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Research article

Processed *Polygonatum cyrtonema* Hua attenuates postpartum depression in rat model by regulating monoamines and hormones

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ABSTRACT

Background: Polygonatum cyrtonema Hua is a traditional Chinese medicinal food herb which can regulate the liver and Qi, nourish the heart and blood, moisten the lungs and nourish the kidneys with the potential to treat emotional diseases. However, few studies have explored the effects of *Polygonatum cyrtonema* Hua on postpartum depression. Therefore, we investigated whether processed *Polygonatum cyrtonema* Hua could improve postpartum depression in rat models by regulating monoamines and hormones.

Methods: Female Sprague-Dawley rats were randomized into normal control (0.9%Nacl), Sham operation (0.9%Nacl), postpartum depression model (0.9%Nacl), fluoxetine (2.5 mg/kg Fluoxetine), low, medium and high dose of processed *Polygonatum cyrtonema* Hua (2.5 g/kg, 5 g/kg, 10 g/kg) groups. Rats in these groups received drug intervention, and then subjected to Open-field test and Forced swimming test. Brain tissues and serum samples were collected and used to quantify levels of monoamines, hypothalamic-pituitary-adrenal axis and serum Estradiol. The status of neuronal cells in hippocampus 1 region was examined through hematoxylin-eosin staining, whereas expression of estrogen receptor α and β was detected by immunohistochemistry.

Results: Rats in the model group showed decreased mobility time, the disorder of neuronal cells in hippocampus 1 area, and decreased concentration of 5-hydroxytryptamine and dopamine in brain tissue, norepinephrine and estradiol in serum as well as estrogen receptor α and β expression. They also exhibited increased adrenocorticotropic hormone, corticosterone and corticotropin releasing hormone in serum. However, the treatment with processed *Polygonatum cyrtonem* Hua or fluoxetine reversed the above abnormalities.

Conclusion: The H group showed significant improvement in postpartum depression in rats, and processed *Polygonatum cyrtonema* Hua can be used as a developing drug for the prevention or treatment of depression.

1. Introduction

Postpartum depression is a neurotic depression [1], which may lead to maternal self-injury, suicide, infant injury and other adverse

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outcomes during the episode [2,3]. Some studies have found that 17.22% of women suffer from postpartum depression worldwide, with the highest prevalence being in Southern Africa regions at 39.96% [4]. Postpartum depression has become a common disease in women [5]. In the period following pregnancy and childbirth, women's hormonal metabolism and neural activity are influenced by social and environmental factors, making them vulnerable to postpartum depression [6–8]. Evidence from previous studies has indicated that the onset of depression is closely related to genetics, neurotransmitters, endocrinology and sex hormones [9,10].

Recent research has demonstrated that abnormal elevations of hypothalamic-pituitary-adrenal (HPA axis) hormones in human serum and cerebrospinal fluid indicate depression [11,12]. Prolonged hyperactivity of the HPA axis in patients with depression may result in excessive secretion of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) causing damage to the hippocampal neurons. This is accompanied by weakening of the negative feedback inhibition of the HPA axis, an effect that increases hyperactivity of the HPA axis [13,14]. Changes in the concentration of amine neurotransmitters such as 5-hydroxytryptamine (5-HT) , dopamine (DA), and norepinephrine (NE) are also closely related to the occurrence of Postpartum depression (PPD) [15,16]. These neurotransmitters mainly regulate the mental, excitability, and mood aspects in humans, and their levels are negatively correlated with the degree of depression [17,18]. Depression can cause damage to nerve cells which changes in estrogen levels can affect brain function and behavior, including emotional regulation and neuroprotection [19,20]. Furthermore, it is important to note that the HPA axis and the HPG axis are not independent of each other; they can exert mutual influence on one another [21]. It has been reported that estrogen can directly affect the level of corticotropin releasing hormone (CRH) through its estrogen α receptor, which in turn influences the HPA axis, leading to depression [22]. Estrogen β receptors are involved in the regulation of amines in the brain [23]. Therefore, there is a close relationship between amine neurotransmitters, HPA axis, estrogen and estrogen receptors, and are macroscopically manifested as behavioral, brain pathologic and other depressive changes.

According to traditional Chinese medicine, the main manifestations of depression are deficiency of the five zang-organs and deficiency of Qi and blood. The five Zang-organs represent five systems which contain all the organs inside the body. The common physiological function of the five zang-organs is to transform and store essence. Qi in the human body is derived from congenital Qi inherited from the parents, food nutrients the food transformed by the spleen and stomach and fresh air inhaled from the natural world by the lung. *Polygonatum cyrtonema* Hua is a medicinal plant derived from Polygonati Rhizoma. It regulates the Yin and Qi, nourishes the heart and blood, moistens the lungs and nourishes the kidneys [24]. Traditional Chinese medicine believes that women belong to Yin, and nourishing Yin nourishes the internal organs and Qi and blood. Women's Qi and blood are crucial for the most critical menstrual, pregnancy, childbirth, and other processes in their lives. Of note, nourishing the middle and benefiting the Qi can improve depression [25]. The homology between medicine and food containing Polygonati Rhizoma was recorded in Herbal Food Medicine [26]. A previous study showed that Polygonati Rhizoma improve the activity times and other related depression indicators in mice [27]. Meanwhile, Polygonati Rhizoma was found to reduce oxidative stress, inflammatory response and damage to hippocampal neurons and synapses [28]. Different processing methods of Polygonati Rhizoma also results in different effects [29,30]. One of the earliest processing methods employed involved wine-steaming Polygonati Rhizoma, which effectively reduced the tingling sensation in the mouth and irritation in the throat commonly experienced after ingestion (hereinafter referred to as processed *Polygonatum cyrtonema* Hua). Therefore, we designed postpartum depression rat models to explore the behavior, changes in hippocampus morphology, brain neurotransmitter levels, serum content of HPA axis and estrogen, hippocampus estrogen receptor expression changes. Through a comprehensive analysis of these parameters, we investigated the impact of processed *Polygonatum cyrtonema* Hua on rats with postpartum depression models.

2. Materials

2.1. Animals

Female sprague dawley (SD) rats, specific pathogen free (SPF) class, 200 ± 20 g (35 d) were used in this study. The rats were obtained from the Laboratory Animal Center of Chongqing Medical University and housed in the Innovative Laboratory Animal House of Chongqing Medical University (animal license number: SCXK2019-0001). Rats were provided clean water and food. All animal procedures were performed in accordance with the principles of medical ethics and the requirements of the Helsinki Declaration and approved by the ChongQing Medical University.

Ethics Committee. Ethical approval number: SCXK2012-0002(Chongqing, China).

2.2. Materials

2.2.1. Drugs

Processed *Polygonatum cyrtonema* Hua was purchased from Chongqing Ansen Pharmaceutical Company (210513, Chongqing, China). Fluoxetine hydrochloride capsules were purchased from Patheon France Lilai Suzhou Pharmaceutical Company (9875A, Jiangxi, China).

2.2.2. Modeling materials and test reagents

Estradiol Benzoate Injection was purchased from Shanghai full woo Biotechnology Zhumadian Company (210106, Shanghai, China). Progesterone Injection was purchased from Zhejiang Xianju Pharmaceutical Company (200901, Zhejiang, China). Gram staining kit was purchased from Beijing Solebo Technology Company (20210406, Beijing, China). Rat adrenocorticotropic hormone (ACTH, 210717A6), Rat Cortisol (Cortisol, 210717C8), Rat CRH (210717CR5), Rat 5-hydroxytryptamine (5-HT, 210717H12), Rat

norepinephrine (NE, 211102 N), Rat dopamine (DA, 210717D6) and Rat estradiol (E2, 211108E) ELISA kits were procured from Jiangsu Jingmei Biotechnology Company (Jiangsu, China). Diaminobenzidine (DAB) chromogenic kit (AFIHC004), phosphate buffered saline (PBS) buffer (AFIHC017), Hematoxylin staining solution (AFIHC006), EDTA buffer (AFIHC010), HRP-Polymer Mouse/Rabbit IHC Kit (AFIHC001), estrogen receptor β (ERβ) (AF04683) and estrogen receptor α (ERα) (AF04782) were bought from YiFang biological (Hunan, China).

2.3. Methods

2.3.1. Animal groups

After one week of adaptive feeding, all rats were subjected to an open-field test [31]. Eligible rats were randomly divided into normal control (Con), sham-operated (Sham) and castration groups. Castrated rats were modelled for postpartum depression, and randomly divided into model group (PPD), fluoxetine group (Flu), low, medium and high dose of processed *Polygonatum cyrtonema* Hua group (L, M, H) after the success of the model. There were 8 rats in each group.

2.3.2. Model

Rats in the castration group were anesthetized, ovaries were located and castration surgery was performed. On days 2–6 after the operation, vaginal smears were examined daily under a light microscope after Gram staining. Within the first 16 days, rats in the Con and Sham groups were subcutaneously injected with 0.3 mL d^{-1} sesame oil, and 0.1 mL d^{-1} sesame oil on the second 7 days. In the castration group, rats were subcutaneously injected with 2.5 μg d^{-1} estradiol injection and 4 mg d^{-1} progesterone injection within the first 16 days, 50 μg d^{-1} estradiol injection on the second 7 days, and the hormone injection was stopped on the 24th day [32]. The rats in the castration group showed depression features after a few days [33], and the OFT (Open field test) was conducted to determine whether the postpartum depression model was successful.

2.4. Drug intervention

The rats in the Con, Sham and PPD groups received normal saline (0.9%NaCl) by gavage. Rats in L, M and H groups were gavaged with processed *Polygonatum cyrtonem* Hua aqueous decoction at the following concentrations: 2.5 g/kg, 5 g/kg, and 10 g/kg according to the conversion of 7 times adult dose. The aqueous decoction was prepared as follows: The water level was about 1 cm higher than the medicinal material. After soaking for 30min, the water decocted for 20min was collected, and then added water and continued to fry for 20 min. The water decocted for two times was filtered, heated, and concentrated to obtain water decocted with a mass ratio of 1:1 between water and processed *Polygonatum cyrtonem* Hua, which was used in the group H. The decoction was diluted 1 and 2 times to obtain the M and L groups. In the Flu group, the concentration of fluoxetine was 2.5 mg/kg through a conversion of 7 times the adult dose. Rats in all groups were fed with 1mL/100 g of rats for 29 days.

2.5. Behavioral testing

2.5.1. OFT

The OFT was conducted using a self-made open box (50*50*40 cm). The surrounding walls were blackened, and the bottom surface was evenly divided into 25 squares of equal area with black lines. Data of horizontal and vertical activities of rats were recorded for each group. Each rat was measured only once, 5min each time.

2.5.2. Forced swimming test (FST)

The FST was performed in a self-made swimming box (56*40*34 cm). After 2 min of acclimation, rats' swimming time and immobility time in 4 min were recorded.

2.6. Test index and method

2.6.1. Determination of serum HPA axis and estrogen content

After behavioral examination, the rats were anesthetized, and 5 mL blood samples were collected from the eyeballs. Blood was centrifuged at 4 °C, 3000R/min, 10min. The supernatant was transferred to newly labeled EP tube, and the concentrations of CRH, ACTH, CORT, NE and E2 were determined using ELISA kits according to the manufacturers' instructions.

2.6.2. Determination of neurotransmitter level in brain tissue

After blood collection from eyes, the rats were immediately neck-broken on an ice tray to collect brain tissue into a liquid nitrogen tank. Next, half of the brain tissue was used to measure 5-HT and DA concentrations using the corresponding ELISA kits according to the manufacturers' instructions.

2.6.3. Observation of pathological changes in the CA1 (hippocampus 1) region of the hippocampus in rats

Three rats were randomly selected from each group to undergo cardiac perfusion fixation. Brain tissue samples were immediately collected. The brain tissue was immediately fixed in formalin solution. The tissue was then removed and dehydrated, embedded, sliced, spread, fished and baked. Next, Gram staining was performed and damage to the nerve cells in the CA1 region of the brain was

observed under a light microscope and photographed.

2.6.4. Detection of ERα and ERβ receptor expression in CA1 region of brain tissue

The brain, sections were boiled, dewaxed, hydrated and blocked in 3% hydrogen peroxide solution. After washing with PBS, the sections were placed successively in the citrate buffer and EDTA buffer and individually heated in a pressure cooker to a boil. 100 μL of non-immune normal goat serum was added and incubated. Subsequently, diluted primary anti-ERα and ERβ (1:100) were added drop-wise, incubated at 4 °C overnight, and washed with PBS buffer. Secondary antibodies were added in drop-wise manner. The samples were incubated and washed with PBS buffer. Hematoxylin was stained after addition of DAB, washed with running water, and differentiated in blue. After dehydration, transparent, neutral gum seal, Image J software was used to calculate positive cell expression data of ERα and ERβ at 50× magnification photos.

2.7. Statistical analysis

Statistical analysis was performed using the SPSS20.0 software. Measurement data were expressed as mean ± standard deviation (±s), and groups were compared with the *t*-test. $P < 0.05$ indicated significant differences, and $P < 0.01$ indicated extremely significant differences. GraphPad Prism 8 software was used to generate graphs.

3. Results

3.1. The general condition of rats

Throughout the adaptive feeding period, the rats exhibited vivacity and activeness, maintaining a regular diet and smooth fur. Moreover, there was a gradual increase in their body weight during this phase. After castration surgery, the daily estrus stages of rats were recorded as shown in Fig. 1(A-D). There was no change in estrous cycle within 5 days, indicating that the castration operation was successful. Rats that were used to establish the model became docile, their fur began to thicken, and they were prone to thirst. Three days after modeling, the model rats exhibited depressive tendency and showed slow movement or appeared immobile in clusters. After 13 days of drug gavage, it was observed that the rats in the treated group became active and their fur gradually regained its luster.

3.2. Effects of different treatments on behavior of postpartum depression model rats

Table 1 shows that after modeling, the vertical scores of rats decreased significantly in the PPD, Flu and L groups ($P < 0.01$) and in the Sham and M groups ($P < 0.05$) compared with scores in the Con group, which confirmed the success of modeling. After gavage treatment, the vertical fraction in the L group was significantly higher compared with that in the PPD and Flu groups ($P < 0.05$).

Analysis of the data presented in Table 2 indicated that after modeling, rats in all groups showed some degree of decrease in

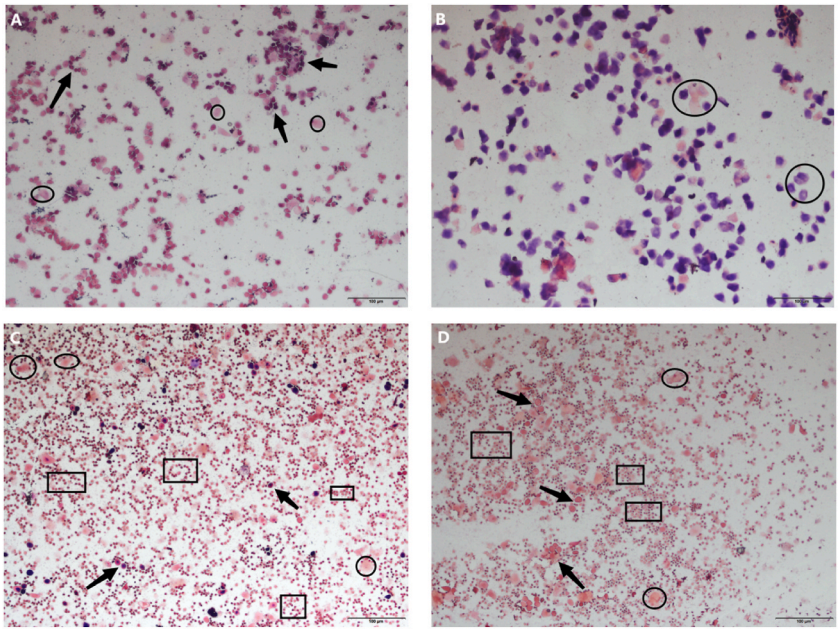

Fig. 1. A: Proestrus B: Estrus C: Anaphase D: Interestrus (ruler: 100 μm). Symbols: black arrow: epithelial cells; circle: keratinised epithelial cells; black box: leukocytes.

horizontal scores, with the PPD group being the most significant compared with rats in the Con group ($P < 0.01$). After treatment, rats in the PPD and Sham groups had significantly lower horizontal scores compared with the Con group ($P < 0.05$).

Table 3 demonstrated that, after modeling, the swimming time of rats in the Flu group was significantly longer compared to that of rats in the Con group ($P < 0.01$). Rats in the L, M, and H groups showed significantly lower swimming time compared to rats in the Flu group ($P < 0.05$). Notably, the swimming time in the L group was significantly increased following treatment compared with rats in the Flu group ($P < 0.05$).

3.3. Effects of different treatments on CA1 region in hippocampal tissue of rats with postpartum depression model

For the Con group, neuronal cells showed a uniform morphology, full cytoplasm, high cell number and structural integrity. In the PPD group, the neuronal cells appeared disorganized and with different sizes. Most cells showed a deep staining and appeared wrinkled, indicating cell atrophy. In certain cells, there were vacuoles and cellular edema, accompanied with blurred cell borders or even cell disappearance, indicating severe damage. Additionally, the nuclei of these cells exhibited slight staining, further confirming the occurrence of significant injury. In the Sham group, neuronal cells showed a uniform morphology and full of cytoplasm, but the cells were slightly scattered compared with those of rats in the Con group. In the Flu group, neuronal cells were restored to a closely aligned state, but the number of cells was lower than that in the Con group, and there was no significant damage. In the L group, neuronal cells were loosely arranged, irregular, and deeply stained and wrinkled, suggesting that the cells had atrophied. Edematous cells could be seen microscopically, and the neurons showed a pale stain-like appearance. In the M group, the arrangement of neuronal cells was restored to an orderly state, and some of the cells were still crinkled, but no vacuolated cells were present. In the group H, neuronal cells showed an intact structure and uniform size, close to cells in the normal controls (Fig. 2).

3.4. Effects of different treatments on neurotransmitter secretion in postpartum depression model rats

The concentration of 5-HT in brain tissues was significantly lower in H and PPD, L and M groups compared to Con group ($P < 0.01$). However, the 5-HT concentrations were significantly higher in Sham, Flu and H groups compared with PPD group ($P < 0.01$). Compared with the Flu group, 5-HT concentrations were significantly decreased in the L and M ($P < 0.01$) and H groups ($P < 0.05$). The concentration of DA was significantly lower in the PPD, L, M and H groups relative to the concentration of the Con group ($P < 0.01$). However, the concentrations of DA were significantly higher in Sham, Flu and H ($P < 0.01$) and L ($P < 0.05$) and M ($P < 0.01$) groups relative to the PPD group. DA concentrations were significantly lower in the L, M, and H groups compared with the Flu group ($P < 0.01$). Compared with the Con group, the concentrations of NE serum were significantly decreased in the PPD and L ($P < 0.01$) and M ($P < 0.05$) groups. It was observed that NE concentrations were significantly higher in the Sham, Flu, M and H ($P < 0.01$) and L ($P < 0.05$) groups than in the PPD group. However, NE concentration in the L group was significantly lower compared with that in the Flu group ($P < 0.01$) (Figs. 3–5).

3.5. Effects of different treatments on hypothalamic-pituitary-adrenal axis hormone secretion in postpartum depression model rats

The results showed that serum ACTH concentration was significantly higher in the PPD, L and M ($P < 0.01$) and H ($P < 0.05$) groups compared with level in the Con group. In contrast, ACTH concentration was significantly lower in the Sham and Flu and the H groups compared with level in the PPD group ($P < 0.01$). The concentration of ACTH was significantly increased in the L, M ($P < 0.01$) and H ($P < 0.05$) groups compared with the Flu group. Serum CORT concentration was significantly higher in the PPD, L and M ($P < 0.01$) and Flu ($P < 0.05$) groups relative to level in the Con group. The CORT concentration in Sham, Flu, L, M and H groups was significantly lower compared with the level in the PPD group ($P < 0.01$). The cortisol concentration was higher in the L group compared with the Flu group ($P < 0.05$). Compared with the Con group, the serum CRH concentration of rats in the PPD, L and M ($P < 0.01$) and H ($P < 0.05$) groups was significantly higher. Lower CRH concentrations were recorded in Sham, Flu, L, M and H groups relative to levels in the PPD group ($P < 0.01$). The concentration of CRH was significantly higher in the L ($P < 0.01$) and M ($P < 0.05$) groups compared with the Flu group (Figs. 6–8).

Table 1
Vertical score of OFT for postpartum depression rat model with different treatments n = 8.

Groups	After modeling (44d)	In treatment (60d)	After treatment (74d)
Con	42.000 ± 16.133	25.333 ± 16.513	23.500 ± 4.231
PPD	10.875 ± 6.105**	10.875 ± 4.883*	17.125 ± 7.160
Sham	25.375 ± 11.351*#	21.500 ± 8.583	11.250 ± 4.787*
Flu	17.143 ± 9.856**	12.375 ± 3.623*	16.000 ± 2.000
L	17.125 ± 9.761**	20.875 ± 10.548	27.375 ± 15.583#^
M	29.500 ± 13.202*##^	21.500 ± 3.891	15.875 ± 4.357
H	36.125 ± 12.017##^^	23.750 ± 10.209#^	21.375 ± 7.708

Note: Compared with the normal control group, *P < 0.05, **P < 0.01; Compared with the model group, #P < 0.05, ##P < 0.01; Compared with fluoxetine group, ^P < 0.05, ^^P < 0.01. The same below.

Table 2
Scores of OFT in postpartum depression in rat models after different treatments (n = 8).

Groups	After modeling(44d)	In treatment(60d)	After treatment(74d)
Con	110.125 ± 35.365	123.333 ± 77.216	103.500 ± 24.040
PPD	71.125 ± 19.037**	48.750 ± 17.758**	60.625 ± 20.121*
Sham	99.625 ± 23.170	98.750 ± 43.100$^{\#}$	58.750 ± 23.641*
Flu	94.125 ± 20.601	71.250 ± 16.140*	74.500 ± 22.469
L	98.750 ± 18.188	83.125 ± 35.073	69.625 ± 36.789
M	96.250 ± 30.103	78.750 ± 13.285*	67.375 ± 24.189
H	94.875 ± 26.941	86.375 ± 30.803	96.125 ± 51.946

Table 3
Swimming results of postpartum depression in rat models treated with different treatments (n = 8).

Groups	After modeling(44d)	In treatment(60d)	After treatment(74d)
Con	158.340 ± 20.728	185.992 ± 20.121	104.898 ± 16.577
PPD	152.586 ± 33.634	201.179 ± 14.169	115.769 ± 19.613
Sham	133.915 ± 23.818	197.658 ± 74.514	95.103 ± 42.150
Flu	196.976 ± 21.000**	211.180 ± 10.589	90.248 ± 28.837
L	159.914 ± 23.026$^{\hat{}}$	195.305 ± 22.479	124.291 ± 16.120$^{\hat{}}$
M	158.568 ± 18.436$^{\hat{}}$	184.560 ± 42.274	135.109 ± 54.478
H	143.304 ± 19.463$^{\hat{}}$	188.531 ± 38.695	111.865 ± 31.809

3.6. Effects of different treatments on estrogen E2 secretion in postpartum depression model rats

The serum E2 concentration was significantly lower in the PPD, L, M ($P < 0.01$) and H ($P < 0.05$) groups compared with levels in the Con group. In addition, E2 concentration was significantly higher in the Sham, Flu, M and H groups compared with the PPD group ($P < 0.01$). However, the E2 concentration was significantly lower in the L group compared with the Flu group ($P < 0.01$) (Fig. 9).

3.7. Effects of different treatments on the expression of estrogen receptors ERα and ERβ in rat models of postpartum depression

In the hippocampal CA1 region of female rats, the expression of ERα in the PPD and L group was significantly lower compared with levels in the Con group ($P < 0.01$). On the other hand, the expression level of Erα was higher in the Flu and M ($P < 0.05$), Sham and H ($P < 0.01$) groups relative to levels in the PPD group. Notably, the expression level of ERα was higher in the H group compared with the Flu group ($P < 0.05$) (Figs. 10, 12). In the hippocampal CA1 region of female rats, ERβ expression was lower in the PPD and L groups than in the Con group ($P < 0.05$). Moreover, ERβ expression was significantly higher in the Sham, Flu and H groups compared with the PPD group ($P < 0.01$). However, ERβ expression was significantly lower in the L group relative to the Flu group ($P < 0.01$) (Figs. 11, 13).

4. Discussion

In this study, a PPD model was constructed to investigate the effects of different concentrations of processed *Polygonatum cyrtonem* Hua on model rats of postpartum depression in terms of behavior, brain morphology, neurotransmitters, HPA axis, estrogen and estrogen receptor. We found no significant difference in chemical indicators between the Con and Sham groups, indicating that surgical manipulation did not any effects on the rats. Moreover, results showed that the vaginal smear estrous cycle in rats did not change within 5 days after ovariectomy, indicating that the procedure was successful. In addition, OFT was inactive in the denuded rats, suggesting the modeling was successful. From the perspective of neurotransmitters, processed *Polygonatum cyrtonem* Hua can effectively increase the cheerful mood, whereas 5-HT, DA, and NE are involved in regulation of the HPA axis, and disruption of the HPA axis may lead to pathological changes in brain tissue morphology, the other processed *Polygonatum cyrtonem* Hua can increase the levels of estradiol via upregulating estrogen receptor expression in the brain. From different perspectives, the effect of processed *Polygonatum cyrtonem* Hua in nourishing the Yin to improve depression levels of PPD model was demonstrated.

Based on the design principle of OFT and the avoidance ability of mice, the behavior of wall sticking in potentially dangerous places or open and unknown places has been widely recognized to be correlated with anxiety and depression to a certain extent [34]. As an indicator of depression, the amount of vertical activity in OFT, also known as vertical score, may reflect the level of interest, which is negatively correlated with depression and anxiety. The lower scores in the post-model rats compared to the Con group indicated the successful modeling of depression in rats. As the depression levels decreased, the rats showed increased interest and showed higher vertical scores, which confirmed the effectiveness of the drug treatment. The results also revealed that OFT had good validity. In addition, the activity of rats in the OFT reflected the exercisability level of rats [35], and there was no significant difference in OFT levels among the groups, indicating that the antidepressants did not significantly improve exercisability, which may be partially explained by tolerance levels of rats.

The FST, also known as the behavioral despair test, evaluates rodent responses to the threat of drowning, and the results are

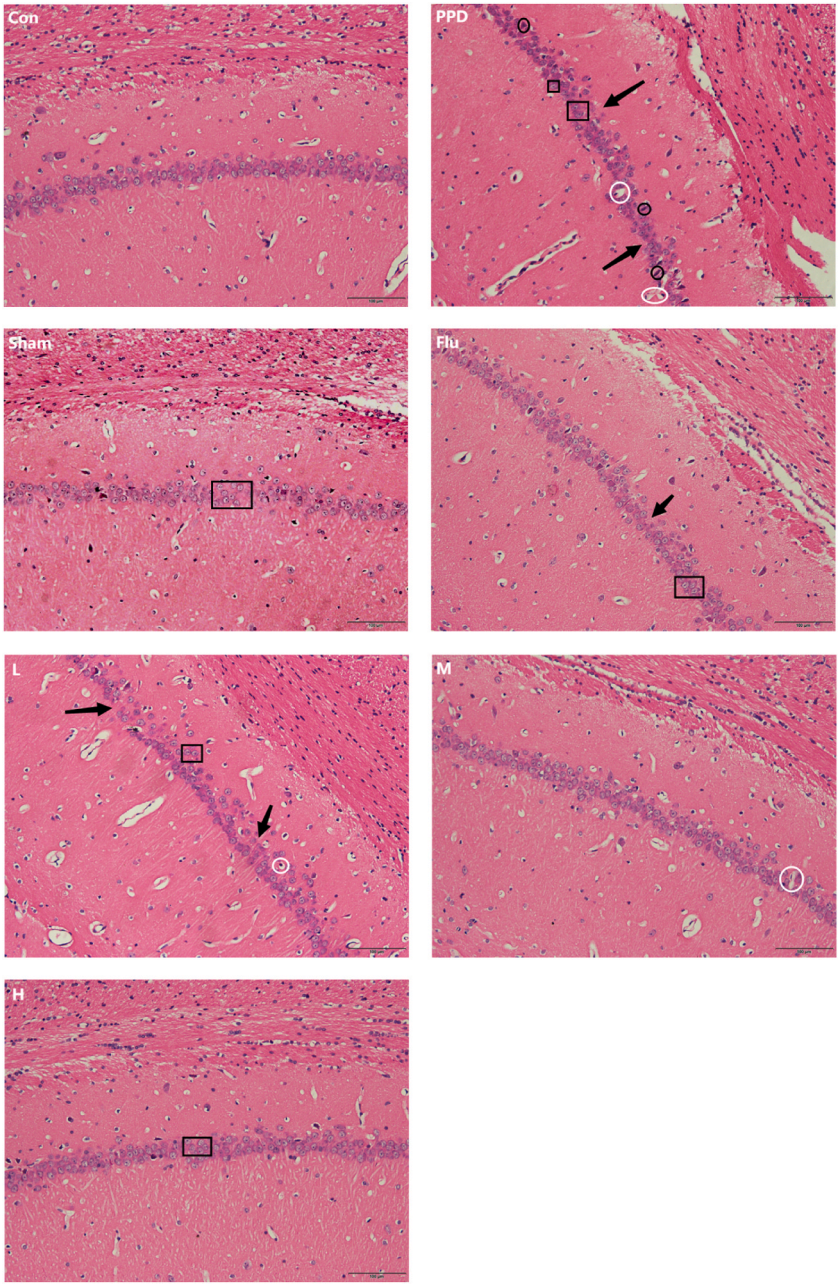

Fig. 2. Morphology of nerve cells in hippocampal CA1 region in the seven groups (scale: 100 μm). Con: control group, Sham: sham-operated group, PPD: model group, Flu: fluoxetine group, L: low dose of processed *Polygonatum cyrtonema* Hua group, M: medium dose of processed *Polygonatum cyrtonema* Hua group, H: high dose of processed *Polygonatum cyrtonema* Hua group Symbols: black arrow: discontinuous neuronal cells; black circle: atrophic neuronal cells; white circle: vacuolated cells; black box: edematous neuronal cells.

interpreted as a measure of susceptibility to negative emotions [36]. Therefore, agents that can increase the swimming time of rats in FST are potential antidepressants. The experimental results showed that the swimming time of L, M, H and Flu groups was higher than that of the PPD group, and this indicated that the treatment had positive effect. Improved enthusiasm of the rats to survive in the desperate situation reflected decreased depression state. In other words, processed *Polygonatum cyrtonem* Hua improved the condition of postpartum depressed rats. However, after treatment, the swimming time of rats decreased in all groups, and this was dependent on the environment or was due to less stable behavior during depression.

The hippocampus regulates neuroendocrine functions and stress emotions, and is sensitive to stress response and can be easily damaged [37]. The hypothesis of "hippocampal neuron regeneration disorder in depression" holds that stress-induced hippocampal

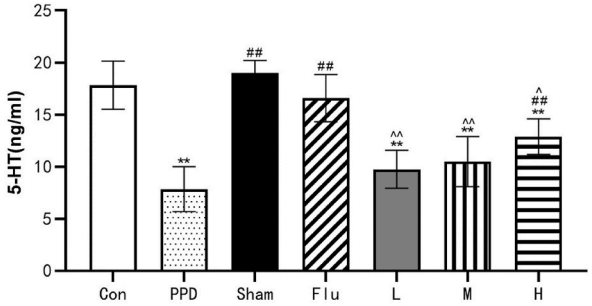

Fig. 3. The content 5-HT in rat models of postpartum depression under different treatments.
Compared with the normal control group, *P < 0.05 **P < 0.01; Compared with the model group, #P < 0.05 ##P < 0.01; Compared with fluoxetine group, ^P < 0.05 ^^P < 0.01.

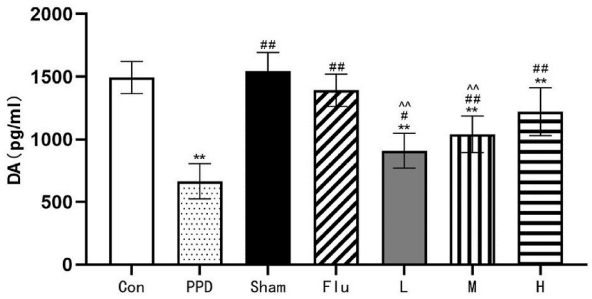

Fig. 4. The content of DA in rat models of postpartum depression under different treatments.

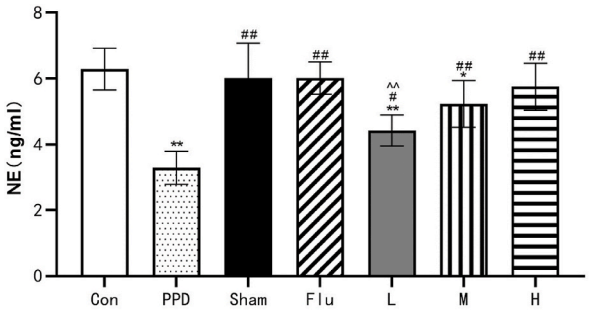

Fig. 5. The level of NE in rat models of postpartum depression under different treatments.

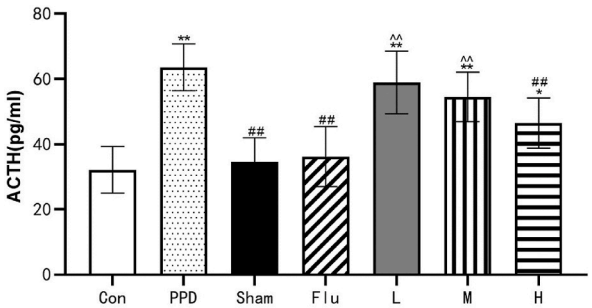

Fig. 6. The content of ACTH in postpartum depression model rats under different treatments.

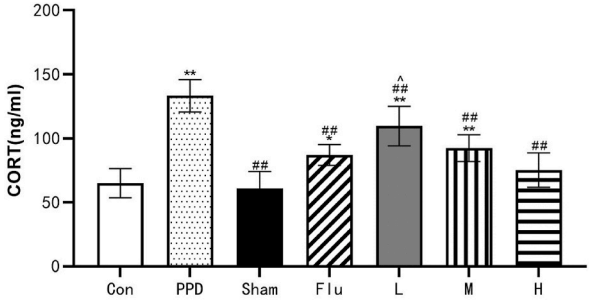

Fig. 7. The content of CORT in rat models of postpartum depression under different treatments.

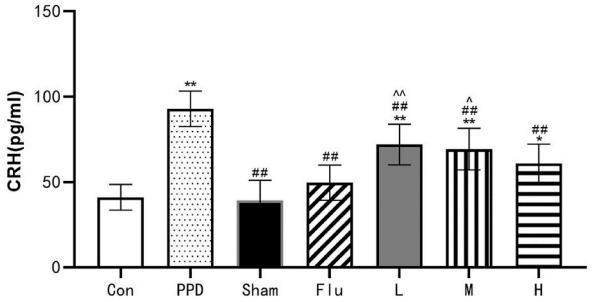

Fig. 8. The content of CRH in rat models of postpartum depression under different treatments.

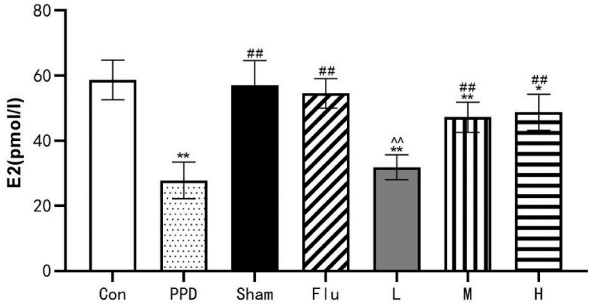

Fig. 9. The level of E2 in rat models of postpartum depression under different treatments.

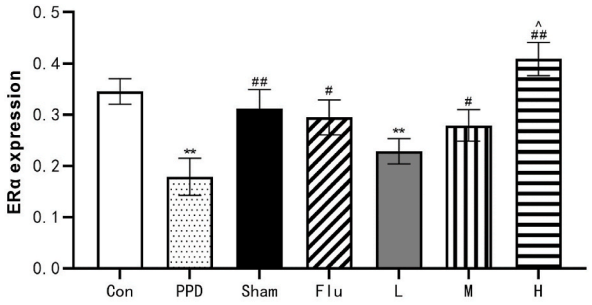

Fig. 10. Expression level of ERα in rat models of postpartum depression under different treatments.

neuron damage and neuronal regeneration disorder together decrease the number and caused structural destruction to hippocampal neurons [38]. In this study, we found that neuronal cells in the Con group and the Sham group were neatly arranged and full of cytoplasm. In comparison, nerve cells in PPD and L groups appeared shrivel, edema, were severely damaged, which confirmed the "hippocampus neuron regeneration depression disorders" hypothesis. The M and H groups had different degrees of recoveries, with the

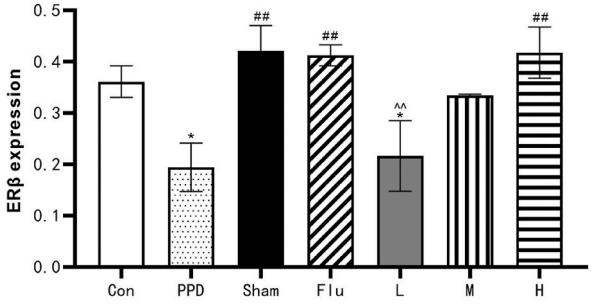

Fig. 11. Expression level of ERβ in rat models of postpartum depression under different treatments.

H group being nearly similar to the Con group. These results indicated that processed *Polygonatum cyrtonem* Hua significantly improved the pathological changes in the hippocampal CA1 region of rats with postpartum depression.

Multiple animal experiments have shown that 5-HT, NE and other substances are generally down regulated in the brain tissue of PPD model animals [39]. This was consistent with results of the present study. Other researchers have demonstrated that the aqueous extract of Polygonati Rhizoma promoted sleep by upregulating GABA receptor and 5-HT receptor expression [40]. In this experiment, we found that processed *Polygonatum cyrtonem* Hua significantly promoted the secretion of 5-HT. 5-HT is a messenger that can induce pleasant emotions which affect almost every aspect of brain activity, and its level are closely related to depression. Meanwhile, NE can be synthesized and secreted by sympathetic postganglionic neurons, adrenergic nerve endings in the brain, and the adrenal medulla. Results shown in our research indicated that the level of monoamine neurotransmitters in the L, M, H and Flu groups were higher compared with levels in the PPD group and gradually returned to the normal level, indicating that processed *Polygonatum cyrtonem* Hua could effectively treat postpartum depression.

Postpartum depression is one of the major causes of impaired HPA axis [41]. Neuroendocrine neurons in the paraventricular nucleus of the hypothalamus can synthesize and secrete CRH, both of which promote the release of ACTH that activates the adrenal cortex to synthesize CORT [42]. Because the hippocampus contains a large number of glucocorticoid receptors [43], abnormal increase in CORT secretion will induce neuronal apoptosis to a certain extent, leading to hippocampal atrophy in patients with depression. This will result in damage to the structure and function of the hippocampus, and induce secretion disorders such as EPI [44]. Monoamine neurotransmitters such as EPI are widely distributed in the central nervous system and participate in the regulation of moods and emotions. It has been reported that elevated HPA axis disorder contributes to depression [45,46]. Our research showed that the HPA axis in the L, M, H and Flu groups was progressively decreased compared with levels in the PPD group, and it was gradually restored to normal levels, indicating that processed *Polygonatum cyrtonem* Hua modulated the HPA axis.

The HPA axis can directly inhibit HPO axis. Estrogen is one of the key hormones associated with the HPO axis. A decrease in serum estrogen levels leads to an overproduction of GnRH, FSH and other hormones by the hypothalamus and pituitary gland due to a negative feedback mechanism. This hyperfunction of the hypothalamus-pituitary-ovary (HPO) axis, in turn, causes dysfunction within the axis [47]. Studies have found that estrogen increases by 1000 times the normal levels shortly before delivery, and then decreases sharply after placental discharge [48]. Ovarian removal, perimenopause, reproductive system tumors and other conditions cause a sudden decrease in estrogen E2, which increases the possibility of depression [49]. Treatment with processed *Polygonatum cyrtonem* Hua resulted in a gradual decrease in estrogen levels in L, M, and H groups to near levels in the Flu and Con groups, indicating that estrogen levels increased with the increase in processed *Polygonatum cyrtonem* Hua concentration. In the brain, the effects of estrogen are mainly mediated by estrogen receptors, and the two subtypes of estrogen receptors that play most of the roles are ERα and ERβ. Decreased expression of estrogen receptor in the brain reduces the neuroprotective effect of estrogen and aggravates the degree of depression [50]. In this study, we found that L, M, H and Flu groups had higher expression levels of ER compared with levels in the PPD group, and the degree of up-regulation of receptor expression in L, M, H groups were positively correlated with the concentration of processed *Polygonatum cyrtonem* Hua. This further demonstrated that processed *Polygonatum cyrtonem* Hua improved depression levels of rats by altering the expression of ER.

5. Conclusion

In conclusion, our study examined behavioral changes, brain tissue morphology, neurotransmitter content, HPA axis activity, estrogen levels, and estrogen receptors in rats with postpartum depression models. The results unequivocally demonstrate that processed *Polygonatum cyrtonem* Hua possesses the ability to ameliorate postpartum depression to a certain degree, particularly at higher concentrations. Based on the theory of traditional Chinese medicine, postpartum depression is characterized by spleen deficiency and changes in Qi and blood, and treatment with processed *Polygonatum cyrtonem* Hua can nourish Qi and Yin. Combined with results of the study, it can be inferred that processed *Polygonatum cyrtonem* can be incorporated into health products or medicine as it is a potential agent for preventing and treating postpartum depression.

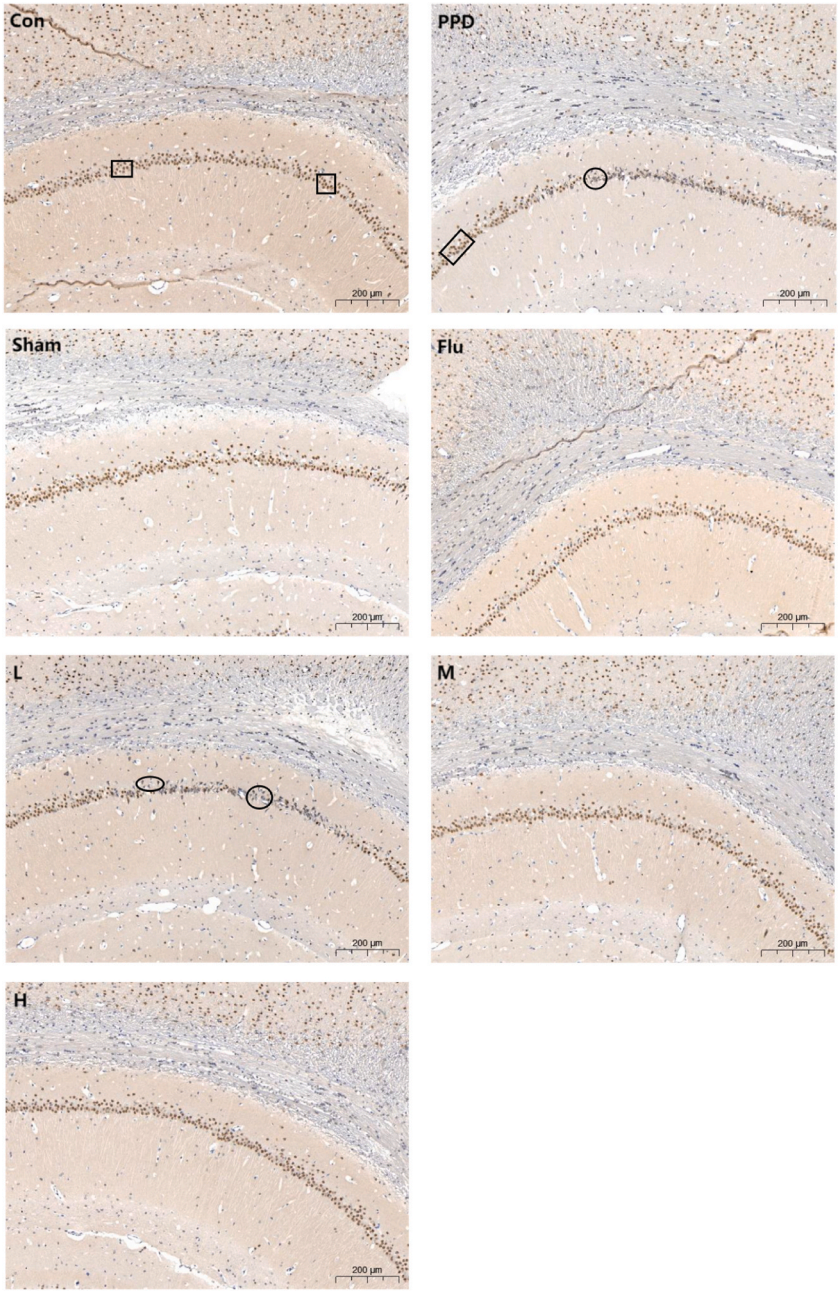

Fig. 12. Expression level of ERα in rat models in hippocampal CA1 region across the seven groups (scale: 200 μm).
Con: control group, Sham: sham-operated group, PPD: model group, Flu: fluoxetine group, L: low dose of processed *Polygonatum cyrtonema* Hua group, M: medium dose of processed *Polygonatum cyrtonema* Hua group, H: high dose of processed *Polygonatum cyrtonema* Hua group Symbols: black box: brownish-yellow positive cells; black circle: non-positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

6. Preparation

[Experimental design]
Timing: [7 days]

1. [Search for literature on the rat model of postpartum depression]
2. [Search for literature on Polygonatum cyrtonem's treatment of depression]
3. [Design experimental process]

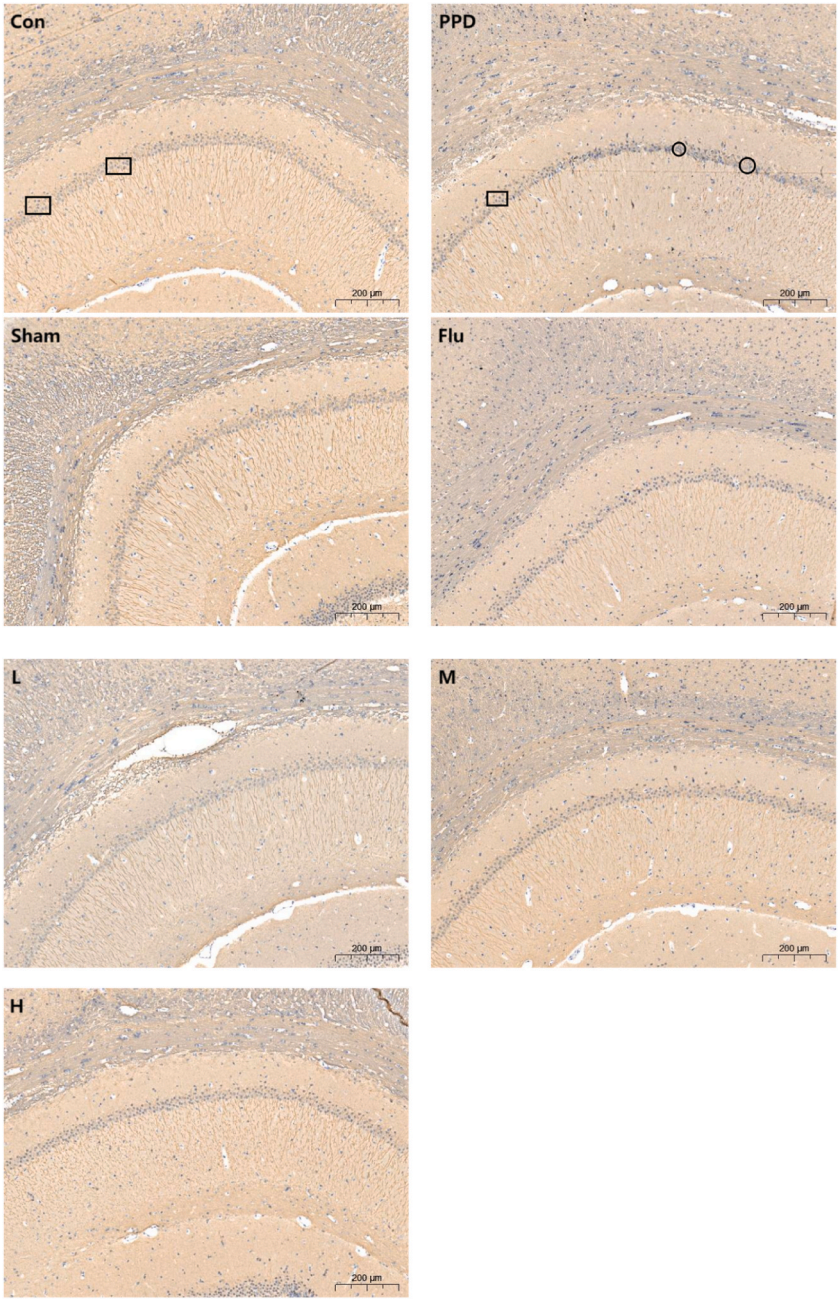

Fig. 13. Expression level of ERβ in the hippocampal CA1 region across the seven groups (scale: 200 μm). Con: control group, Sham: sham-operated group, PPD: model group, Flu: fluoxetine group, L: low dose of processed *Polygonatum cyrtonema* Hua group, M: medium dose of processed *Polygonatum cyrtonema* Hua group, H: high dose of processed *Polygonatum cyrtonema* Hua group Symbols: black box: brownish-yellow positive cells; black circle: non-positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[Procurement of reagents and rats]
Timing: [5 days]

7. Step-by-step method details

[Animal model]
Timing: [37 days]

1. [Adaptive feeding]
2. [Castration operation]
3. [Record estrous cycle change]
4. [Establishment of Postpartum depression model in rats through Hormone Cessation Testing]

   [Treatment and testing]
   Timing: [30 days]

5. [Behaviors (OFT, FST)]
6. [Gavage treatment: Con, Sham, PPD: 0.9%Nacl; Flu: fluoxetine; L, M, H: 2.5 g/kg, 5 g/kg, 10 g/kg Polygonatum cyrtonem aqueous decoction]
7. [Indicator detection]

a. [Serum testing: HPA axis, NE, E2]
b. [Brain tissue detection: 5-HT, DA]
c. [brain histomorphology: CA1 Hippocampus]
d. [Immunohistochemistry: Estrogen receptor α and β]

8. Limitations

The sample size is not large enough, so the sample may have limitations and low universality.

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Data availability statement

The data used to support the findings of this study are included within the article.

CRediT authorship contribution statement

Xiao-hong Zhu: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. **Jia-li Zhang:** Writing – original draft, Validation, Methodology. **De-hua Li:** Writing – original draft, Validation, Formal analysis. **Zhong-qiang Wang:** Writing – original draft, Validation, Formal analysis. **Yan-ku Liu:** Writing – original draft, Validation, Investigation. **Jing-xian Fan:** Writing – original draft, Formal analysis, Data curation. **Shang-ren Jiang:** Writing – original draft, Validation, Formal analysis. **Xin-ran Li:** Software. **Xian-yuan He:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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