

Paeoniflorin exhibits antidepressant activity in rats with postpartum depression *via* the TSPO and BDNF-mTOR pathways

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Postpartum depression (PPD) is the most common type of puerperal mental syndrome and affects maternal physical and mental health and even the growth and development of infants. Paeoniflorin exerts a potential antidepressive effect; however, the functional roles and potential mechanisms of paeoniflorin in PPD are still largely unknown. PPD rat models were prepared by withdrawing hormone-simulated pregnancy (HSP), and subjects were treated with paeoniflorin and fluoxetine or plasmids. The sucrose preference test (SPT), forced swimming test (FST) and tail suspension test (TST) were used to monitor depression-like behavior in rats. A radioimmunoassay was utilized for estradiol (E₂) and progesterone (P) measurements. ELISA was performed to detect serum corticosterone (Cor), hippocampal allopregnanolone (Allo), IL-1 β and TNF- α levels. Expression of the E₂ receptors ER α and ER β was detected by qPCR. Western blotting was used to detect TSPO, BDNF and mTOR phosphorylation. Paeoniflorin drastically increased the sucrose preference of rats while decreasing the immobility time in the FST and TST in PPD models. Moreover, paeoniflorin intervention upregulated serum E₂, hippocampal Allo, ER α , and ER β levels but degraded P, serum Cor, IL-1 β , TNF- α and ER α /ER β levels. Mechanistically, paeoniflorin promoted TSPO and BDNF-mTOR pathway activation in PPD rats. Furthermore, suppression of TSPO or the BDNF-mTOR pathway partially reversed the effects of paeoniflorin on depression-like behaviors, hormone levels, and inflammatory cytokine release. Paeoniflorin may improve symptoms of PPD by regulating the TSPO and BDNF-mTOR pathways, indicating that paeoniflorin may be an effective anti-PPD and antidepressant drug, providing evidence for the future treatment of PPD.

Key words: paeoniflorin, TSPO, BDNF-mTOR, postpartum depression

INTRODUCTION

Postpartum depression (PPD) is the most common type of mental syndrome in the puerperium and is usually accompanied by extreme sadness and despair, which can lead to a lack of energy, anxiety, irritability and even suicide and infanticide (O'Hara and McCabe, 2013; Kiryanova et al., 2017). Approximately 13–19% of new mothers have experienced PPD, and the incidence continues to increase annually (Xia et al., 2016). In addition, PPD may also have an unfavorable effect on the baby's behavior, emotion and cognitive development,

which can cause adverse consequences for family and society (Halligan et al., 2007; Feldman et al., 2009). At present, the exact biological pathogenesis of PPD is unclear, making its prevention and treatment difficult, and no effective treatment has been found thus far. Therefore, conducting in-depth research on the etiology and pathogenesis of PPD is of important practical significance for a deeper understanding of the occurrence, development, outcome and design of new therapeutic drugs.

BDNF is a well-known neurotrophic factor, the imbalance of which is closely related to affective disorder

ders, and its role in depression has also attracted much attention (Erickson et al., 2012; Haile et al., 2014). In past research on the role of BDNF in depression, BDNF and the mammalian target of rapamycin (mTOR) signaling pathway were found to be important for the antidepressant effect of ketamine. It was also found that BDNF could regulate the activation of mTOR, while the fast-acting antidepressant effect of ketamine could be blocked by BDNF knockdown and the selective mTOR protein antagonist rapamycin (Li et al., 2010; Autry et al., 2011). This finding suggested that activation of the BDNF-mTOR signaling pathway may be one way that antidepressants exert pharmacological effects. Previous studies found that BDNF was notably reduced in the serum of PPD patients (Tan et al., 2018), although its specific function remains unclear.

Transporter protein (TSPO) is an 18 kDa protein mainly distributed in the outer mitochondrial membrane of steroid-synthesizing cells in the central and peripheral nervous systems (Culty et al., 1999), the ligand of which has anxiolytic and antidepressant effects without the conspicuous side effects of traditional benzodiazepines (Rupprecht et al., 2009; Costa et al., 2012). Related studies have found that the anxiolytic and antidepressant activity of the TSPO ligand compound YL-IPA08 has important value in PPD treatment (Ren et al., 2020), suggesting that TSPO may become a new target for PPD treatment.

Paeoniflorin, an amorphous glucoside, is the main active component of total glycosides in the roots of *Paeonia lactiflora* Pallas plants (Li et al., 2017). Past studies have shown that paeoniflorin exhibits potential neuroprotective, anti-ischemic, antioxidant and anti-inflammatory effects and can alleviate depression-related behaviors (Li and Li, 2015; Hu et al., 2019). However, research on PPD has not yet been reported, and whether it is related to the regulation of the TSPO and BDNF-mTOR pathways needs further exploration. In summary, we hypothesize that paeoniflorin could improve PPD by modulating the TSPO and BDNF-mTOR pathways.

METHODS

Animal model

Female Sprague-Dawley (SD) rats (220–250 g) were purchased from the Animal Research Center of Wuhan University (Wuhan, China) and kept in an incubator with a temperature of 25°C and a relative humidity of 65% under a light-dark cycle of 12 h. All rats could drink and eat ad libitum. All animal treatments were carried out in strict accordance with the guidelines for the care and use of laboratory animals of the National

Institutes of Health. This study was also approved by the animal ethics committee.

PPD models were established in rats after withdrawing hormone simulation pregnancy (HSP). Two days before the experiment, rats in each group were subjected to the open field test in turn, and rats with depression tendency were excluded. Then, bilateral ovaries of rats were resected and ligated. Vaginal cytology smears were performed 2–5 days after the operation to determine the changes in the estrous cycle, and unqualified rats were excluded. One week after ovariectomy, the rats were injected subcutaneously with 0.1 mL E₂ (2.5 µg) and 0.2 mL P (4 mg) every day for 16 days followed by 0.1 mL E₂ (50 µg) per day for one week to simulate hormone secretion levels during pregnancy. The animals in the sham operation group were injected subcutaneously with 0.3 mL of sesame oil every day for the first 16 days and 0.1 mL of sesame oil every day for the next 7 days. The injection was stopped on the 24th day. No treatment was performed in the control group. After the model was established, the animals received paeoniflorin (Solaibao Biotechnology, Beijing, China) 20 mg/kg dose and fluoxetine 3.0 mg/kg (Changzhou Lilly Biotechnology Co., Ltd.) once a day by oral gavage for 2 weeks. The behavioral test was performed 1 h after the last dose or model establishment, and subjects were dissected immediately after the behavioral test. Six mice in each group participated in the experiment.

Construction and injection of shRNA lentiviral plasmids

Lentivirus containing the green fluorescent protein gene was commercially obtained, which was a package containing four BDNF-targeted or TSPO-targeted shRNAs and a nontargeting vector as a negative control (vehicle) (Hu6-MCS-CMV-EGFP, GV115, GENECHM, China). BDNF- or TSPO-targeting shRNA lentiviruses were transfected into primary cultured neurons according to the manufacturer's instructions. The transfection efficacy of shRNAs was determined by fluorescence microscopy. Mice were then anesthetized with 5% chloral hydrate and fixed on a stereotaxic frame. A lentiviral suspension containing 1×10^9 TU/mL was injected bilaterally into the hippocampus at a rate of 0.2 µL/min (total volume 2 µL).

Sucrose preference test (SPT)

Before the test, rats were given sugar water training to measure the baseline level of the syrup bias of each group of rats. After fasting for 24 h, subjects were given

en two preloaded and quantified bottles of water: one contained 2% sucrose solution, and the other contained tap water. After 12 h of free drinking, consumption was calculated by weighing the drinking bottles. Sucrose preference was calculated by the following formula: (sucrose preference rate) = ((sucrose intake) / (sucrose intake + water intake)) × 100.

Forced swimming test (FST)

In short, rats were individually placed in a glass cylinder (diameter 20 cm, height 40 cm) containing 25 cm deep water at a temperature of approximately 28°C, guided to preswim for 15 min, and then forced to swim again after 24 h under the same experimental conditions, during which the immobility time of rats in the tank within 5 min was recorded. Immobility was defined as the time the rat floated in the water without struggling or the time required to keep its head only above the water. Conventional antidepressants reduced the immobility time in this test.

Tail suspension test (TST)

Subjects were suspended separately from the tail 15 cm above the floor by adhesive tape (2 cm from the end). Then, a video camera was used to record each rat, and the immobility time in the last 4 min of the 6 min test was calculated and reported in seconds. The rats were defined as not moving when they were passively suspended and completely at rest. Conventional antidepressants reduced immobility time in this test.

Radioimmunoassay for the detection of E₂ and P

After the behavioral test, the rats were anesthetized with 10% chloral hydrate (30 mg/kg), and blood samples were taken from the abdominal aorta and then centrifuged at 1500 ×g at 4°C for 15 min. Serum was collected and stored at -20°C for E₂ and P level detection in each group of rats. The kits were provided by the China Institute of Atomic Energy Science, and the instrument was a Maglumi automatic chemiluminescence tester. The operation process was in accordance with the instructions of the kits.

ELISA

After the mice were anesthetized, blood was collected from the eyeballs of the mice, left standing at room

temperature for 2 h, refrigerated at 4°C. The next day, the blood was centrifuged at 1500 ×g for 15 min at a low temperature, and the serum was collected and refrigerated at 4°C for later use. Mice were then sacrificed by cervical dislocation, and the hippocampal fraction was dissected and frozen in liquid nitrogen. Subsequently, it was weighed and cut into pieces (1–3 mm) and prepared into a 10% homogenate with an ultrasonic cell crusher (Shanghai Billion Instrument Co., Ltd., Shanghai, China). Later, the homogenate was centrifuged at 1500 ×g at 4°C for 15 min and tested with supernatant. ELISA kits were utilized to detect serum Cor (Elabscience, Wuhan, China), hippocampal Allo (Raybiotech, Norcross, GA, USA), IL-1β (Shanghai Yanjin Biotechnology Co., Ltd., Shanghai, China), and TNF-α (R&D Systems, Minneapolis, MN, USA) levels. In brief, the dilution and sample addition of the standard products were carried out in strict accordance with the instructions of ELISA kits, and the processes of incubation, washing, enzyme addition, color development, and termination of the reaction were conducted in sequence. After zero-setting of the microplate reader, the OD values of serum Cor and Allo, IL-1β and TNF-α in the hippocampus of mice in each group were measured sequentially at a wavelength of 450 nm. The concentration of each factor in the sample was then calculated from the standard curve.

Quantitative real-time polymerase chain reaction (qPCR) detection

Hippocampal tissue fragments were digested with trypsin and washed with PBS, and TRIzol reagent (Invitrogen, CA, USA) was added to extract RNA, which was reverse-transcribed into cDNA with the iScript™ cDNA synthesis kit (Bio-Rad, CA, US). After transcription, the synthesized cDNA was subjected to qPCR analysis using an UltraSYBR Mixture kit (CWBio, Beijing, China) on an Applied Biosystems 7500 qPCR System (Life Technologies). The qPCR conditions were as follows: 95°C for 10 min, 95°C for 10 s, 56°C for 30 s, and 72°C for 32 s, for a total of 40 cycles. GAPDH served as an internal reference. The relative expression level of target genes was calculated using the relative quantification 2^{-ΔΔCt} method to determine fold-change. All samples were run in triplicate. The primers used were as follows:

ERα forward: 5'-AGCAACAGCATCGCCGTCTG-3',
ERα reverse: 5'-AGCATCTCCAGCAGGTCAT-3';
ERβ forward: 5'-ATCTCCTCCCAGCAGCAGTCAG-3',
ERβ reverse: 5'-AGCATCTCCAGCAGGTCAT-3';
GAPDH forward: 5'-GCAAGTTCAACGGCACAG-3',
GAPDH reverse: 5'-GCCAGTAGACTCCACGACAT-3'.

Western blot

RIPA buffer (Beyotime, Shanghai, China) was utilized to extract the total protein from hippocampal brain tissue from each group, and protease and phosphatase inhibitors were added. After grinding with liquid nitrogen and centrifuging at 12,000 rpm for 30 min at 4°C, the supernatant protein was collected and stored at -80°C. The concentration of the sample was determined with a BCA kit (KeyGEN, Nanjing, China) and was uniformly 2 µg/µL. Then, 20 µL of protein was separated by 10% SDS-PAGE electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membrane. After 1 h of blocking with 5% BSA, the following antibodies were applied: TSPO (Abcam, ab108489, 1:500), BDNF (Abcam, ab108319, 1:5000), p-mTOR/mTOR (Cell Signaling Technology, #5536, #2983, 1:1000) and GAPDH (Cell Signaling Technology, #5174, 1:1000). After incubation with the antibody overnight at 4°C, the blots were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 h. The immunoreactive bands were visualized using an ECL kit (KeyGEN) on a Bio-Rad Chemi-Doc MP system (Bio-Rad). ImageJ software was used to quantify the immunoblots.

Statistical analysis

The data are presented as the means ± standard deviation (SD). All statistical analyses were conducted using SPSS Statistics 23 software, and graphs were drawn with GraphPad 8 software. Group comparisons were performed with Student's *t* test (for comparisons between two groups) or one-way analysis of variance (ANOVA) with the Tukey *post hoc* test (for comparisons between multiple groups) after the confirmation of normal distribution using the Shapiro-Wilk normality test and equal variances via the Brown-Forsythe test. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Hormone withdrawal after HSP-induced PPD in rats

After establishing a rat PPD model, several comparisons were conducted on different indicators. The results of depression-related behaviors included that the sucrose preference rate of the model group was significantly lessened (Fig. 1A; $F_{(2,15)}=8.25$, $p < 0.001$), while TST ($F_{(2,15)}=18.70$, $p < 0.001$) and FST ($F_{(2,15)}=13.33$, $p < 0.001$) immobility time were increased (Fig. 1B, C). The detection

of E_2 and P by radioimmunoassay showed that the serum E_2 level ($F_{(2,15)}=10.75$, $p < 0.001$) was drastically decreased, and the P level ($F_{(2,15)}=12.55$, $p < 0.001$) was significantly increased in PPD rats (Fig. 1D, E). ELISA showed that the serum Cor level ($F_{(2,15)}=18.17$, $p < 0.001$) of the model group was markedly increased compared with the control group and sham group. The level of Allo ($F_{(2,15)}=9.53$, $p < 0.001$) was lessened in the hippocampal fraction, while that of IL-1β ($F_{(2,15)}=10.72$, $p < 0.001$) and TNF-α ($F_{(2,15)}=16.47$, $p < 0.001$) was upregulated (Fig. 1F-I). Furthermore, the expression of ERα ($F_{(2,15)}=11.84$, $p < 0.001$) and ERβ ($F_{(2,15)}=12.38$) in the hippocampus of the model group was drastically reduced, while the ratio of ERα/ERβ ($F_{(2,15)}=8.68$, $p < 0.001$) increased significantly (Fig. 1J-L). Collectively, hormone withdrawal after HSP successfully induced PPD in the rat models.

The effect of PPD on the activation of the TSPO and BDNF-mTOR pathways

To analyze the effect of PPD on the activation of the TSPO and BDNF-mTOR pathways, western blotting was used to detect the protein levels of TSPO, BDNF, and p-mTOR in the hippocampus. The results of the above proteins showed that the TSPO ($F_{(2,15)}=13.06$, $p < 0.001$), BDNF ($F_{(2,15)}=13.25$, $p < 0.001$) protein and p-mTOR/mTOR ($F_{(2,15)}=17.42$, $p < 0.001$) levels in the hippocampus of PPD rats were drastically downregulated compared with those in the control or sham group (Fig. 2A-D). The above results indicated that the TSPO and BDNF-mTOR pathways were inactivated in PPD rats.

The improvement effect of paeoniflorin on PPD in rats

To analyze the improvement effect of paeoniflorin on PPD, PPD rats were treated with paeoniflorin and fluoxetine. After the intervention, paeoniflorin dramatically ameliorated the depression symptoms of PPD rats, as evidenced by the SPT (Fig. 3A; $F_{(4,25)}=12.00$, $p < 0.001$), TST (Fig. 3B; $F_{(4,25)}=13.21$, $p < 0.001$) and FST (Fig. 3C; $F_{(4,25)}=13.57$, $p < 0.001$). In addition, paeoniflorin or fluoxetine treatment clearly upregulated serum E_2 levels ($F_{(4,25)}=14.06$, $p < 0.001$) but significantly decreased P levels ($F_{(4,25)}=18.45$, $p < 0.001$) (Fig. 3D and E). Serum Cor ($F_{(4,25)}=15.42$, $p < 0.001$), IL-1β ($F_{(4,25)}=8.95$, $p < 0.001$) and TNF-α ($F_{(4,25)}=16.32$, $p < 0.001$) levels were markedly decreased, and the level of hippocampal Allo ($F_{(4,25)}=14.70$, $p < 0.001$) was notably increased in PPD rats by paeoniflorin or fluoxetine treatment (Fig. 3F-I). Moreover, qPCR assays showed that paeoniflorin drastically increased the hippocampal ERα ($F_{(4,25)}=11.59$, $p < 0.001$) and

ER β ($F_{(4,25)}=19.54$, $p<0.001$) expression levels but reduced the ER α /ER β ratio ($F_{(4,25)}=13.84$, $p<0.001$) (Fig. 3J-L). Taken together, paeoniflorin relieved PPD in rats.

The effect of paeoniflorin on the activation of the TSPO and BDNF-mTOR pathways in rats with PPD

To analyze the effect of paeoniflorin on the activation of the TSPO and BDNF-mTOR pathways in PPD rats, western blotting was performed to detect the protein levels of TSPO, BDNF, and p-mTOR in the hippocampus. The results showed that TSPO ($F_{(4,25)}=10.04$, $p<0.001$), BDNF ($F_{(4,25)}=17.06$, $p<0.001$) proteins and p-mTOR/

mTOR ($F_{(4,25)}=12.49$, $p<0.001$) levels were drastically up-regulated by paeoniflorin or fluoxetine in comparison with the model group (Fig. 4A-D). These results demonstrated that paeoniflorin could activate the TSPO and BDNF-mTOR pathways in PPD rats.

Inhibition of TSPO or BDNF partially reverses the effect of paeoniflorin on PPD

To determine whether paeoniflorin could improve PPD by activating the TSPO and BDNF-mTOR pathways, TSPO or BDNF was inhibited in PPD rats. The results of the SPT, FST and TST showed that the inhibition

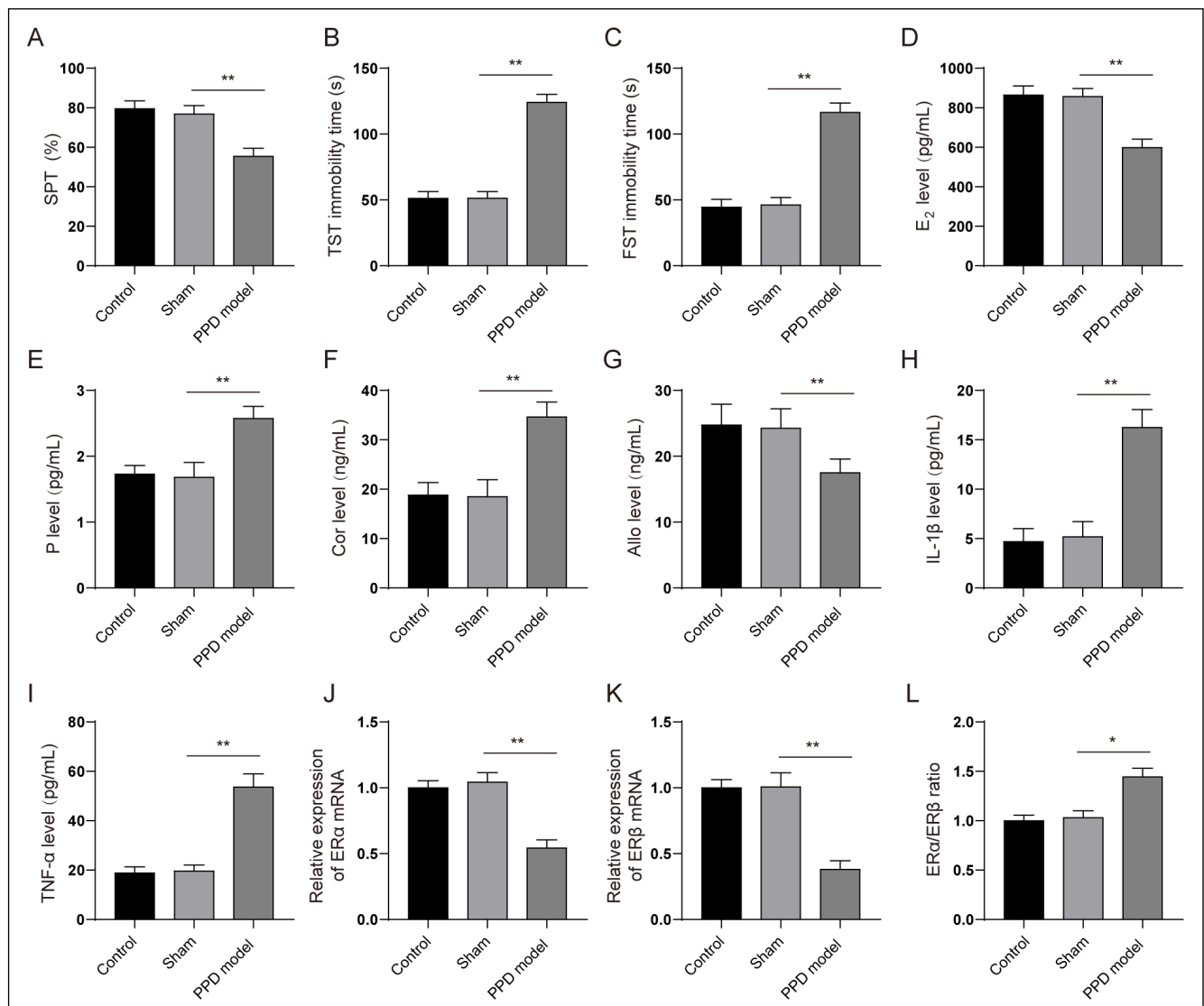


Fig. 1. Hormone withdrawal after HSP-induced PPD in rats. (A-C) SPT, TST and FST assessed depression-like behaviors in rats; (D-E) Radioimmunoassay detected serum E₂ and P levels; (F-I) ELISA was performed to measure serum Cor, hippocampal Allo, IL-1 β , and TNF- α levels in rats; (J-L) Hippocampal ER α , ER β , ER α /ER β ratio was determined *via* qPCR. Comparisons were conducted using one-way ANOVA with *post hoc* analysis. $n=6$. * $p<0.05$; ** $p<0.01$.

of TSPO or BDNF upregulated the immobility time in the TST ($F_{(5,30)}=10.73$, $p<0.001$) and FST ($F_{(5,30)}=13.92$, $p<0.001$), which was reduced by paeoniflorin, and downregulated the elevated sucrose preference rate ($F_{(5,30)}=13.28$, $p<0.001$) (Fig. 5A-C). Moreover, inhibition of TSPO or BDNF downregulated the elevated serum E_2 level ($F_{(5,30)}=11.01$, $p<0.001$) and upregulated the P lev-

el ($F_{(5,30)}=17.43$, $p<0.001$) in paeoniflorin-treated PPD rats (Fig. 5D and E). ELISA showed that inhibition of TSPO or BDNF upregulated paeoniflorin-reduced serum Cor levels ($F_{(5,30)}=18.74$, $p<0.001$), reduced hippocampal Allo levels ($F_{(5,30)}=15.95$, $p<0.001$), and increased IL- 1β ($F_{(5,30)}=19.40$, $p<0.001$) and TNF- α ($F_{(5,30)}=19.83$, $p<0.001$) levels (Fig. 5F-I). Furthermore, TSPO or BDNF

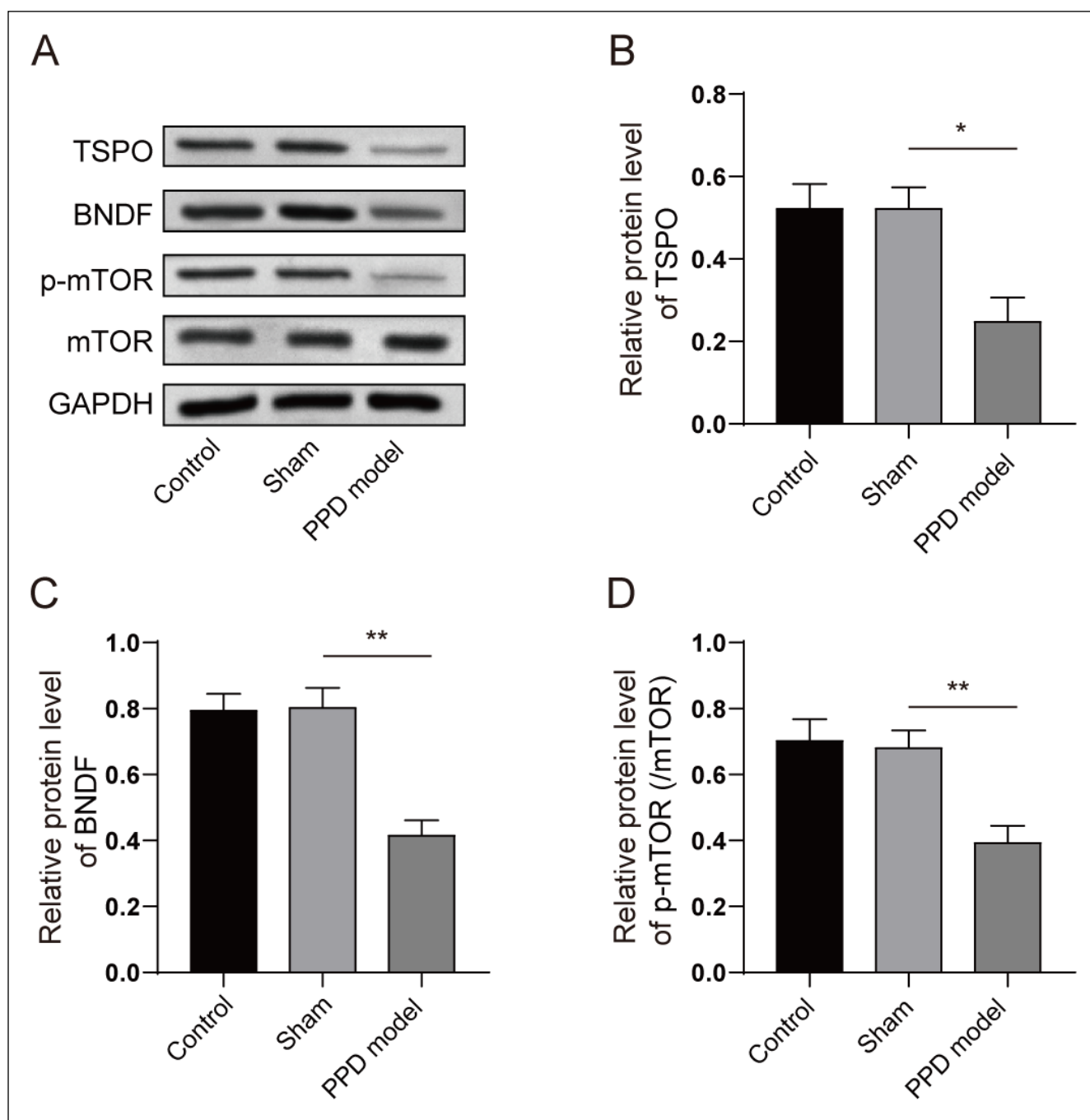


Fig. 2. The effect of PPD on the activation of TSPO and BDNF-mTOR pathways in rats. (A) Western blot was used to detect the expression of TSPO, BDNF, mTOR, and p-mTOR proteins in the hippocampus of PPD rats; (B) Quantification analysis of TSPO protein; (C) Quantification analysis of BDNF protein; (D) Quantification analysis of p-mTOR protein. Comparisons were conducted using one-way ANOVA with *post hoc* analysis. $n=6$. * $p<0.05$; ** $p<0.01$.

suppression decreased the elevated hippocampal ER α ($F_{(5,30)}=16.82$, $p<0.001$) and ER β ($F_{(5,30)}=19.51$, $p<0.001$) expression induced by paeoniflorin and significantly increased the ER α /ER β ratio ($F_{(5,30)}=17.44$, $p<0.001$) (Fig. 5J-L). Together, paeoniflorin may improve PPD in rats by activating the TSPO and BDNF-mTOR pathways.

DISCUSSION

PPD is a serious mental illness and the most common complication of childbirth, which has negative

impacts on mothers who have just given birth. Suicides caused by PPD account for approximately 20% of postpartum deaths (Lindahl et al., 2005). However, the pathogenesis of PPD is very complicated and has not been fully elucidated. In this study, a PPD model was established to evaluate the effect of paeoniflorin on PPD. Rats treated with HSP mimicked the high levels of E₂ and P in the third trimester of pregnancy, and these levels dropped rapidly after delivery. The decline in circulating ovarian steroids caused subjects to develop symptoms of PPD (Stoffel and Craft, 2004; Furuta et al., 2013). In this study, the antide-

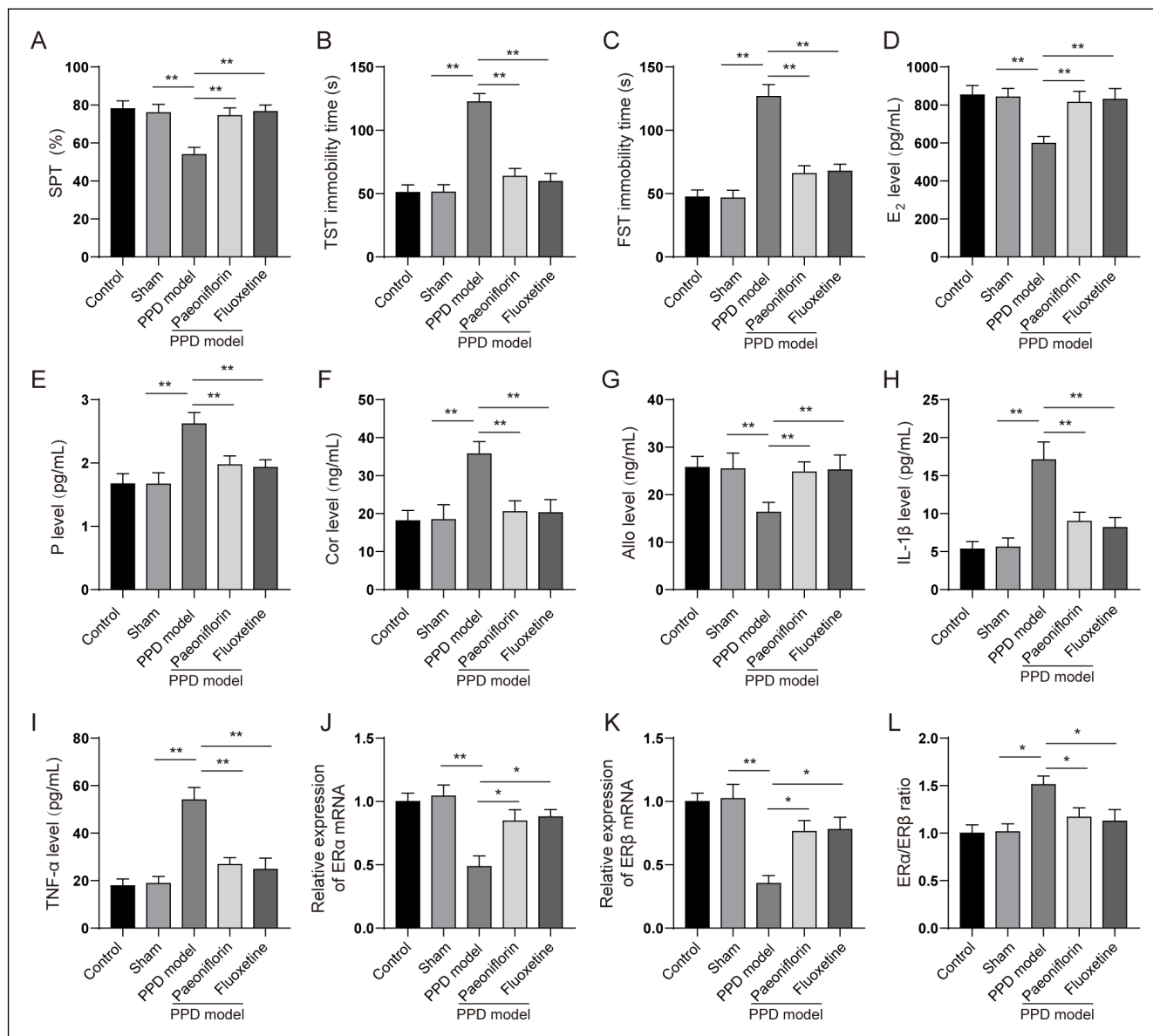


Fig. 3. The improvement effect of paeoniflorin on PPD in rats. (A-C) SPT, TST and FST assessed depression-like behaviors of PPD rats after paeoniflorin intervention; (D-E) Radioimmunoassay detection of serum E₂ and P levels in PPD rats treated with paeoniflorin; (F-I) Serum Cor, hippocampal Allo, IL-1 β , and TNF- α levels were assessed via ELISA; (J-L) qPCR was used to measure hippocampal ER α , ER β expression, and ER α /ER β ratio. Comparisons were conducted using one-way ANOVA with *post hoc* analysis. n=6. * $p<0.05$; ** $p<0.01$.

pressant activity and mechanism of paeoniflorin in PPD rat models were evaluated by detecting depressive-like behavior, hormone levels, cytokine release, and TSPO and BDNF-mTOR pathway activation. The results showed that paeoniflorin plays a positive role in improving depression in PPD rats, the mechanism of which may be achieved by TSPO and BDNF-mTOR pathway activation.

Fluoxetine, an antidepressant, was used as a positive control for paeoniflorin drug administration in this study (Volz and Laux, 2000). Current studies have found that fluoxetine can also relieve many symptoms of premenstrual dysphoria, which is closely related to ovarian hormones, including P (Su et al., 1997). Paeoniflorin is the main biologically active ingredient in *Paeonia lactiflora* Pallas (Zhou et al., 2020). Previous stud-

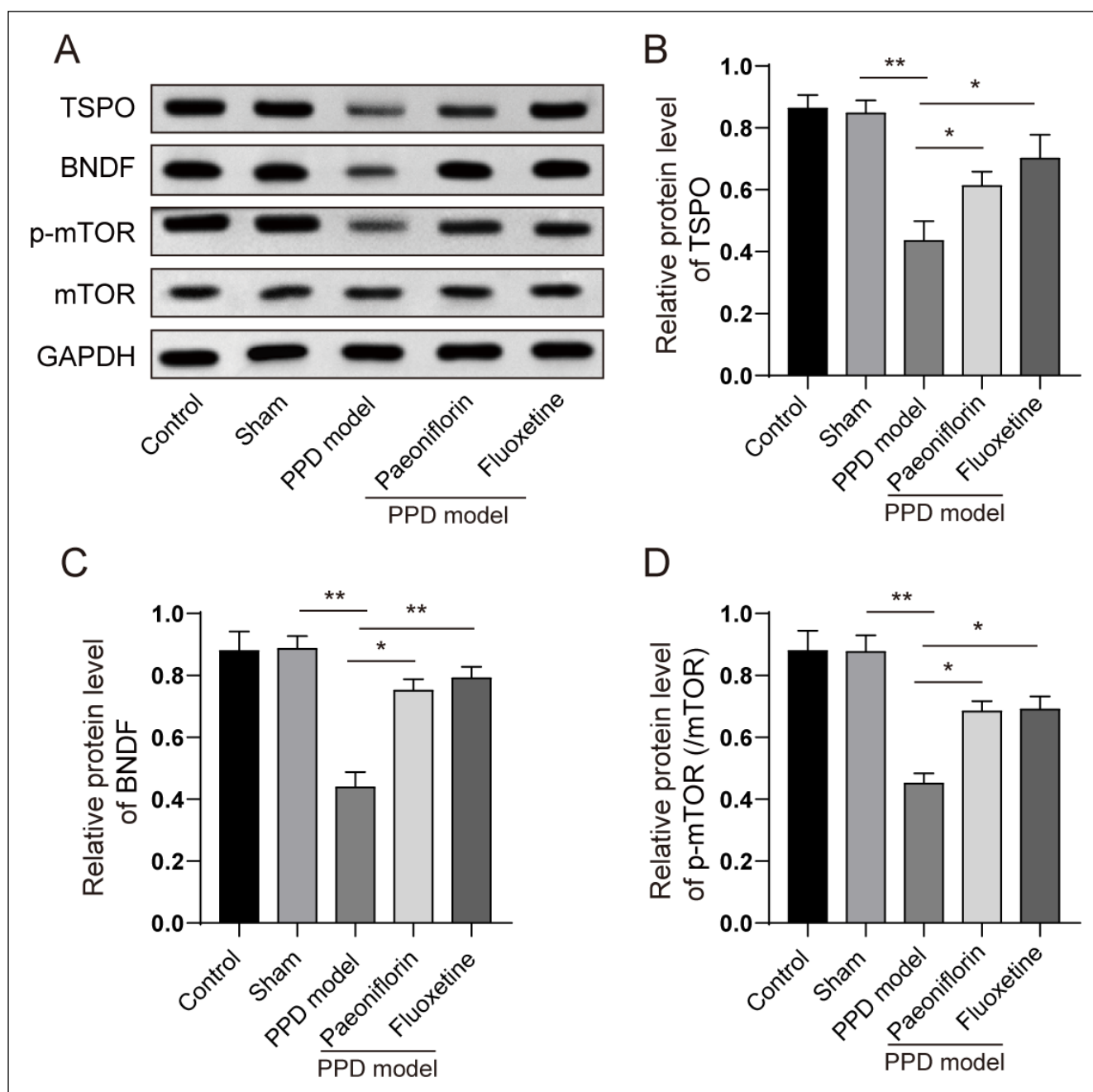


Fig. 4. The effect of paeoniflorin on the activation of TSPO and BDNF-mTOR pathways in PPD rats. (A) Western blot to detect the expression of TSPO, BDNF, mTOR, and p-mTOR proteins in the hippocampus of rats; (B) Quantification analysis of TSPO protein; (C) Quantification analysis of BDNF protein; (D) Quantification analysis of p-mTOR protein. Comparisons were conducted using one-way ANOVA with *post hoc* analysis. n=6. *p<0.05; **p<0.01.

ies have found that paeoniflorin can improve central nervous system diseases, such as depression (Bai et al., 2021). Additionally, paeoniflorin can inhibit the activation of spinal microglia and exert anti-inflammatory effects (Hu et al., 2018). Previous studies have found that paeoniflorin can improve chronic stress-induced depression-like behavior in a mouse model by affect-

ing the ERK1/2 pathway (Tang et al., 2021). However, whether paeoniflorin can improve PPD has not yet been reported. The SPT, FST and TST are well-established models for evaluating depression in animal models (Wang et al., 2008). After establishing PPD rat models, we observed that the sucrose preference of PPD rats was drastically reduced, and the immobility time of

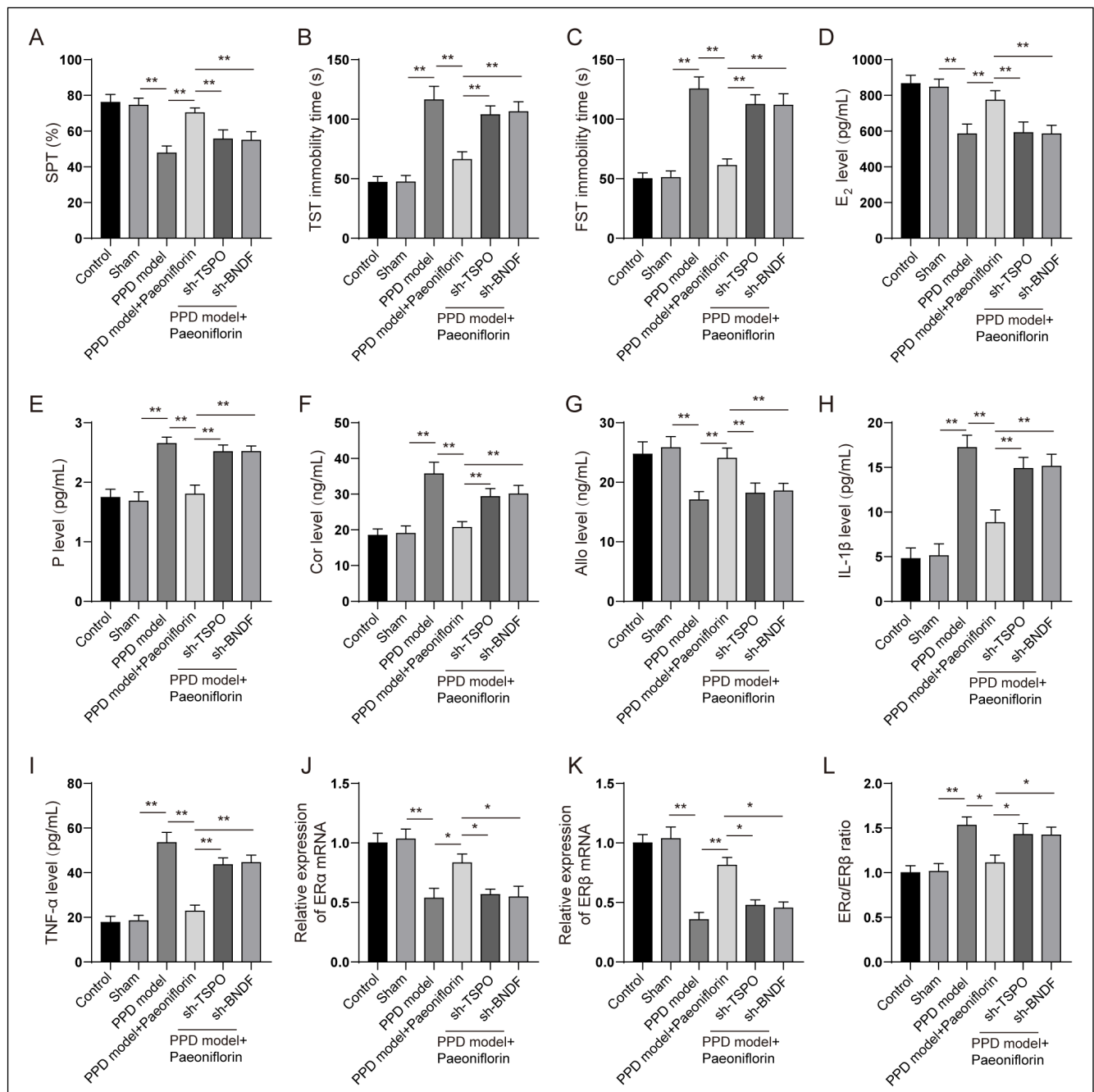


Fig. 5. Inhibition of TSPO or BDNF partially reverses the improvement effect of paeoniflorin on PPD. (A-C) SPT, TST and FST were performed to evaluate depression-like behaviors of rats; (D-E) Radioimmunoassay was used to detect serum E₂ and P levels in rats; (F-I) ELISA detection of serum Cor, hippocampal Allo, IL-1β, and TNF-α levels; (J-L) qPCR detection of hippocampal ERα, ERβ expression, ERα/ERβ ratio. Comparisons between groups were performed using one-way ANOVA with *post hoc* analysis. n=6. **p*<0.05; ***p*<0.01.

the FST and TST increased notably. In these behavioral tests, paeoniflorin enhanced the sucrose preference and reduced the immobility time of PPD rats. In addition, paeoniflorin showed comparable results to fluoxetine treatment, indicating that paeoniflorin can function as an effective PPD antidepressant. E_2 exerted an antidepressant effect by regulating transmitter release and nerve excitability and could inhibit the reuptake of 5-HT in the postsynaptic membrane by promoting the synthesis of 5-HT (Li et al., 2020). Moreover, E_2 acted on the autonomic nervous system and the ascending reticular activation system, which regulated the excitability of the central nervous system and mental and psychological activities (Li et al., 2019). Some studies have suggested that the cause of PPD is a sharp decline in the release of hormones from the fetus and placenta in mothers after childbirth. The decrease in maternal adrenal hormones leads to a decline in maternal sex hormones and then causes maternal neurotransmitter secretion abnormalities, resulting in abnormal excitement and inhibition of the maternal brain, ultimately leading to PPD (Hu et al., 2019). In our study, when the PPD model was established, the E_2 level in the serum of rats was drastically reduced, while the P level was significantly increased, indicating that paeoniflorin could effectively improve PPD. $ER\alpha$ and $ER\beta$ are E_2 receptors that can mediate the genotypic effects of E_2 and exert gene regulatory effects by regulating the transcription of specific target genes (Weyant et al., 2001). We also found that after establishment of the PPD model, the expression levels of $ER\alpha$ and $ER\beta$ in the hippocampus of rats were notably decreased, while after paeoniflorin intervention, the levels were significantly restored. Previous studies have shown that E_2 can produce two different physiological effects through $ER\alpha$ and $ER\beta$ (Jacobson et al., 2015), thus indicating that changes in $ER\alpha$ and $ER\beta$ can target E_2 , thereby affecting PPD.

TSPO is related to neurodegenerative and psychiatric diseases. Decreased expression of TSPO was observed in patients with comorbid anxiety and depression or bipolar disorder (Abelli et al., 2010; Arbo et al., 2015). Our data showed that TSPO levels were downregulated in PPD rat models, which suggested that PPD may also cause a decrease in TSPO levels. In addition, we found that paeoniflorin could restore the level of TSPO in PPD rats, and inhibiting the level of TSPO could reverse the effect of paeoniflorin on improving PPD. This suggested that TSPO may be a mechanism by which paeoniflorin relieves PPD. BDNF, a neurotrophic factor, is essential for placental development during pregnancy and is associated with major depression (Nagahara and Tuszynski, 2011; Christian et al., 2016). Studies have shown that BDNF is reduced during pregnancy and postpartum with depressive symptoms (Fung et

al., 2015). Decreased BDNF levels are considered to be the underlying pathological mechanism of impaired neurogenesis in depression (Jiang et al., 2012). It was reported that BDNF and another mammalian target of rapamycin (mTOR) play an important role in the development of certain mental diseases (such as depression) (Autry et al., 2011; Kim et al., 2012). mTOR exists in the synaptic area in neurons, where it regulates the synthesis of locally translated proteins and is essential for different forms of synaptic plasticity (Ni et al., 2020). We found that BDNF was downregulated in the PPD models, and the activation of mTOR was also drastically inhibited. In addition, we found that paeoniflorin reversed the downregulation of BDNF and the inhibition of mTOR phosphorylation in PPD rats, indicating that paeoniflorin may improve PPD by regulating the BDNF-mTOR pathway. Considering previous research results, paeoniflorin could ameliorate depression-like behavior by inhibiting the pyroptosis CASP-11/GSDMD pathway or the inflammatory TLR4/NF- κ B signaling pathway (Bai et al., 2021; Tian et al., 2021). Whether the same functional pathways exist in PPD is a topic of our future research directions.

CONCLUSIONS

In summary, we studied the effect of paeoniflorin on the treatment of PPD and its molecular mechanism. The findings proved that paeoniflorin may improve symptoms of PPD by activating the TSPO and BDNF-mTOR pathways.

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