recorded during the ten days. After the experiment, the changes of histological pathologic in the hippocampus were examined, and the \( \gamma \)-aminobutyric acid concentration of the rat hippocampus was detected using ELISA kit. Results: Pathological results indicated that GWBS improved pyramidal cells in hippocampus keeping the disordered arrangement. In comparison of the model group, the body weights of the weastern medicine group and the GWBS group apparently increased. Besides, the retention time of the light box was significantly prolonged and the concentrations of \( \gamma \)-aminobutyric acid in the hippocampus were significantly promoted in the two groups. The differences above were with statistical significance (\( P<0.05 \)). In addition, the sucrose consumption values were improved markedly. Conclusion: GWBS, the agent of relieving the depressed liver and resolving phlegm, makes a difference in treatment of anxiety and depression rats and its mechanism may be related to the increase of GABA concentration in hippocampus.
1. Introduction
Anxiety and Depression is a clinical symptom of mood disorder. Anxiety and Depression is a type of mood disorder characterized by significant and persistent low mood, slow thought, cognitive impairment, decreased will, and physical symptoms [1].

WHO’s latest statistics on the global prevalence of Anxiety and Depression and dysthymia is 12.8%, which predicts that Anxiety and Depression will become the world’s second medical disease in 2020 [2].

At present, modern medicines, such as tricyclic antidepressant, selective serotonin reuptake inhibitors and so on, have been used to treat anxiety and depression. The drugs with long treatment cycle also have obvious side effects and the high price can compel some patients to halt treatment [3].

GWBS which origins from Three Causes——Diseases and Syndrome (a famous traditional Chinese medicine masterpiece) consists of Wendan Decoction, Bupleurum and Scutellaria Baicalensis Georgi. It has a significant effect on the treatment of anxiety and depression. (Clinical observation of GWBS in the treatment of post-stroke anxiety and depression, GWBS in the treatment of 30 cases of insomnia with anxiety, clinical study of GWBS combined with clomipramine in the treatment of anxiety and depression in college students).

The basic research of GWBS on anxiety and depression is mostly focused on the phenomenon observation. In this study, anxiety and depression model rats were induced by chronic unpredictable stress to be used to observe the effect on behavior and the inhibitory neurotransmitter of GWBS in anxiety and depression rats.

2. Materials and methods

2.1. Animals
The male SD rats, SPF grade and body weight (180 ~ 200)g, were housed in a temperature and humidity-controlled environment.

2.2. Experimental Materials

2.2.1. Drugs and reagents. Experimental reagent: rat γ-aminobutyric acid (GABA) assay kit was from Shanghai Fanke Biotechnology Co., Ltd., 96T; diazepam tablets were obtained from Jining Ankang Pharmaceutical Co., Ltd., Chinese medicine quasi-word H10970219; Bupleurum; Astragalus; Qing Pinxia; medlar; Zhuru; dried tangerine peel; glutinous rice; raw licorice (Guangdong side Tianjiang Fenjian granule).

2.2.2. Experimental equipment. Constant temperature water bath, bench top centrifuge 1 set, electronic needle treatment instrument, microplate reader were provided.

2.3. Methods

2.3.1. Grouping and administration. Twenty male Sprague-Dawley rats were divided into 4 groups: blank control group, model group, western medicine group (0.26mg·kg⁻¹) and GWBS group (11.5 g·kg⁻¹). Except for the blank control group and the model group which were administered with distilled water at 0.4 ml/kg/d, the other groups were given corresponding drugs at 2 ml/kg/d by gavage.

2.3.2. Model preparation. The anxiety and depression model rats were induced by the chronic unpredictable emotional stress with a little change [4]. Except for the blank control group, the other three groups were received 7 days of unpredictable stress including nine kinds of stimulation-foot electric shock, water deprivation for 1h, fasting for 1h, 24h light, 24h dark, co-cage, single cage, tilt and mixed cage (the rats were grabbed randomly 1 or 2 rats from each of two cages). These methods were randomly assigned to seven days, and the rats received 2 or 3 kinds of stress stimulation a day.
2.4. Observations

2.4.1. Light microscopy. After the rats were sacrificed, the light microscopy specimens were immediately fixed in newly prepared 10% neutral formalin for 48h, followed by routine paraffin embedding and sectioning (3µm). Hematoxylin-eosin (HE) staining,

2.4.2. Body weight. Record daily body weights of rats.

2.4.3. Sucrose consumption test. The rats were trained to adapt to sugar drinking water in the laboratory before the experiment: both bottles were filled with 1% sucrose in the first 24 hours, while one bottle was filled with 1% sucrose and the other was filled with tap water in the second 24 hours with two bottles in each cage. Then carried out the sucrose consumption test after being fast 24 hours .At the same time, 200ml 1% sucrose and tap water 200ml respectively were given to each group of rats. One hour later, two bottles of water were taken away to record the consumption [5].

2.4.4. Light-dark box test. The light-dark box (45cm×27cm×27cm) was composed of two parts separated (the light box and the dark box) by a wooden board. A 7.5cm×7.5cm hole on the wooden board was used for rats to go through, and an illuminating device was installed in the upper part of the light box. At the beginning of the experiment, the rats were placed in the center of light box with its back towards the compartment. The residence time of rats in the light box during 5 minutes were observed [6].The experiment was based on the rats' exploration and curiosity about the unknown environment. If the depressive symptoms of rats were alleviated, the minutes of exploring behaviors in the light box would increase.

2.4.5. GABA. The concentration of GABA in rat hippocampus was strictly determined according to the ELISA kit instructions.

2.5. Statistical analyses
Data are presented as the mean ±SD. Differences were evaluated using Statistical Package for Social Science 21.0. Statistical analysis was performed using One-way ANOVA followed by least-significant difference (LSD). \( P<0.05 \) was considered to be statistically significant.

3. Results

3.1. Histopathological results of rat hippocampus (HE staining)
The hippocampal vertebral cells of normal rats were arranged neatly and in a regular shape. The vertebral cells of the hippocampus in the model group were scattered and the cytoplasmic staining was eosinophilic. After the intervention of western medicine or GWBS, the vertebral cells were arranged neatly. (Figure 1)

![Blank control group, Model group, GWBS group, Western medicine group](image)

**Figure 1** Histopathological results of rat hippocampus HE (×200)

3.2. Effect of GWBS on the general state of anxiety and depression model rats
On the first day of the experiment, differences in weights among the groups have no statistical significance.
On the seventh day of the experiment, Compared with the model group, the weights of the weastern medicine group and the GWBS group distinctly increased with statistical difference ($P<0.05$) (Table 1).

**Table 1** Effect of GWBS on the body weights of anxiety and depression model rats

| Group                | First day (g) | Seventh day (g) |
|----------------------|---------------|-----------------|
| Blank control group  | 206.00±11.36  | 256.00±10.82    |
| Model group          | 205.33±11.06  | 183.00±4.00*    |
| Western medicine group | 207.00±3.00  | 208.33±11.24#   |
| GWBS group           | 205.33±8.14   | 212.33±2.52#    |

**Note:** Compared with control groups, *$P<0.05$; Compared with the model groups, # $P<0.05$.

3.3. Effect of GWBS on the sucrose consumption values of anxiety and depression model rats

Relative to the model group, the sucrose consumption values of the weastern medicine group and the GWBS group preference significantly increased (Table 2)

**Table 2** Effect of GWBS on the sucrose consumption values of depression model rats

| Group               | Sucrose consumption values (ml) |
|---------------------|---------------------------------|
| Blank control group | 15                              |
| Model group         | 6                               |
| Western medicine group | 11                           |
| GWBS group          | 10                              |

3.4. Effect of GWBS on the retention time of light-dark box in anxiety and depression model rats

Relative to the model group, the retention time of the weastern medicine group and the GWBS group significantly increased with statistical difference ($P<0.05$) (Table 3).

**Table 3.** Effect of GWBS on the retention time of light-dark box in model rats

| Group                | the retention time of the light box (s) |
|----------------------|----------------------------------------|
| Blank control group  | 135.33±43.65                           |
| Model group          | 14.06±1.73*                            |
| Western medicine group | 262.67±32.58#                       |
| GWBS group           | 292.00±13.86#                          |

**Note:** Compared with control groups, *$P<0.05$; Compared with the model groups, # $P<0.05$

3.5. Effect of GWBS on GABA concentration in hippocampus of anxiety and depression model rats

Compared with the model group, the concentration of GABA in the hippocampus of diazepam and GWBS groups significantly increased with statistical difference ($P<0.05$). (Table 4)

**Table 4.** Effect of GWBS on GABA in hippocampus of depression model rats

| Group                 | GABA ($\mu$mol/m) |
|-----------------------|-------------------|
| Blank control group   | 3.17±0.06         |
| Model group           | 2.07±0.09*        |
| Western medicine group | 3.17±0.16#        |
| GWBS group            | 2.91±0.15#        |

**Note:** Compared with control groups, *$P<0.05$; Compared with the model groups, # $P<0.05$. 


4. Discussion

That GWBS can be applied to improve the disordered arrangement of pyramidal cells in hippocampus was proved by pathological data.

The phenomenon that body mass of most patients with anxiety and depression have reduced in clinical observations is related to the loss of appetite. Similar to this condition, the weights of animals with anxiety and depression also generally decrease in some extent. In the aspect of weights, no difference was observed among the groups of rats in the experiment before the anxiety and depression model rats were induced. In addition, Chinese medicine treatment reversed the weight reduction of the model group after administration. A conclusion has been drawn that GWBS makes a difference in the weight restoration of anxiety and depression rats.

In the sucrose consumption test, the sucrose consumption values and the percentage of preference for the sucrose have been considered as effective indicators used to demonstrate the pleasant sensation of rats [7].

In comparison of the model group, the sucrose consumption values of the two treatment groups were increased apparently, which can indicate that GWBS has markedly therapeutic effect on anxiety and depression rats.

As an important inhibitory neurotransmitter in the mammalian central nervous system, GABA is considered to play a regulatory role in many sites such as cerebral cortex, thalamus, and hippocampus. It is said that the imbalance of GABAergic nervous system function is the main factor leading to anxiety and depression. That the GABA of the central nervous system acts on the GABA receptor and inhibits the activity of nerve cells in a depressed state by inhibiting the postsynaptic potential through changing the chloride ion permeability of the nerve cell membrane is generally believed [8]. In the end of the experiment, the results of hippocampal tissue test showed Chinese medicine treatment had improved the concentrations of GABA significantly in the anxiety and depression model rats. All the evidences above suggest that GWBS makes a difference in treatment of anxiety and depression rats and its mechanism may be related to the increase of GABA concentration in hippocampus.

Acknowledgments

Supported by a project grant from Science and technology project of Henan Province (Grand No.162102310466), Key scientific research projects of Henan Province College sand universities (Grand No.16A360010), The Young Core Teacher of Henan Province (Grant No. 2016GGJS-080).

References

[1] Jiang K.D., Psychiatry. M. Beijing: People's Medical Publishing House, 2005, 123 -124.
[2] Dennis C.L., Dowswell T. Interventions (other than pharmacological, psychosocial or psychological) for treating antenatal Depression. J. Cochrane Database Syst Rev, 2013, 7, 6795-6802.
[3] Zhao Y.X., Chen Y. Effects of ginkgo ketone ester on behavior and inflammatory cytokines in depressed rats. J. Pharmacology and Clinics of Chinese Materia Medica, 2015, 31, 88-92.
[4] Roman O, Seres J, Pometlova M, et al. Neuroendocrine or behavioral effects of acute or chronic emotional stress in Wisata Kyotoa and spontaneously hypertensive (SHR)rats, J. Endocrine regulations, 2004, 38, 151-155.
[5] Deng Zuwei, Yuan Yuping, Lv Longfei. Effect of Ganoderma lucidum spore oil on behavioral science of depressive mice and its neurophysiology. J. Chinese Pharmaceutical Journal, 2017, 52, 1325-1330.
[6] Shan Xin, Kong Zhouyang, Wang Sujuan, etc. study on the anti-anxiety activity of spider incense extract and Total Valeriana. J. Chinese Traditional and Herbal Drugs, 2016, 4, 1361-1365.
[7] Willner P. Validity reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. J. Psychopharmacology, 1997, 134, 319-329.
[8] OuYang J.Y., Hu Z.Y., Chu Y. et. Effects of gamma-aminobutyric acid on nitric oxide synthase
and nitric oxide levels in the frontal cortex of emotional stress rats. *J. Journal of Third Military Medical University*, 2013, 35, 385-389.