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Original Research Article

Puerarin mitigates symptoms of depression in ovariectomized female rats by regulating hippocampal cAMP-CREB-BDNF signaling pathway

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Abstract

Purpose: *To investigate the effect of puerarin on the symptoms of depression in ovariectomized female rat model, and the mechanism of action involved.*
Methods: *Ninety healthy female Sprague Dawley (SD) rats were assigned to five groups: sham, model, low-dose puerarin, medium-dose puerarin, and high-dose puerarin groups, with 18 rats per group. Changes in the levels of dopamine (DA), norepinephrine (NA), 5-hydroxytryptamine (5-HT), and apoptosis-related proteins were determined and compared.*
Results: *Compared with the sham group, vertical and horizontal scores, and levels of DA, NA, 5-HT, Bcl-2, p-CREB and BDNF in the model group were significantly decreased. On the other hand, duration of immobility in forced swimming was prolonged, while the concentrations of caspase-1 and ASC increased significantly ($p < 0.05$). Compared with the model group, with increase in pueraria dose, vertical and horizontal scores, and expression levels of DA, NA, 5-HT, Bcl-2, p-CREB and BDNF of rats were gradually increased, duration of immobility in forced swimming was gradually shortened, and the expression levels of Caspase-1 and ASC w decreased gradually, relative to model group ($p < 0.05$).*
Conclusion: *Puerarin mitigates depression-like behavior of ovariectomized-depression rats by activating cAMP-CREB-BDNF signaling pathway. Thus, this compound is a potential new lead in the development of drugs for the treatment of depression in ovariectomized women.*

Keywords: *Puerarin, cAMP-CREB-BDNF signaling pathway, Ovariectomized, Depression rats*

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INTRODUCTION

Depression is a common chronic neuropsychiatric disease characterized by repeated attacks, high morbidity and disability, as well as high rates of recurrence and suicide [1]. It is estimated that depression affects more than 122 million people worldwide, and about 800,000 of these people die by suicide each year. In addition, most patients with depression have mental cognitive dysfunction which affects attention, executive function, as well as learning and memory, all of which have serious impacts on the quality of life of patients.

The pathogenesis of depression is still not clear,

although it is believed that it may be the result of interactions of multiple factors such as viral infection, environmental stress, emotional trauma and neuro-developmental abnormalities [2]. The perimenopausal period is the most sensitive period of female emotional disorders. During this period, women's mood is extremely unstable, and they may show irritability, insomnia, and even depression, anxiety and other symptoms [3]. Studies have found that perimenopausal depression not only brings about emotional changes, it also aggravates physical diseases and increases the incidence of osteoporosis and diabetes [4]. Therefore, it will be of great significance to develop a safe way of treating perimenopausal depression. At present, tricyclic antidepressants, monoamine oxidase inhibitors and other drugs are mainly used in clinical treatment of depression. However, antidepressants take a long time to exert their effects, and they have certain side effects. Thus, they do not completely relieve the symptoms in patients with major depression. Puerarin is an isoflavone compound extracted from *Pueraria*. It dilates tubular blood vessels and improves microcirculation. In recent years, studies have found that puerarin has a strong anti-depression effect [5]. However, its mechanism of action in the treatment of perimenopausal depression is still unclear. In this study, a model of depression was established in ovariectomized female rats, and the rats were used to determine the effect of puerarin on depression, as well as the associated mechanism.

EXPERIMENTAL

Animals

Ninety healthy female Sprague Dawley (SD) rats were obtained from Zhejiang Weitong Lihua Experimental Animal Technology Co. Ltd. [production license SCXK (Zhejiang) 2020-0002, usage license SYXK (Zhejiang) 2019-0003)]. The rats had a mean body weight of 217 ± 13 g. All rats were kept in laboratory at mean temperature of 23 ± 2 ℃ and relative humidity of 55 ± 10 % in a 12 h light/12 h dark environment. They were allowed *ad libitum* access to feed and water.

Main equipment and reagents

The major reagents and equipment used, and their sources (in brackets) were: H&E staining kit (Solarbio Bioscience & Technology Co. Ltd); PCR detection kit (Shanghai Hengyuan Biotechnology Co. Ltd); mouse anti-human CREB monoclonal antibody (Beijing Aorui Dongyuan Biotechnology Co. Ltd); rabbit anti-human BDNF polyclonal antibody (Shanghai Hengfei Biological Technology Co. Ltd); puerarin (Jiangxi Zhongshan Pharmaceutical Co. Ltd, batch no. 20184384, specifications: 2 mL: 100mg); constant temperature water bath box (Tianjin Hengao Science and Technology Development Co. Ltd, Model: HWT-6B); paraffin slicing machine (Shenyang Hengsong Technology Co. Ltd, Model: HS-S7220-B); -80 ℃ ultra-low temperature refrigerator (Beijing Alice Biotechnology Co. Ltd, Model: DW-86L626), and low-temperature, high-speed centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co. Ltd, model BH1200R).

Establishment of ovariectomized female rat depression model

The rats were anesthetized and fixed in supine position. The hair was shaved below the last ribs on the dorsal face of each rat, and the shaved surface was routinely disinfected. A small incision was slowly made about 2 cm from the spine to the abdominal cavity. The ovaries were clipped, and the fallopian tubes were ligated. The ovaries on both sides of the rats were removed, then sutured layer by layer and disinfected. This study received approval from the Animal Ethical Committee of Beijing Luhe Hospital Affiliated to Capital Medical University according to "Principles of Laboratory Animal Care" (NIH, 1985) [6].

The rats were assigned to five groups: sham, model, low-dose puerarin, medium-dose puerarin, and high-dose puerarin groups, with 18 rats per group. In the sham operation group, only para-ovarian adipose tissue was removed.

Rats in the sham group were fed normal feed and water, without any stimulation, while those in model and each puerarin group were subjected to multiple stress factors i.e., electric shock on the plantar, using rat passive avoidance box; 30 volts, 3 times/min, 10 sec at a time at 10-sec intervals, in a total of 15 times; heat stress (rats were placed in a self-made water bucket of height 16 cm, with 45℃ hot water and water depth of 14 cm, and the rats were removed after 5 min); swimming in ice-cold water (rats were placed in a self-made water bucket of height 16 cm, with 5 ℃ cold water and water depth of 14 cm, and the rats were removed after 5 min). One stress was randomly selected every day for a total of 21 days.

After three weeks of stimulation, the rats were given their respective drugs. Rats in the sham operation and model groups received normal saline via gavage, while those in the low-dose, medium-dose and high-dose puerarin groups

received puerarin at doses of 50, 100 and 200 mg/kg, respectively, once daily for 4 weeks. Rats weights and behavioral changes were compared amongst the groups 21 days after stress.

Open field test

The open field experiment was carried out on the 20th day of stress. The rats were put into an open field behavior experimental box, and their behaviors were monitored for 10 min. The experimental box was cleaned thoroughly at the end of the experiment. Horizontal movement scores (3 to 4 feet in a grid at the same time, was scored 1 point; walking through a grid was scored 1 point, walking more than 8 cm along the line was scored 1 point), and vertical movement scores (both forefeet off the ground was scored 1 point) of rats in both groups were recorded.

Forced swimming test

The rats were placed in a glass tank containing water at a temperature of about 25 ℃, allowed to swim for 5 min, and the duration of immobility was observed within 3 min. The experimental box was cleaned thoroughly after an experiment with each rat.

Histopathological changes in the hippocampus were compared amongst the groups of rats. Six rats were taken from each group, and their hippocampal tissues were extracted and routinely made into paraffin sections which were then dewaxed with xylene, dehydrated with gradient alcohol, subjected to hematoxylin dye staining for 15 min, rinsed with phosphate buffer, treated with eosin dye for 3 min and rinsed again with phosphate buffer. The sections were dried at room temperature, dehydrated, cleared, sealed using neutral gum, and examined microscopically.

The concentrations of dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in hippocampus of rats in each group were determined. After the rat behavior experiment, 6 rats were taken from each group and fasted for 10 h. The hippocampal tissues of the rats were subjected to high performance liquid chromatography-electrochemical analysis, using 3, 4-dihydroxybenzoic acid as internal standard. The hippocampus of each rat was isolated on ice, homogenized with homogenization buffer, and the supernatant was filtered through a 0.22-μm filter, followed by quantitative analysis with a chemical detection instrument. The results were displayed on a chromatographic software and calculated with a computer. The expression levels of apoptosis-related protein Bcl-2, Caspase-1, ASC, *p*-CREB and BDNF in rat hippocampal tissues in each group were assayed with immunoblotting method. Brain tissue of each rat was lysed in lysis buffer and crushed on ice. The lysate was centrifuged, and the supernatant was stored in an ultra-low temperature refrigerator at -80 ℃. Following determination of lysate protein contents, the proteins were resolved with SDS-PAGE, followed by transfer to PDVF membranes which were sealed by incubation with 5 % non-fat milk for 1 h. Then, the membranes were incubated overnight at 4 ℃ with primary antibodies, followed by rinsing and incubation with HRP-linked secondary antibody for 1 h at room temperature. Image analysis was performed using an Image software.

Statistics

Measured data are presented as mean ± SD. Independent sample *t*-test was employed for two-group comparison of measurement data, while single-factor multivariate comparison was used for comparison amongst multiple groups. The SPSS22.0 software package was used for statistical analysis of data. Values of $p < 0.05$ indicated statistically significant differences.

RESULTS

Variations in rat weight

Before stress, body weight was comparable amongst the groups. However, relative to sham operation rats, marked reduction in body weight was seen in model rats. However, rat weights increased gradually with increase in dose of puerarin, when compared with model rats ($p < 0.05$). These data are shown in Table 1.

Table 1: Variations in rat weights (mean ± SD, n = 18)

Group	Before stress	21 days after stress
Sham	222.10±3.37	352.62±14.60
Model	221.96±3.65	300.42±19.83
Low-dose puerarin	221.74±3.22	316.84±15.57
Medium-dose puerarin	221.65±4.45	322.22±13.72
High-dose puerarin	221.42±3.18	331.76±11.35
F	0.10	28.73
P-value	0.983	<0.001

Behavioral changes

There were marked reductions in vertical and horizontal scores in the model rats, and the duration of immobility in forced swimming was prolonged, relative to sham operation rats ($p < 0.05$). With increase in puerarin dose, the vertical

and horizontal scores of rats were gradually increased, and the duration of immobility in forced swimming was gradually shortened, relative to model group ($p < 0.05$). These results are shown in Table 2.

Histological and pathological changes in rat hippocampus

In the sham group, the neurons were high in number and arranged neatly, with intact morphology, and the nucleoli were clear. In the model group, the normal structure of neurons disappeared, the cell distribution was decreased, and the nucleoli were smaller and pyknotic. In the puerarin group, the hippocampal tissue was gradually improved with increase in puerarin dose, with the high-dose puerarin group being similar to the sham operation group. These results are presented in Figure 1.

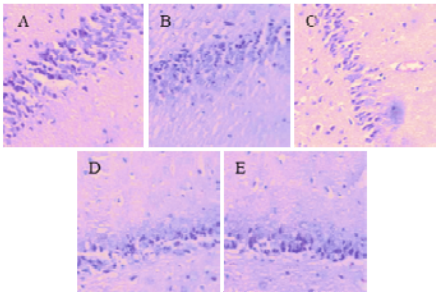

Figure 1: Histological and pathological changes in the hippocampus in rats. A: sham; B: model; C: low-dose puerarin; D: medium-dose puerarin, and E: high-dose puerarin groups

Variation in DA, NA and 5-HT levels

The levels of DA, NA and 5-HT in brain tissue of rats in the model group were significantly decreased, relative to the sham group ($p < 0.05$). However, with increase in puerarin concentration, the levels of DA, NA and 5-HT were gradually increased, relative to model group ($p < 0.05$).

Expression levels of apoptosis-related proteins in hippocampal tissues of rats

Figure 2 shows that the hippocampal expression level of apoptosis-related protein Bcl-2 in model rats was significantly decreased, but the expressions of caspase-1 and ASC were markedly up-regulated, relative to sham**.** With increase in puerarin concentration, the Bcl-2 protein level was gradually increased, while those of ASC and caspase-1 were gradually decreased, relative to model rats *(p* < 0.05).

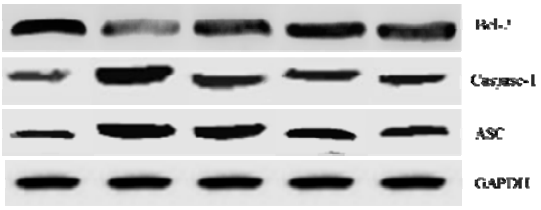

Figure 2: Comparison of expression levels of apoptosis-related proteins in hippocampal tissues of rats

Table 2: Behavioral changes in rats (mean ± SD, n = 18)

Group	Number through the grid (points)		Immobility time in forced swimming (s)
	Horizontal score	Vertical score	
Sham	99.87±5.42	37.25±5.56	80.56±10.34
Model	37.36±1.25	17.15±1.13	125.89±5.54
Low-dose puerarin	42.22±3.36	19.57±3.71	113.52±9.43
Medium-dose puerarin	50.41±2.51	23.25±1.69	106.72±4.26
High-dose puerarin	62.85±6.12	25.83±2.74	97.43±6.82
F	656.23	97.28	90.13
P-value	<0.001	<0.001	<0.001

Table 3: Changes in DA, NA and 5-HT expression levels in each group of rats (mean ± SD, n = 6)

Group	DA (ng/mg)	NA (ng/mg)	5-HT (ng/mg)
Sham	1.47±0.12	1.74±0.04	0.73±0.03
Model	0.59±0.05	0.58±0.03	0.31±0.05
Low-dose puerarin	0.69±0.06	0.69±0.04	0.39±0.03
Medium-dose puerarin	0.77±0.05	0.81±0.06	0.43±0.02
High-dose puerarin	0.85±0.08	1.01±0.04	0.48±0.07
F	122.73	685.90	79.13
P-value	<0.001	<0.001	<0.001

Expression levels of p-CREB and BDNF in the hippocampus of rats

As presented in Figure 3, the expression levels of p-CREB and BDNF in the hippocampus of model group were significantly decreased, relative to sham group. However, with increase in puerarin concentration, the expression levels of p-CREB and BDNF were gradually increased, when compared to model rats.

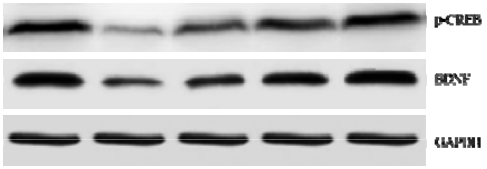

Figure 3: Expression levels of p-CREB and BDNF in rat hippocampal tissue

DISCUSSION

Depression is a chronic disease caused by various factors. Its main clinical features are marked and persistent depression, impaired activity, and slow thought and cognitive function. The onset of depression is generally relatively slow, during which loss of appetite, loss of sleep quality, hallucinations, and delusions may occur. In severe cases, patients may even lose their desire to live [7]. Socio-economic developments have led to sustained increases in all kinds of pressure. These also increase the incidence of depression, leading to a series of family and social problems and economic losses. Therefore, medical experts pay a lot of attention to the treatment of depression.

Perimenopausal depression is a common type of depression in women. At present, psychotherapy and biological therapy are used for the treatment of depression. The biological therapy includes electrotherapy and drug therapy, while drug therapy is the preferred method for the treatment of depression, although most drugs for depression are expensive and prone to drug resistance [8]. In traditional Chinese medicine, there is an early understanding of depression, which was often classified as "depression syndrome". Studies have found that the treatment of depression with traditional Chinese medicine can result in obvious clinical efficacy, with minimal adverse reactions, and it does not easily result in drug resistance [9].

Puerarin is the major component of extract of *Pueraria*, known as "the good medicine of the earth". It dilates blood vessels, promotes blood circulation, removes blood stasis, improves microcirculation, and exerts anti-oxidant and neuroprotective effects. In addition, puerarin produces antidepressant effects [10]. However, its mechanism of action in the treatment of depression is not fully understood. In this study, the mechanism involved in puerarin-induced mitigation of depression symptoms in ovariectomized female model rats was determined.

The hippocampus is a key region of the brain which is responsible for learning, memory and affective disorders. Decreases and increases in neuronal regeneration in the hippocampus are important factors involved in occurrence of, and recovery from depression, respectively [11]. Studies have found that depression is not a complete functional mental disorder, and in most patients, it is accompanied by changes in brain tissue, while the hippocampus is involved in the generation of emotional behavior, autonomous activities and endocrine integration and depression [12].

In this study, chronic stimulation was used to establish a depression model of ovariectomized rats, and the open-field test and forced swimming test were used to measure behavioral changes in these rats. The results showed that chronic stress caused changes in the hippocampal structure of rats, resulting in the loss of hippocampal neurons, leading to loss of interest, reduced activity and other symptoms in rats. However, puerarin significantly reduced the pathological changes in hippocampal neurons in depressive rats, and mitigated the depressive symptoms.

The pathogenesis of depression is complex, and the monoamine neurotransmitter hypothesis and neuroendocrine function hypothesis have been put forward to explain it. In the monoamine neurotransmitter hypothesis, low levels of monoamine neurotransmitters such as DA, NA and 5-HT play an important role in the occurrence of depression [13]. The results of this study showed that puerarin enhanced the levels of DA, NA and 5-HT in brain tissue of ovariectomized, depressed rats. This may be due to the fact that puerarin increased the levels of monoamine neurotransmitters or cAMP in the rat brains.

The BDNF is a growth factor of 5-HT cholinergic neurons. It enhances synaptic connection, and it affects the plasticity of neurons as well as synthesis of neurotransmitters and neurotrophic factors. It has been shown that decrease in brain BDNF level increased the sensitivity of hippocampal neurons to traumatic stress, resulting in the atrophy and even death of

hippocampal neurons [14]. The up-regulation of expression of BDNF may have an antidepressant effect, possibly through binding to its tyrosine kinase receptor B to promote the phosphorylation of CREB, thereby increasing the expression of BDNF. Other studies have shown that CREB promoted the survival of nerve cells and increased synaptic plasticity by increasing the expression of BDNF gene and anti-apoptotic gene Bcl-2 [15-17]. The results of this study showed that puerarin promoted the expressions of p-CREB and BDNF in the hippocampus, and activated the cAMP-CREB-BDNF signaling pathway, thereby exerting an anti-depressive effect.

CONCLUSION

Puerarin mitigates depression-like behavior in ovariectomized female rats by activating cAMP-CREB-BDNF signaling pathway. This finding provides a potential lead in the development of drugs for the treatment of depression in ovariectomized women.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Authors' contribution

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Rui Liu and Min Li designed the study, supervised the data collection, and analyzed the data. Rui Liu interpreted the data and prepared the manuscript for publication. Yubo Li, Zheng Wang, Jinfeng Li and Yuan Liang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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