

Shuyu capsules relieve liver-qi depression by regulating ERK-CREB-BDNF signal pathway in central nervous system of rat

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Abstract. The purpose of this study was to investigate the possible therapeutic mechanism of Shuyu capsules in liver-qi depression. Liver-qi depression rats were prepared based on chronic unpredictable mild stress (CUMS) and delayed constraint. Rats were gavaged with Shuyu capsule, fluoxetine, Radix Bupleuri and Radix Paeoniae Alba to construct rat models. Body weight test, sucrose preference test and open-field test were applied to test rat models. Western blot analysis and quantitative real-time PCR was applied to determine the relative expression of extracellular signal-regulated protein kinase (ERK), cyclic AMP response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) in hippocampus and frontal lobe tissues. ELISA was used to detect the content of BDNF in serum. Body weight, sugar intake and total distance were significantly decreased in depression group compared with control. The four drugs significantly increased levels of these factors. Compared with control group, ERK, CREB and BDNF expression were significantly decreased in depression group in both hippocampus and frontal lobe tissues at both mRNA and protein level. Shuyu capsule and fluoxetine group showed a significant increase in the expression of ERK, CREB and BDNF at mRNA, p-ERK and p-BDNF at protein level. Compared with Radix Paeoniae Alba, Radix Bupleuri were better in the rescue of ERK, CREB and BDNF expression. In conclusion, the pathogenesis of liver-qi depression associated with lower expression of ERK, CREB and BDNF

in hippocampus and frontal. Shuyu capsule and main constitution alleviated the depressive-like behaviors and reversed the disruptions of the p-ERK, p-CREB and BDNF in stressed rats.

Introduction

Depression disorders are the most severe psychiatric disorders worldwide. The symptoms include retardation of thinking, hypopraxia and downcast mood (1). Many factors are considered to be related with pathogenesis of depression disorder, including internal secretion, nervous system and immune system. Earlier studies presented that liver-qi depression may be related to signal transduction after receptors related to synaptic plasticity, gene transcriptional regulation and target gene expression alteration (2).

Signal transduction system plays a key role in directing signal into cells, and is the target of anti-depression drugs. The pathways involved in depression disorder include cAMP pathway, mitogen-activated protein kinase (MAPK) pathway and CaMK pathway (3-5), of which MAPK pathway plays a key role in the brain-derived neurotrophic factor (BDNF) signal transduction. Extracellular signal-regulated protein kinase (ERK1/2), a number of MAPK family, transmits extracellular stimulus signals into cells, such as neurotransmitter, neurotrophic factors and nerve growth factor (6,7). Besides it involves in pathophysiology of various nervous system diseases by regulating gene expression, synaptic reorganization, axon growth and excitability of neurons (8). Phosphorylation of ERK could activate cyclic AMP response element binding protein (CREB), which is an endonuclear regulatory factor, regulating transcription by self-phosphorylation, and playing a key role in regulating and maintaining emotion and memory (9,10). BDNF is a downstream target of CREB, and is a representative number of neurotrophic factors, functioning in hippocampus and frontal lobe tissues by regulating neuroplasticity (11,12). In an earlier study, we found a significant difference of ERK, CREB and BDNF between liver-qi depression rats and normal rats, indicating that ERK-CREB-BDNF pathway may participate in the pathogenesis of liver-qi depression (13). It is more likely a putative treatment target for liver-qi depression.

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The anti-depression drugs can be divided into five groups, including monoamine oxidase inhibitor, tricyclic antidepressant, selective noradrenaline reuptake inhibitor and selective 5-HT reuptake inhibitor. But long-term intake may induce toxic and side-effect and untoward effect. While Chinese medicine in treating depression showed good curative effect and relative lower recurrence rate. Thus, treatment of depression disorder by using Chinese medicine attracts increased attention.

Shuyu capsule is a kind of Chinese medicine developed by our research group, mainly containing active principle from Radix Bupleuri, rhizoma cyperi, Radix Paeoniae Alba and liquorice. It has been studied in a rat model of premenstrual syndrome depression that Shuyu capsules rectified abnormal 5-HT₃AR and 5-HT₃BR expression and 5-HT₃ channel current changes in a rat model (14). But no pathway alterations was revealed related to depression. Thus in this study, we aimed to analyze the influence on ERK-CREB-BDNF signal pathway of Shuyu capsule and its component.

Materials and methods

Ethics statement. All animal experiments were approved by the Ethics Committee of Taishan Medical University (Tai'an, China).

Animals and reagents. Healthy Wistar male rats (n=48, 150±20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were housed in animal room at 22±2°C. Sterilized diet and water were freely accessed, with an adaptive breeding for 2 weeks. Rats were divided into six groups according to cardinal number of sugar water preference test and open-field test, including control group, model group, Shuyu group, Radix Bupleuri group, fluoxetine group and Radix Paeoniae Alba group, 8 rats in each group.

Shuyu capsule was obtained from Qingdao Haichuang Center for Innovative Biomedical Research (Qingdao, China). Clinical batch no. 2008L11169. Fluoxetine capsule was obtained from Eli Lilly Suzhou Pharmaceutical Co., Ltd. (Suzhou, China). Clinical batch no. J20100016. Radix Bupleuri extraction and Radix Paeoniae Alba were obtained from Qingdao Haichuang Center for Innovative Biomedical Research.

Sucrose preference test. Sucrose preference test was performed according to Moriyama *et al* (15). Two drinking spouts (water and 1% sucrose) were provided to rats after water starvation for 24 h. Bottles were exchanged every 1 h. Sucrose preference was calculated as: Sucrose preference = [sucrose consumption (ml)/water consumption (ml) + sucrose consumption (ml)] x 100%.

Open-field test. Open-field test was performed according to Zhu *et al* (16). Before the experiments, rats were allowed an adaptive activity. Rats were placed in the center of the arena, and the activity of rats was recorded, including the number of vertical activity, total journey, the time spend in the center of arena. The total journey was considered as an index of this test.

Stress design. Liver-*qi* depression rats were prepared based on chronic unpredictable mild stress (CUMS) and

delayed constraint (17,18). Depression rats were prepared by providing 2-3 random types of stimulus every day, lasting for 4 weeks. The stimulus included cage inclining (45°), continuous illumination, dirty cage (pouring 200 ml water in cage), empty bottle, limiting food, white noise, strobe light, smell (mothball), foreign matter (block, a strip of cloth), rotation and constraint (four legs restrained by adhesive plaster).

Rats model. Control group rats were prepared without any stimulus, and given a gavage of normal saline (1 ml/200 g). Model group were prepared by giving a gavage of normal saline (1 ml/200 g) to depression rats. Shuyu group was prepared by giving a gavage of Shuyu capsule (4.08 mg/kg, about 8-fold dosage of human) to depression rats. Radix Bupleuri group was prepared by giving a gavage of Radix Bupleuri capsule (4.08 mg/kg) to depression rats. Fluoxetine group was prepared by giving a gavage of fluoxetine (0.72 mg/kg/day) to depression rats. Radix Paeoniae Alba group was prepared by giving a gavage of Radix Paeoniae Alba (36 mg/kg/day) to depression rats.

Model evaluation and sampling. Rats were anesthetized using 3% chloral hydrate (0.3 ml/100 g). Blood was obtained from ostatica, half was stored in Ep tubes treated with heparin in advance. The rest blood was centrifuged at 1200 x g, 4°C for separating serum. All samples were stored at -70°C.

Western blot analysis and ELISA. Blood was obtained from ostatica after rats were anesthetized using 3% chloral hydrate (0.3 ml/100 g). Hippocampus and frontal lobe tissues were obtained from head after rats were sacrificed. All samples were stored at -70°C.

Western blot analysis was performed according to standard protocols. Total protein was extracted using RIPA lysate. Protein concentration was measured with BCA protein assay kit (Pierce, Bonn, Germany) according to the manufacturer's instructions. Total cellular proteins were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes (EMD Millipore, Billerica, MA, USA). After blocking in 5% skim milk for 1 h, the membranes were incubated with primary antibodies (β-actin, A1978, 1:2,000; Sigma-Aldrich, St. Louis, MO, USA) (ERK, cat. no. 9102, 1:2,000; p-ERK, cat. no. 9101, 1:2,000; CREB, cat. no. 9197, 1:150; P-CREB, cat. no. 9191, 1:150; all from Cell Signaling Technology, Inc., Danvers, MA, USA) (BDNF, cat. no. ab108383, 1:1,200; Abcam, Cambridge, MA, USA) overnight at 4°C, followed by the incubation with goat anti-rabbit secondary antibodies (ERK, 1:2,000; CREB 1:150; BDNF 1:1,200) for 2 h at room temperature. Protein bands were visualized on X-ray film using enhanced chemiluminescence ECL substrate (Pierce).

The levels of BDNF were detected with enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction.

Quantitative-real time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized from RNA samples using a RevertAid First-Strand cDNA Synthesis kit (Takara, Dalian, China).

qRT-PCR was performed with SYBR Premix ExTaq (Takara) with a LightCycler 480 apparatus (Roche Molecular Systems, Inc., Pleasanton, CA, USA). β -actin was used for internal control. The relative expression of genes was calculated by $2^{-\Delta\Delta C_q}$ method. The sequences of primers were: ERK forward, 5'-GTGAAGTTTCATTTCCAATCCGC-3' and reverse, 5'-GGGACATCACCCCTCACTTAC-3'; CREB forward, 5'-CCATCCACTCCTGTGTCATCT-3' and reverse, 5'-CCTTGTAATCCTCTTCCATCA-3'; BDNF forward, 5'-CACCCGCGAGTACAACCTTC-3' and reverse, 5'-CCCA TACCACCATCACACC-3'.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). All data are presented as mean \pm SD. A value with $P < 0.05$ indicated statistically significant difference.

Results

Body weight analysis. Body weight was measured once a week after model rats were made, as shown in Fig. 1. In week 4, body weights were significantly decreased in liver-qi depression model rats ($P < 0.01$) in comparison with control group, but no significance were found in drug group (Table I). Compared with depression model group, body weights were significantly increased in Shuyu group and fluoxetine group ($P < 0.01$), and no significant difference was found in Radix Paeoniae Alba group and Radix Bupleuri group.

Sugar preference test. Sugar consumption was tested once a week in the six groups (Fig. 2). In week 4, sugar consumption was significantly decreased in depression group in comparison with normal control ($P < 0.05$), and significant increase was found in drug groups in comparison with depression group ($P < 0.01$), indicating that depression rats lacked pleasant sensation, which could be improved by fluoxetine, Shuyu capsule, Radix Bupleuri extraction and Radix Paeoniae Alba extraction (Table II).

Open-field test. Total journey of rats in open-field was recorded once a week (Fig. 3). In week 4, total journey in depression group was significantly decreased compared with control group ($P < 0.01$), and no significant difference were found in drug groups (Table III). Compared with depression group, total journey in fluoxetine group ($P < 0.01$), Shuyu group ($P < 0.01$) and Radix Bupleuri group ($P < 0.05$) was significantly increased, while no significant increase was found in Radix Paeoniae Alba group.

Detection of ERK, BDNF and CREB in hippocampus and frontal lobe tissues. Expression levels of BDNF, ERK and CREB in hippocampus and frontal lobe tissues were detected at both mRNA and protein levels.

ERK expression. ERK expression was detected in hippocampus and frontal lobe tissues in all groups. In hippocampus tissues (Fig. 4A and B), ERK expression was significantly decreased compared with control group ($P < 0.05$) at both mRNA and protein level. After gavaged with Shuyu capsule or fluoxetine, ERK expression was significantly increased ($P < 0.01$), nearly to the normal group. Although in Radix

Table I. Comparison of weight (mean \pm SD) in rats in week 4.

Group	Before modeling	After modeling
Control group	172.7 \pm 2.215	258.7 \pm 4.030
Depression model group	170.1 \pm 2.493	225.7 \pm 3.290 ^a
Shuyu group	174.2 \pm 1.914	251.3 \pm 4.882 ^b
Fluoxetine group	175.0 \pm 2.270	252.1 \pm 5.692 ^b
Radix Bupleuri group	170.5 \pm 3.312	245.3 \pm 4.393 ^c
Radix Paeoniae Alba group	169.8 \pm 4.704	244.3 \pm 6.218 ^c

^aCompared with control group, significant difference was $P < 0.01$. ^bCompared with depression model group, there was significant difference, $P < 0.01$ and ^ccompared with depression model group, there was significant difference, $P < 0.05$.

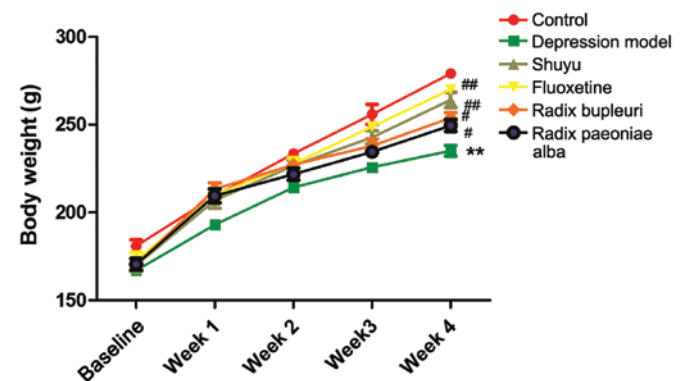


Figure 1. Body weight comparison. Body weight was significantly decreased in depression models compared with control group ($P < 0.01$). Compared with depression models, there is significant increase in the drug groups ($P < 0.05$). ^aCompared with depression models, $P < 0.05$. ^{##}Compared with depression models, $P < 0.01$. ^{**}Compared with control group, $P < 0.01$.

Bupleuri group and Radix Paeoniae Alba group ERK expression was increased, it was still less than it in control group ($P < 0.05$). In frontal lobe tissues (Fig. 4C and D), similar results were obtained, except for the Radix Bupleuri group and Radix Paeoniae Alba group. No significant difference was found in the two groups compared with depression model group.

BDNF expression. BDNF expression was detected in hippocampus and frontal lobe tissues in all groups. In hippocampus tissues (Fig. 5A and B), BDNF expression was significantly decreased compared with control group ($P < 0.01$) at both mRNA and protein level. After gavaged with Shuyu capsule or fluoxetine, BDNF expression was significantly increased ($P < 0.01$), and in Radix Bupleuri group and Radix Paeoniae Alba group, it was also increased ($P < 0.05$). In frontal lobe tissues (Fig. 5C and D), similar results were obtained, except for the Radix Bupleuri group and Radix Paeoniae Alba group. No significant difference was found in the two groups compared with depression model group.

CREB expression. CREB expression was detected in hippocampus and frontal lobe tissues in all groups. In hippocampus tissues (Fig. 6A and B), CREB expression was significantly decreased compared with control group ($P < 0.01$) at both mRNA and protein level. After gavaged with

Table II. Sugar consumption was tested in week 4 (mean \pm SD).

Groups	Before modeling	After modeling
Control group	0.8137 \pm 0.02404	0.8755 \pm 0.02270
Depression model group	0.7822 \pm 0.04440	0.5507 \pm 0.04922 ^a
Shuyu group	0.7992 \pm 0.01608	0.8182 \pm 0.04202 ^b
Fluoxetine group	0.7734 \pm 0.03438	0.8329 \pm 0.02393 ^b
Radix Bupleuri group	0.7902 \pm 0.02464	0.7652 \pm 0.02632 ^b
Radix Paeoniae Alba group	0.7760 \pm 0.04704	0.7930 \pm 0.02401 ^b

^aCompared with control group, there was significant difference, $P < 0.01$. ^bCompared with depression model group, there was significant difference, $P < 0.01$.

Table III. Total journey of rats in open-field test.

Groups	Before modeling	After modeling
Control group	13,327 \pm 756.5	9,786 \pm 654.8
Depression model group	12,585 \pm 801.2	5,447 \pm 356.4 ^a
Shuyu group	12,732 \pm 669.1	9,320 \pm 531.9 ^b
Fluoxetine group	13,073 \pm 810.2	9,646 \pm 718.6 ^b
Radix Bupleuri group	12,336 \pm 980.8	9,057 \pm 643.0 ^c
Radix Paeoniae Alba group	13,097 \pm 794.3	9,024 \pm 589.4

^aCompared with control group, there was significant difference, $P < 0.01$.

^bCompared with depression model group, there was significant difference, $P < 0.01$, and ^c $P < 0.05$.

Table IV. Concentration of BDNF in peripheral blood.

Groups	Concentration (ng/ml)
Control group	162.3 \pm 14.54
Depression model group	116.5 \pm 4.627 ^a
Shuyu group	161.6 \pm 8.025 ^b
Fluoxetine group	161.34 \pm 8.286 ^b
Radix Bupleuri group	158.8 \pm 6.206 ^b
Radix Paeoniae Alba group	161.1 \pm 6.764 ^b

^aCompared with control group, there was significant difference, $P < 0.05$.

^bCompared with depression model group, there was significant difference, $P < 0.05$. BDNF, brain-derived neurotrophic factor.

Shuyu capsule ($P < 0.01$) fluoxetine ($P < 0.01$), Radix Bupleuri ($P < 0.05$) or Radix Paeoniae Alba ($P < 0.05$), BDNF expression was significantly increased at mRNA level. At protein level (Fig. 6B), Shuyu capsule ($P < 0.01$) fluoxetine ($P < 0.01$), Radix Bupleuri ($P < 0.05$) showed a significant increase of p-CREB/CREB. In frontal lobe group, all the drug groups showed significant increase of CREB compared with depression model group at mRNA level (Fig. 6C). At protein level, only Radix Paeoniae Alba group didn't present significant increase of p-CREB/CREB (Fig. 6D).

BDNF concentration in peripheral blood. BDNF concentration was detected in peripheral blood of all groups using

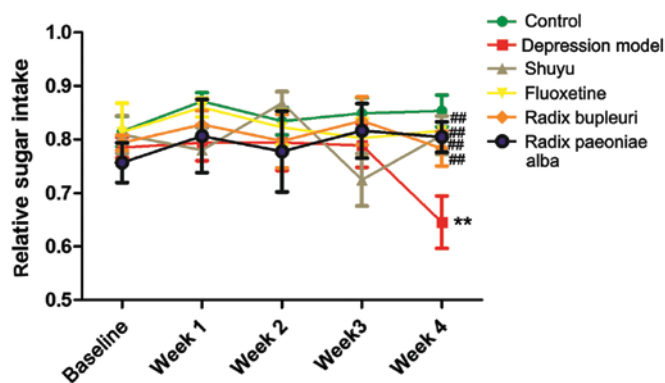


Figure 2. Sugar preference test. Compared with control group, sugar intake was significantly decreased in depression models ($P < 0.01$). Compared with depression models, sugar intake was significantly increased in all the drug groups ($P < 0.01$). **Compared with control group, $P < 0.01$. ##Compared with depression models, $P < 0.01$.

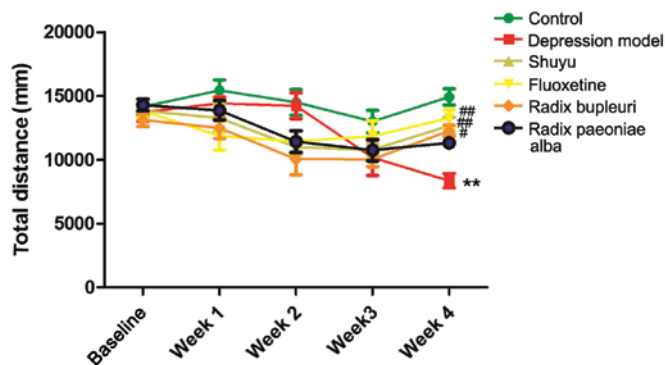


Figure 3. Total distance test. Depression models showed significantly decreased total distance ($P < 0.01$). Except for Radix Paeoniae Alba, the other three drugs significantly increased total distance ($P < 0.05$). **Compared with control group, $P < 0.01$. #Compared with depression models, $P < 0.05$. ##Compared with depression models, $P < 0.01$.

ELISA. Compared with normal control, BDNF concentration was significantly decreased in depression rats ($P < 0.05$), and it was significantly increased in the four drug groups compared with depression model group ($P < 0.05$) (Table IV).

Discussion

CUMS is a typical method to construct liver-qi depression models (19). In this study, we constructed liver-qi depression rat models by combining CUMS and delayed constraint stimulus. In the procedures, rats were randomly given several stimuli, which was similar with human depression formation mechanism (20).

In this study, we applied open-field test, weight and sucrose preference test to evaluate depression rat models, of which open-field test is the most important method to detect rats' excitability and environmental adaptation (21). Open-field test showed that total journal of depression models were significantly decreased compared with control group, and it was significantly increased in all the drug groups. These findings indicated that Shuyu capsule and its component may effectively increased adaptive capacity and excitability in depression rats.

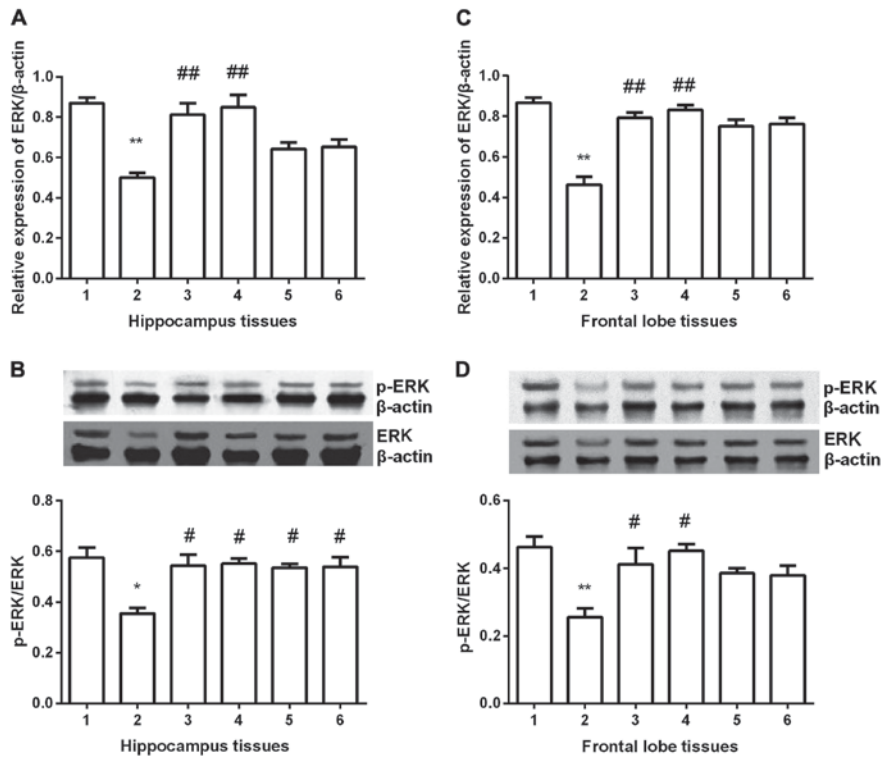


Figure 4. Expression of ERK in hippocampus and prefrontal cortex tissues. ERK expression was significantly decreased in depression group ($P < 0.01$) and Shuyu capsule and fluoxetine significantly increased ERK expression at mRNA level (A and C). Western blot analysis showed that p-ERK/ERK was significantly decreased and Shuyu capsule and fluoxetine significantly increased p-ERK/ERK in both tissues ($P < 0.05$) (B and D). Radix Bupleuri and Radix Paeoniae Alba significantly increased p-ERK/ERK only in hippocampus tissues ($P < 0.05$) (B). **Compared with control group, $P < 0.01$. #Compared with depression models, $P < 0.05$. ##Compared with depression models, $P < 0.01$. ERK, extracellular signal-regulated protein kinase; p-, phosphorylated; 1, control group; 2, depression group; 3, Shuyu group; 4, fluoxetine group; 5, Radix Bupleuri; 6, Radix Paeoniae Alba.

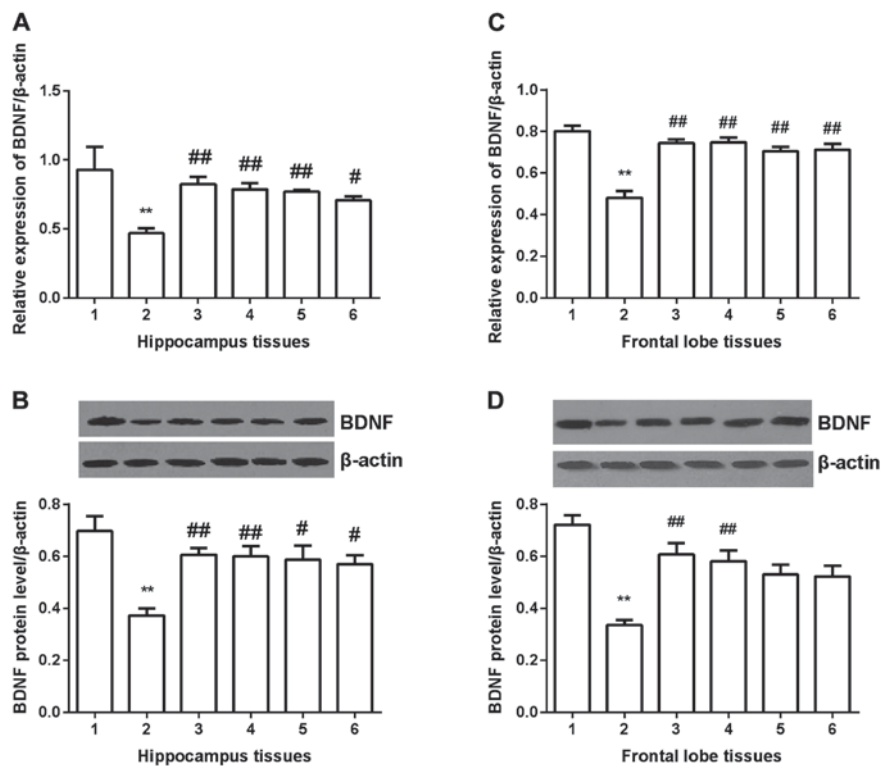


Figure 5. Expression of BDNF in hippocampus and prefrontal cortex tissues. BDNF expression was significantly decreased at both mRNA and protein level. All the drugs significantly increased BDNF expression at mRNA level (A and C). At protein level, in hippocampus tissues, all the four drugs significantly increased BDNF expression (B), but in prefrontal cortex tissues, only Shuyu capsule and fluoxetine significantly increased BDNF expression (D). **Compared with control group, $P < 0.01$. #Compared with depression models, $P < 0.05$. ##Compared with depression models, $P < 0.01$. BDNF, brain-derived neurotrophic factor; 1, control group; 2, depression group; 3, Shuyu group; 4, fluoxetine group; 5, Radix Bupleuri; 6, Radix Paeoniae Alba.

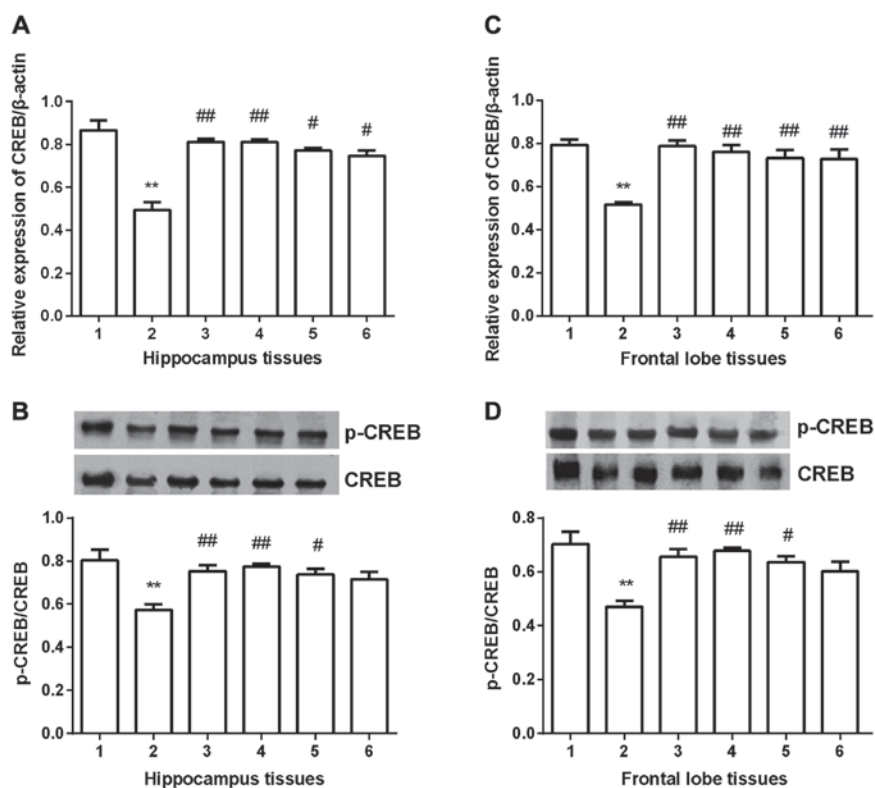


Figure 6. Expression of CREB in hippocampus and prefrontal cortex tissues. At mRNA level, CREB expression was significantly decreased in depression group compared with depression group and all the four drugs significantly increased its expression (A and C). Western blot analysis showed that p-CREB/CREB was significantly decreased in depression group compared with control group, and only Radix Paeoniae Alba did not significantly increase p-CREB/CREB (B and D). **Compared with control group, $P < 0.01$. #Compared with depression models, $P < 0.05$. ##Compared with depression models, $P < 0.01$. CREB, cyclic AMP response element binding protein; p-, phosphorylated; 1, control group; 2, depression group; 3, Shuyu group; 4, fluoxetine group; 5, Radix Bupleuri; 6, Radix Paeoniae Alba.

Although fluoxetine dramatically increased adaptive capacity and excitability in rats, long-term medication may induce overexcitation in central nervous system (22).

Food-intake and digestion of rats were detected by weight. Many depression patients showed decreased appetite and digestion ability, thus it is an important index in evaluating depression rats. In this study, weight increment in depression models was significantly decreased compared to other groups ($P < 0.05$), indicating that depression rats showed obviously decreased appetite, but fluoxetine and Shuyu capsule effectively improved the situation.

Sucrose preference test was used to detect pleasant sensation of rats. The preference coefficient is low when pleasant sensation to environment is lost (23). In the present study, sucrose preference in drug groups was significantly increased compared with depression rats, indicating that capsules and components showed effective performance in increasing of pleasant sensation.

The mechanism of depression is closely associated to the central nervous system. In this study, we chose hippocampus and frontal lobe as subject due to the two districts regulating motion and behavioristics via variety of pathways (24). According to the posthumous autopsy report of depression patients, hippocampus was atrophy to some extent (25). Earlier study showed that hippocampus and fascia dentata volume was much smaller in patients with Alzheimer disease compared with normal samples, which suggested that the shrinking of hippocampus induced cognitive function obstacle, resulting

in depression (26). Prefrontal cortex functions importantly in the emotion process as it is found that left prefrontal cortex compensatory conducting emotion stimulus in the analysis of magnetoencephalogram in depression patients. Magnetic stimulation interference across cranium showed that prefrontal lobe provided attention in the coding and extracting memory (27). Thus prefrontal lobe performs important roles in regulation of emotion and cognitive function.

ERK belongs to the MAPK family, which participates in the physiology activity, including signal transmission and recognition, cell growth and development, and proliferation (28). Signals are transmitted through MAPK signal pathway via three kinase cascades. ERK pathway is the classical MAPK signal pathway, and the reaction chain mainly consists of three protein kinases. ERK pathway regulates cell proliferation, differentiation or apoptosis through the Ras/Raf/MEK/ERK pathway (29,30). In this study, we found that expression of ERK was significantly lower than other groups at both mRNA and protein level, which suggested that liver-*qi* depression disorder may be related to the lower expression of ERK.

CREB is widely distributed in hippocampus and cerebral cortex (31). Dephosphorylation of CREB means it has no transcriptional activity. CREB is phosphorylated if signals stimulate and recognize the corresponding site of CREB and then activate target gene transcription. CREB-defective rats showed dysfunction in neuro plasticity in mice, mainly in the memory function related with auditory sense (32). Dysmnnesia is improved after endogenous CREB expression is increased,

indicating that CREB could obviously promote nerve excitability caused by cerebral injury (33). In this study, CREB expression was significantly decreased in depression group. It is increased in Shuyu group, fluoxetine group and Radix Bupleuri group nearly to the normal level. It is suggested that these three drugs increased CREB expression and showed anti-depression effect by improving depression, emotion and behavior.

BDNF is a member of neurotrophic factor family. Earlier study showed that long-term chronic stress could reduce BDNF level in cerebrum limbic system, and further induce atrophy of hippocampus and prefrontal cortex (34). BDNF functions in the nerve-protective and anti-depression mechanism by promoting hippocampus proliferation and differentiation via TrkB regulation, and by increasing antioxidase and scavenging free radical (35). BDNF is also found in peripheral nervous system, which may be related to the ability of across hemato-encephalic barrier. In depression patients and rats, BDNF expression is decreased and can be rescued by anti-depression drugs, indicating that BDNF may be the target of anti-depression drugs, but it is uncertain. In this study, we identified BDNF expression was significantly decreased in depression rats compared with normal control. The expression was rescued when rats were gavaged with drugs. Thus we suggested that anti-depression drugs exerts anti-depression function by increasing BDNF expression at both mRNA and protein level.

In this study, fluoxetine, Shuyu capsule, *Radix Paeoniae Alba* and Radix Bupleuri were used to detect the effect on depression rats. Radix Paeoniae Alba and Radix Bupleuri are two mainly components in capsule. Radix Bupleuri showed effective promotion on the expression of ERK, CREB and BDNF, indicating that it is the main effective constituent.

There are some limitations in this study: i) In addition to hippocampus and prefrontal cortex tissues, inferior colliculus, amygdaloid nucleus and corpus striatum also involved in the regulation of depression. Further study is needed to investigate on these encephalic regions. ii) We prepared liver-qi depression models by combining CUMS and delayed constraint. Classic evaluation method can be used on depression model, but there is still controversy in evaluating liver-qi depression model. iii) To investigate the active composition, we analyzed the effect of Radix Bupleuri and Radix Paeoniae Alba on depression rats, but did not extract the effective monomer, which still needs more research.

Acknowledgements

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