Rutin Treats Perimenopausal Depression Rats through Allopregnanolone Mediated mRNA Expression of Gabra4, Gabrb2 in the Prefrontal Cortex

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Abstract

Objective

We determined whether the pathogenesis of perimenopausal depression (PMD) is associated with allopregnanolone (3α,5α-THP, ALLO) mediated GABAA receptors subunits expression changes in different brain regions. Simultaneously, we aimed to investigate the therapeutic efficacy and intervention mechanisms of the flavonoid rutin in treating PMD.

Methods

The PMD rat model was established by ovariectomy surgery followed by chronic unpredictable mirutin stress processes. ALLO was administered via intraperitoneal injection to increase ALLO levels in PMD rats, while rutin was administered via oral gavage for PMD treatment. Behavioral assessments, including open-fierutin test, sucrose preference test, and forced swimming test, were conducted to evaluate depressive-like behaviors in rats. ELISA was employed to measure the levels of E2, 5-HT, NE, ALLO, and GABA in the serum. Quantitative PCR was used to assess the mRNA expression of Gabra4, Gabrb2, and Gabrd in the prefrontal cortex, hippocampus, hypothalamus, and amygdala.

Results

The PMD rats exhibited depressive-like behavior, with decreased levels of E2, 5-HT, NE, ALLO, and GABA in the serum. The mRNA expression of Gabra4 and Gabrb2 increased in the prefrontal cortex, hippocampus, and hypothalamus of PMD rats, while Gabrd showed a increase in the hypothalamus and amygdala. ALLO improved depressive-like behavior and increased serum levels of E2, 5-HT, NE, and ALLO in PMD rats. ALLO acted on PMD rats, reduced mRNA expression of Gabra4 and Gabrb2 in the prefrontal cortex, increased mRNA expression of Gabrd in the prefrontal cortex, elevated mRNA expression of Gabra4 and Gabrd in the hippocampus, and decreased Gabrb2 mRNA expression in the hypothalamus.Rutin improved depressive-like behavior in PMD rats, increased serum levels of 5-HT and ALLO, and decreased mRNA expression of Gabra4 and Gabrb2 in the prefrontal cortex.

Conclusion

ALLO-mediated mRNA expression of Gabra4, Gabrb2 in the prefrontal cortex, and Gabrb2 in the hypothalamus is one of the pathological mechanisms in PMD. ALLO can improve depressive symptoms in PMD rats. Rutin (8.65 mg/kg) exerts a therapeutic effect on PMD by upregulating serum ALLO levels, subsequently downregulating mRNA expression of prefrontal cortex Gabra4 and Gabrb2.

1. Introduction

Depression is an emotional disorder, and the probability of females experiencing depression is twice that of males[1]. The perimenopausal period is a susceptible time for depression. In China, the detection rate of depressive symptoms in perimenopausal women is 36.0–41.6%[2]. A meta-analysis found that the prevalence of depression during the perimenopausal period is 42.47% in India[3].The perimenopausal period refers to the time in women from the onset of menopause to within 12 months after menopause. During this stage, ovarian function begins to decline, and women often exhibit symptoms such as depressive emotions, melancholy, chest tightness, palpitations, hot flashes, etc. Due to continuous pressure from various aspects such as family and society, depressive emotions are difficult to self-relieve, gradually developing into (perimenopausal depression, PMD)[4].

The pathogenesis of PMD primarily involves various factors such as epigenetic changes, monoamine neurotransmitter and receptor hypothesis, glial cell-induced neuroinflammation, estrogen receptor, interaction between the HPA axis and HPG axis, and the microbiota-gut-brain axis[5].The specific pathogenic mechanisms of PMD remain unclear, and exploring novel research directions may represent the optimal approach to elucidate the precise etiology of PMD. Allopregnanolone (3α,5α-THP, ALLO), a neurosteroid derived from progesterone and cholesterol, undergoes cyclic fluctuations during the female physiological cycle and plays a crucial role in emotional disorders in women[6]. Research indicates that aberrant fluctuations in ALLO levels cause premenstrual anxiety and depressive emotions[7]. The reduction in ALLO levels is considered a crucial pathogenic mechanism in postpartum depression, and Brexanolone injection (GABA-A receptor-modulating allopregnanolone) is employed as a therapeutic medication for postpartum depression[8, 9]. ALLO acts as a positive allosteric modulator of GABAA receptors (GABAAR), modulating the chloride ion channel of GABAAR to regulate overall inhibitory activity[10]. As a result, ALLO exhibits anxiolytic and antidepressant effects. ALLO-mediated expression of GABAAR subunits is one of the pathological mechanisms underlying various emotional disorder in women, including premenstrual dysphoric disorder and postpartum depression. Sun[11] discovered that ALLO mediated sensitivity of GABAAR α4 subunit plays a significant role in premenstrual dysphoric disorder. The biological mechanisms underlying postpartum depression are elucidated by numerous studies indicating that significant fluctuations in ALLO levels can induce alterations in the expression of GABAAR subunits[12]. Upon reviewing literature related to PMD, we identified a close correlation between ALLO levels and the onset of PMD. The serum levels of ALLO were found to decrease in perimenopausal women[13], and PMD-afflicted women exhibited lower ALLO levels compared to their healthy perimenopausal counterparts[14]. Simultaneously, a study identified a decrease in mRNA levels of GABRG2, possibly associated with an increased susceptibility to PMD[15]. Up to the present, there is a notable absence of research reports investigating the role of ALLO in mediating the expression of GABAAR subunits within the pathological mechanism of PMD.

Rutin, a flavonoid compound with sedative, anticonvulsant, and antidepressant properties, is found in various natural plants including Forsythia suspensa, Hypericum monogynum, buckwheat, and sophora flower buds[16].Ibrahim M Ibrahim[17] discovered that rutin can alleviate depressive behavior in mice subjected to chronic stress. Arun Parashar[18] discovered that rutin exhibits antidepressant effects by protecting neurons in the hippocampal region of mice with depression. Rutin has been found to exhibit

antidepressant effects by inhibiting monoamine oxidase expression, attenuating HPA axis hyperactivity, improving monoaminergic neurotransmitter and brain-derived neurotrophic factor levels, and influencing cholinergic, glutamatergic, and GABAergic systems, ultimately alleviating cognitive impairments and promoting neuroprotection[16, 19, 20]. Existing evidence indicates that rutin has demonstrated potential as a neuroprotector both in vivo and in vitro. However, there is currently no study demonstrating the therapeutic efficacy of rutin in treating PMD.

2. Methods

2.1 Study design`

The focus of this study is to explore the pathological mechanisms of PMD and investigate the pharmacological efficacy as well as the intervention mechanisms of rutin. This study is comprised of three experiments. Experiment one involves the establishment of PMD rat model and the exploration of the pathological mechanisms of PMD. Experiment two aims to validate the preliminary hypotheses regarding the pathological mechanisms by administering ALLO to PMD rats. Experiment three focuses on investigating the therapeutic efficacy and intervention mechanisms of rutin in treating the PMD rats. Figure 1 illustrates the diagram of the study design.

2.2 Animals and establishment of PMD rats

Specific pathogen free (SPF) grade female SD rats (8 weeks orutin, 200-300g) was used in this study, animal license number: SCXK(Beijing)2019-0008. All animals were purchased from the BEIJING HFK BIOSCIENCE CO.,LTD (Beijing, China), and housed at 21 ± 2℃ and 45 ± 10% relative humidity under a 12:12 h light/dark cycle with food and water available ad libitum. The animals were habituated to maintenance conditions for 1 week and handled daily to eliminate the human factor.

The PMD rat model was established by ovariometry (OVX) followed by chronic unpredictable mirutin stress (CUMS) processes[21]. After 1 week of adaptive feeding, rats were anesthetized using a 3% sodium pentobarbital intraperitoneal injection. Subsequently, the rats were depilated, and an incision was made in the lower third of the abdomen to locate the uterus, which was Y-shaped. We then removed both ovaries and sutured the incision[22]. For the sham control group, the operation was the same as the bilateral ovarian ablation, but only fat around the bilateral ovaries was removed.

1–2 weeks after OVX, CUMS treatment was administered. Briefy, the rats were exposed to stress stimuli, including water deprivation (24 h), food deprivation (24 h), white noise (15 min), wet cage (24 h), odor (mothball, 12 h), restricted food (1 h), empty bottle (1 h), strange items (12h), crowded living (12 h), tail nipping (1 min), restricted activity (2 h), continuous light (24 h), which lasted for 5 weeks. During the intervention, the operators need to ensure that stress is unpredictable. One or two stress stimulation was randomly given every day, and the same stress did not appear again within 3 days.

2.3 Drug treatment regimen

PMD rats were injected (intraperitoneally) with either ALLO (10 mg/kg in sesame oil, once each morning (between 9:00 AM and 10:00 AM) over a 48-h period for a total of three injections during this period. Control group rats were given the same number of injections of vehicle (sesame oil), rats were behavioral tested 0.5 h after the final hormone injection[11].

Drug treatment (i.g.) was administered 0.5h earlier than stresses every morning.The rats (experiment 3) were randomly into allocated six groups: Sham (distilled water), model (PMD + distilled water), positive control (PMD + Fluoxetine, 1.8mg/kg/day), low-dose rutin (HPLC ≥ 98%) (PMD + rutin, 8.65mg/kg/day), medium-dose rutin (PMD + rutin, 39.325mg/kg/day), and high-dose rutin (PMD + rutin, 70mg/kg/day).

2.4 Behavioral Assessments

2.4.1 Open fierutin test,OFT

The Open-Fierutin Test (OFT) protocol was performed as previously described[11]. An opaque laboratory box was divided into 9 squares of equal size. Each rat was gently placed in the center of the test area, observed for 6 min, and recorded the total distance and rest time of rats by camera. After each test, the test box was cleaned with 75% ethanol.

2.4.2 Sucrose preference test,SPT

The hallmark symptom of depression is pleasure deficiency, quantified by measuring the reward sweet taste solution (saccharin or sucrose) in rodents[23]. Before the formal experiment, rats were subjected to 3-day adaptive training. On the first day, two bottles of 1% sucrose water were placed in each cage. On the second day, one bottle of 1% sucrose water and one bottle of water were placed in each cage. On the third day, all rats were fasted and water deprived for 24 hours. After 24 h, a formal experiment was conducted, giving each rat one bottle of 1% sucrose water and one bottle of water. Each bottle was filled with 100ml, and one hour later, the water bottle was removed and the amount of sugar and water consumed by the rats was read. The sucrose preference rate was calculated, which was sugar consumption/(sugar consumption + pure water intake) × 100%.

2.4.3 Force swimming test, FST

The Force swimming test (FST) protocol was performed as previously described[24]. Each rat was individually placed into a transparent glass container with a depth of 50 cm, ensuring that the limbs and tail of the rat courutin not touch the bottom of the container. The water temperature was maintained at 25°C. The total duration of the experiment was 6 minutes, the first 2 minutes served as an adaptation period for the rat, while the subsequent 4 minutes constituted the formal experiment. The cumulative immobility time of each rat was observed. After the completion of each rat's test, the water was replaced, and the glass container was thoroughly cleaned to avoid any impact on subsequent experiments.

2.5 Serum of ELISA

Determination of 5-HT, NE, E2, ALLO, and GABA in rat serum using enzyme-linked immunosorbent assay (ELISA) content. Rat whole blood samples were collected using sterile tubes, placed at room temperature for 0.5 h, and centrifuged at (3500 rpm) for 20 min. The supernatant was extracted, or the samples were stored at − 80°C, but repeated freeze–thaw shourutin be avoided. Using a SpectraMax iD5, absorbance at 450 nm was used to determine the levels of 5-HT, NE, E2, ALLO, and GABA expression in serum.

2.6 Quantitative PCR

Total RNA was extracted from the prefrontal lobe, hypothalamus, amygdala, and hippocampus using the RNA Isolation Kit V2 (RC112-01 50 rns, Vazyme, China) and transcribed into cDNA using HiScript RT SuperMix for qPCR (R323, Vazyme, China). RT-qPCR was performed using an Quantstudio 3 and ChamQ SYBR aPCR Master Mi (Q311, Vazyme, China). The relative mRNA expression level of each target gene was calculated using the 2 − ΔΔCT method. The primers for the gene fragment were designed as follows:

GapdhF: 5'GAAGGTOGOTGTGAACGCAT3'

GapdhR: 5'CCCATTTGATGTTAGCGGGAT3'

Gabra4F: 5'GACCGTGTACTTTCACCTCAGA3'

Gabra4R: 5'ATGCTTAGGGTGGTCATCGT3'

Gabrb2F: 5'TGGACCTAAGGCGGTATCCA3'

Gabrb2R: 5'GACTGCATTGTCATCGCCAC3'

GabrdF: 5'CCAAGTCTGCCTGGTTCCAT3'

GabrdR: 5'TAGCTCTCCAGGTCCAGCAT3'

2.7 Statistical Analyses

Data are shown as mean ± standard deviation (SD). All analyses were carried out using the Graph pad prism 9.0.0 software. One-way ANOVA and t tests were used where necessary to analyze differences between mean values. The significance levels were set at: *$p < 0.05$, **$p < 0.01$,***$p < 0.001$.

3. Results

3.1 Pathological mechanism and establishment of PMD rats

3.1.1 Behavioral Assessments and ELISA

Behavioral assessments revealed that compared to the sham group, the OVX + CUMS group exhibited a significant decrease in total distance of the OFT and sucrose preference rate ($p < 0.05$, $p < 0.01$; Fig. 2A,

2C). Moreover, total rest time in the OFT and the immobility time in the FST significantly increased ($p < 0.01$; Fig. 2B, 2D). ELISA results demonstrated that compared to the sham group, the OVX + CUMS group exhibited a significant decrease in serum levels of E2, 5-HT, NE, ALLO, and GABA ($p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.05$; Fig. 2E-2I).

3.1.2 Quantitative PCR

Quantitative PCR results indicated that compared to the sham group, the OVX + CUMS group exhibited a significant increase in mRNA expression of Gabra4 in the prefrontal cortex, hippocampus, and hypothalamus ($P < 0.01$, $P < 0.001$, $P < 0.01$; Fig. 3A-3C); mRNA expression of Gabrb2 in the prefrontal cortex, hippocampus, and hypothalamus showed a significant increase ($P < 0.01$, $P < 0.001$, $P < 0.01$; Fig. 3E-3G), as did the mRNA expression of Gabrd in the hypothalamus and amygdala ($P < 0.05$, $P < 0.01$; Fig. 3K, 3M).

3.2 The effect of ALLO on PMD rats

3.2.1 Behavioral Assessments and ELISA

Following ALLO intervention, the vehicle - ALLO group exhibited a significant reduction in immobility time of FST ($P < 0.05$, Fig. 4A) and a significant increase the levels of E2, 5-HT, NE, and ALLO in serum compared to the vehicle group ($P < 0.05$, Fig. 4B-4E).

3.2.2 Quantitative PCR

Quantitative PCR results revealed that compared to the vehicle group, the vehicle - ALLO group exhibited a significant decrease in Gabra4 mRNA expression in the prefrontal cortex ($P < 0.05$, Fig. 5A). There was a significant increase in Gabra4 mRNA expression in the hippocampus ($P < 0.05$, Fig. 5B). Moreover, there was a significant decrease in Gabrb2 mRNA expression in the prefrontal cortex and hypothalamus ($P < 0.05$; Fig. 5E, 5G), while there was a significant increase in Gabrd mRNA expression in the prefrontal cortex and hippocampus ($P < 0.01$, $P < 0.05$; Fig. 5I, 5J).

3.3 The treatment effect and mechanism of rutin on PMD rats

3.3.1 Behavioral Assessments

Behavioral assessments revealed that compared to the model group, the fluoxetine, rutin (Low), rutin (Medium), and rutin (High) groups showed a significant increase in total distance of the OFT ($P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.01$; Fig. 6A) and a significant decrease in total rest time of the OFT ($P < 0.05$; Fig. 6B); the rutin (Low) group exhibited a significant increase in sucrose preference rate ($P < 0.01$; Fig. 6C); the rutin (Low) and rutin (Medium) groups showed a significant decrease in immobility time of the FST ($P < 0.05$, $P < 0.01$; Fig. 6D).

3.3.2 ELISA

ELISA results indicated that compared to the model group, the fluoxetine group exhibited a significant increase in serum E2 levels ($P < 0.05$; Fig. 7A); both the fluoxetine and rutin (Low) groups showed a significant increasethe levels of 5 -HT in serum ($P < 0.05$; Fig. 7B), and the rutin (Low) group exhibited a significant increase the levels of ALLO in serum ($P < 0.01$; Fig. 7D).

3.3.3 Quantitative PCR

Quantitative PCR results showed that compared to the model group, the rutin (Low) group exhibited a significant decrease in mRNA expression of Gabra4 and Gabrb2 in the prefrontal cortex ($P < 0.01$; Fig. 8A, 8B); the rutin (Medium) and rutin (High) groups showed a significant increase in Gabrb2 mRNA expression in the hypothalamus ($P < 0.01$, $P < 0.001$; Fig. 8C).

4. Discussion

Depression stands out as a leading cause of heightened self-harm and suicide rates, significantly compromising the well-being and overall health of women in the perimenopausal stage[25]. The exploration of the pathogenesis and therapeutic drugs of PMD are urgently needed. While there are various hypotheses concerning the pathogenesis of PMD, there is a medical consensus that ovarian dysfunction is a crucial factor contributing to the development of PMD[26]. Hence, while estrogen has emerged as a primary research direction for PMD, the controversy surrounding its impact on female depression and the significant side effects of estrogen replacement therapy have impeded the progress of estrogen-focused investigations into the pathogenesis of PMD[27]. ALLO, as a metabolite of progesterone and a positive allosteric modulator of GABAAR, plays a crucial role in female neurocognitive disorders such as postpartum depression and premenstrual dysphoric disorder. Scholars in the field of PMD research have gradually recognized the significance of ALLO in the context of PMD[28].

Compared to the Sham group, the OVX + CUMS group exhibited a significant decrease in serum levels of E2, 5-HT, and NE, accompanied by depressive-like behavior. The observed changes are consistent with the clinical presentation of PMD patients, indicating the successful establishment of PMD rats in this study. The significant decrease in ALLO and GABA levels in the serum of OVX + CUMS group aligns with previous clinical research findings[14, 29–31]. As a positive allosteric modulator of GABAAR, ALLO is likely to be involved in the pathogenesis of PMD by regulating the expression of GABAAR subunits. Therefore, we employed Q-PCR to assess the mRNA expression of Gabra4, Gabrb2, and Gabrd in brain regions. The study revealed a significant upregulation of Gabra4 and Gabrb2 mRNA expression in the prefrontal cortex, hippocampus, and hypothalamus of the OVX + CUMS group, along with a significant increase in Gabrd mRNA expression in the hypothalamus and amygdala. Many laboratories have demonstrated that ALLO withdrawal leads to a transient increase in the expression of GABAAR α4 subunit in various brain regions of the hippocampus in female mice, as well as in an in vitro neuronal system[32, 33]. The above evidence supports the findings of this study. GABAR β2 subunit is associated with neuropsychiatric disorders including bipolar disorder, epilepsy, autism spectrum disorder, Alzheimer's disease, depression, and premenstrual dysphoric disorder[34]. Compared to wild-type mice, Gabrb2 knock-out mice exhibit lower levels of depression[35, 36]. In comparison to normal young mice, perimenopausal

mice exhibit a reduced mRNA expression of GABAAR β2 in the prefrontal cortex[37]. Furthermore, there is no significant difference in prefrontal cortex GABAAR β2 mRNA expression between perimenopausal mice and those subjected to chronic unpredictable stress[37]. Clinical investigations have observed a notable reduction in mRNA transcription levels of the GABAAR β2 subunit in the prefrontal cortex among individuals suffering from depression and mood disorders[38]. Due to GABAARβ2 expression being subject to epigenetic regulation, and given that epigenetic regulation varies with development, genotype, and disease states, the expression of GABAARβ2 in different mood disorders is not consistent[39, 40]. Many studies identifie a close association between the GABAAR δ subunit and depressive emotions. In comparison to the Sham group, the OVX + CUMS group showed no significant difference in Gabrd mRNA expression in the prefrontal cortex and hippocampus, while there was a significant increase in the hypothalamus and amygdala brain regions. Zhang[24] found that the expression of the δ subunit showed no significant difference between liver-qi stagnation syndrome rats with premenstrual dysphoric disorder and normal rats, moreover, overexpression of the δ subunit in normal rats did not induce depressive behavior. Currently, there is no research reporting the relationship between Gabrd and PMD, and most literature suggests that the decrease in δ subunit expression is associated with depressive emotions[37]. Therefore, we speculate that the GABAAR δ subunit may not be involved in the occurrence and development of PMD.The significant increase in mRNA expression of the δ subunit in the hypothalamus and amygdala in this study may be related to the decrease in ALLO. A study found that compared to the pregnancy period, postpartum mice showed an increasing trend in the expression of GABAAR δ subunit[41].

The existing evidence preliminarily indicates that ALLO and the GABAergic system are associated with the pathogenesis of PMD. To further validate the hypothesis that the decrease in ALLO levels mediates changes in the mRNA expression of GABAAR subunits, leading to the onset of PMD, this study administered ALLO through intraperitoneal injection to increase ALLO levels in PMD rats. And than we observed depressive-related indicators, ALLO levels in serum, and changes in GABAAR subunit mRNA expression in the brain regions of PMD rats. The study findings indicate that intraperitoneal injection of ALLO increased the serum ALLO levels in PMD rats. This improvement in ALLO levels was associated with amelioration of depressive-like behavior in rats and an increase in serum levels of E2, 5-HT, and NE, suggesting that ALLO has a therapeutic effect on PMD. Following ALLO intervention, a significant decrease in Gabra4 mRNA expression was observed in the prefrontal cortex, and a significant increase in Gabra4 mRNA expression was noted in the hippocampus of PMD rats. Furthermore, there were significant reductions in Gabrb2 mRNA expression in the prefrontal cortex and the hypothalamus, while significant increases in Gabrd mRNA expression were observed in the prefrontal cortex and hippocampus. Many studies have also found that ALLO can enhance the expression of α4 and δ subunits in the hippocampus of female rats[42–44].Based on relevant literature and the results of previous studies, we believe that ALLO-mediated expression of Gabra4, Gabrb2 in the prefrontal cortex, and Gabrb2 in the hypothalamus is one of the pathological mechanisms of PMD. At the same time, the α4 and δ subunits in the prefrontal cortex and hippocampus may be potential targets for the therapeutic effects of ALLO on PMD. Interestingly, we found a significant increase in hippocampal Gabra4 mRNA expression in PMD rats, and

even after ALLO intervention, Gabra4 mRNA expression remained significantly elevated. Therefore, further research is needed to explore the underlying reasons for this observation.

Rutin, a monomer derived from traditional Chinese herbal medicine, exhibits antidepressant effects with minimal toxic side effects[45]. This study revealed that rutin treatment significantly ameliorated depressive-like behavior in PMD rats, elevated serum levels of 5-HT and ALLO, and concurrently reduced the mRNA expression of Gabra4 and Gabrb2 in the prefrontal cortex. The optimal therapeutic effect for PMD was observed in the low-dose group of rutin (8.65 mg/kg). Based on preliminary research, we propose that rutin exerts its therapeutic effects on PMD by upregulating ALLO levels in the serum, subsequently mediating the downregulation the mRNA expression of Gabra4 and Gabrb2 in the prefrontal cortex.

This study, for the first time, investigated the mRNA expression of ALLO-mediated GABAAR α4, β2, and δ subunits in multiple brain regions of PMD rats. By pinpointing the brain regions and subunits regulated by ALLO, it has provided numerous clues and insights for subsequent research into the pathogenesis of PMD.Simultaneously, it was discovered that rutin possesses therapeutic effects on PMD, offering a novel option for clinical treatment of PMD. However, this study has several limitations. For instance, it only investigated the mRNA expression of ALLO-mediated GABAAR α4, β2, and δ subunits in emotion-related brain regions. Subsequent research should continue to explore the protein expression of these subunits mediated by ALLO. The overall functional expression of GABAARs is crucial in the pathogenesis of PMD. Various GABAARs contain α4, β2, and δ subunits, with the α4β2δGABAR receptor having the closest association with depression and ALLO. Therefore, future studies should emphasize the impact of ALLO on the function of α4β2δGABAR.

Conclusion

ALLO-mediated mRNA expression of Gabra4, Gabrb2 in the prefrontal cortex, and Gabrb2 in the hypothalamus is one of the pathological mechanisms in PMD. ALLO can improve depressive symptoms in PMD rats. Rutin (8.65 mg/kg) exerts a therapeutic effect on PMD by upregulating serum ALLO levels, subsequently downregulating mRNA expression of prefrontal cortex Gabra4 and Gabrb2.

Declarations

Data availability

Data will be made available on request.

Author Contribution

Conceptualization, Jieqiong Wang and Jie Gao; animal experintment, Zhan Gao, Xufeng Yu, Li Geng, and Jing Zhao; data curation, Zhan Gao and Mingzhou Gao; formal analysis, Zhan Gao, Dongmei Gao and Ya Sun; funding acquisition, Mingqi Qiao, Jieqiong Wang, and Mingzhou Gao; methodology, Tingting Song;

writing original draft, Zhan Gao and Mingzhou Gao; writing—review and editing, Jieqiong Wang and Jie Gao. All authors have read and agreed to the published version of the manuscript.

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Ethics Approval The animal study was reviewed and approved by the Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine.

Consent to Participate Not applicable.

Consent to Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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Figures

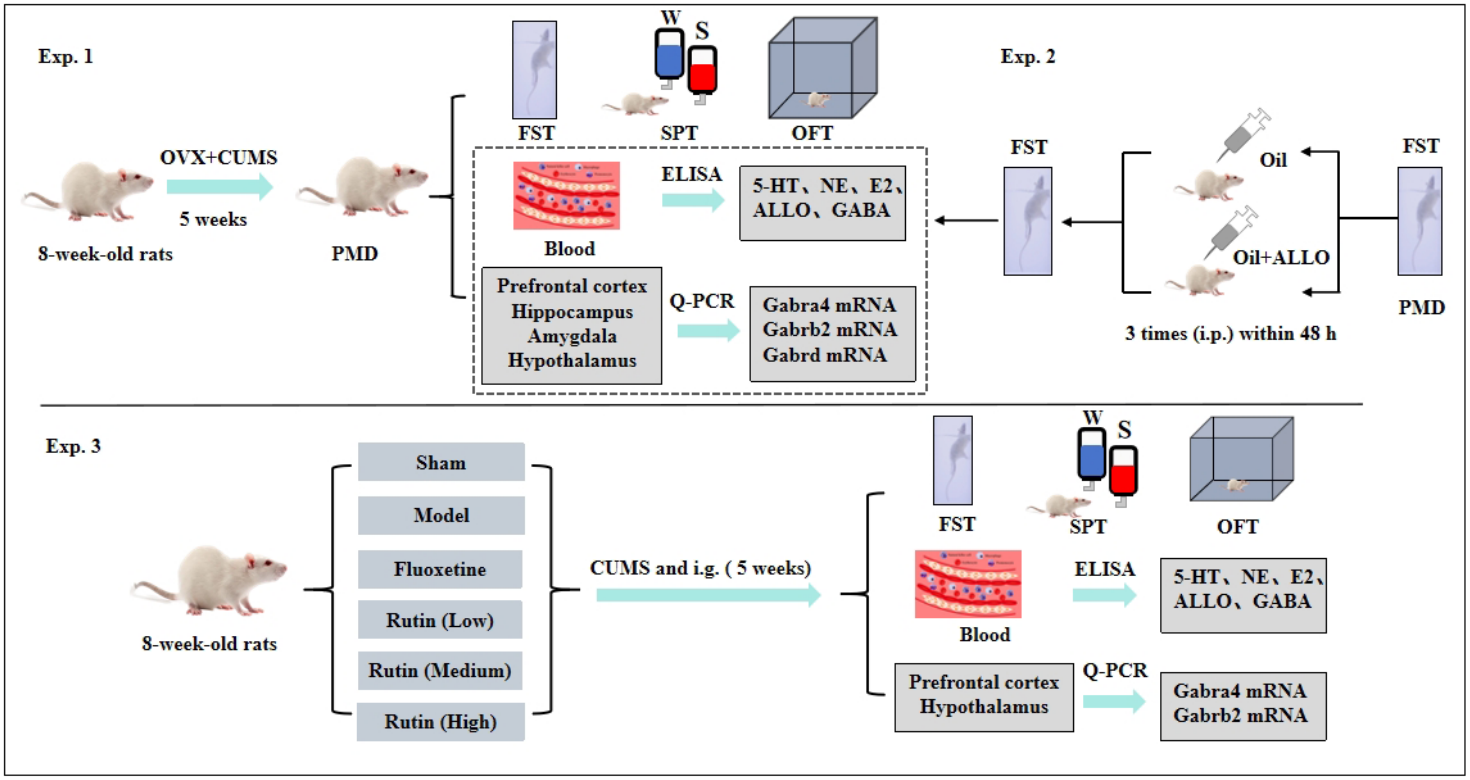

Figure 1

Flow diagram of our study design

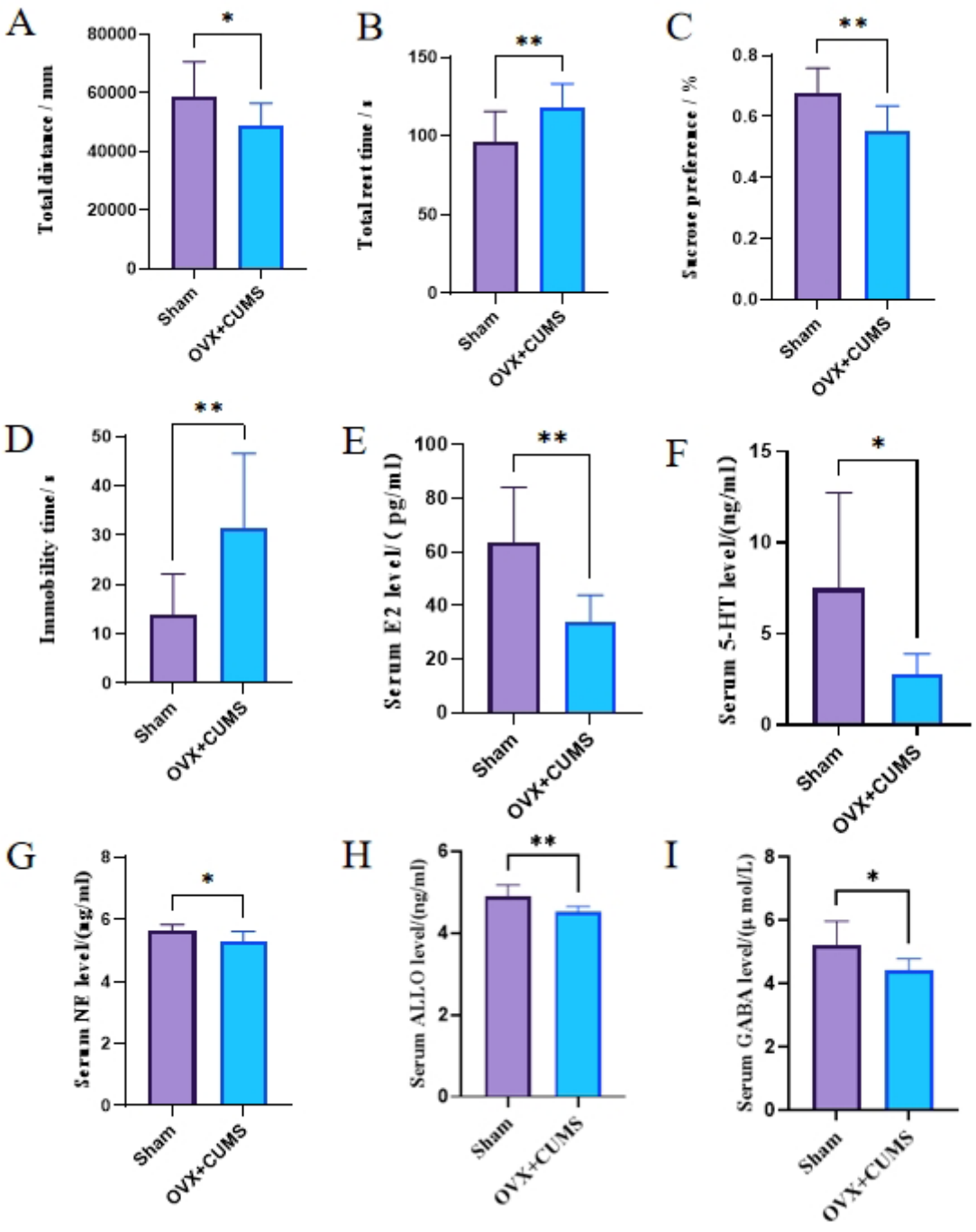

Figure 2

Behavioral Assessments and ELISA results of PMD rats. (A, B) The total distance and total rest time of the OFT; (C) Sucrose preference in the SPT; (D) Immobility time for the FST; (E-I) Levels of E2, 5-HT, NE, ALLO, and GABA in the serum, respectively. *p < 0.05, **p < 0.01.

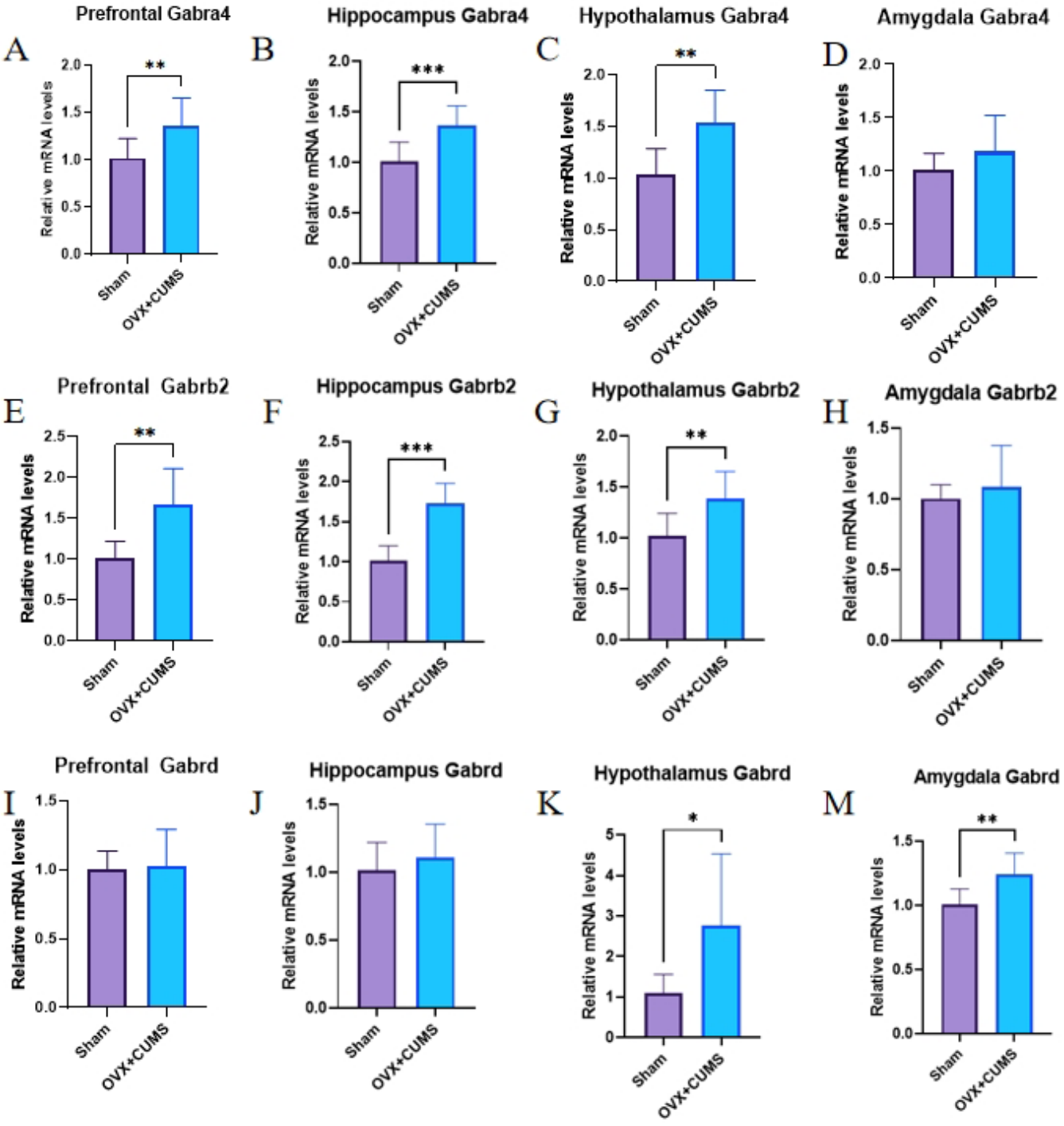

Figure 3

GABAAR subunits mRNA expressions in the brains of PMD rats. (A-D) the mRNA expression of α4 in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; (E-H) the mRNA expression of β2 in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; (I-M) the mRNA expression of δ in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; *p < 0.05, **p < 0.01,***p < 0.001.

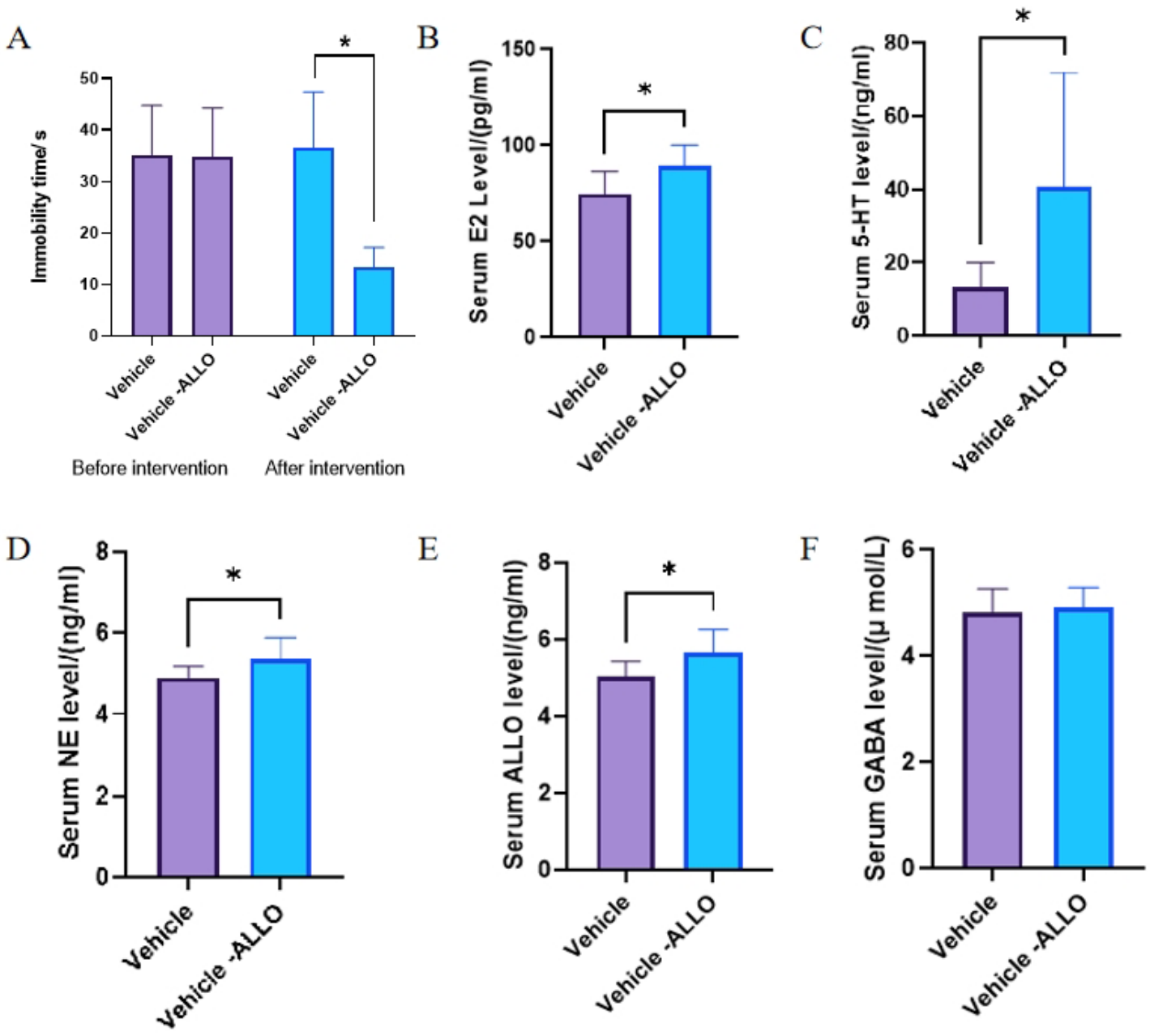

Figure 4

Effect of ALLO on behavioral assessments and ELISA in PMD rats. (A) Results of FST before and after ALLO intervention; (B-F) Levels of E2, 5-HT, NE, ALLO, and GABA in the serum, respectively. *p < 0.05.

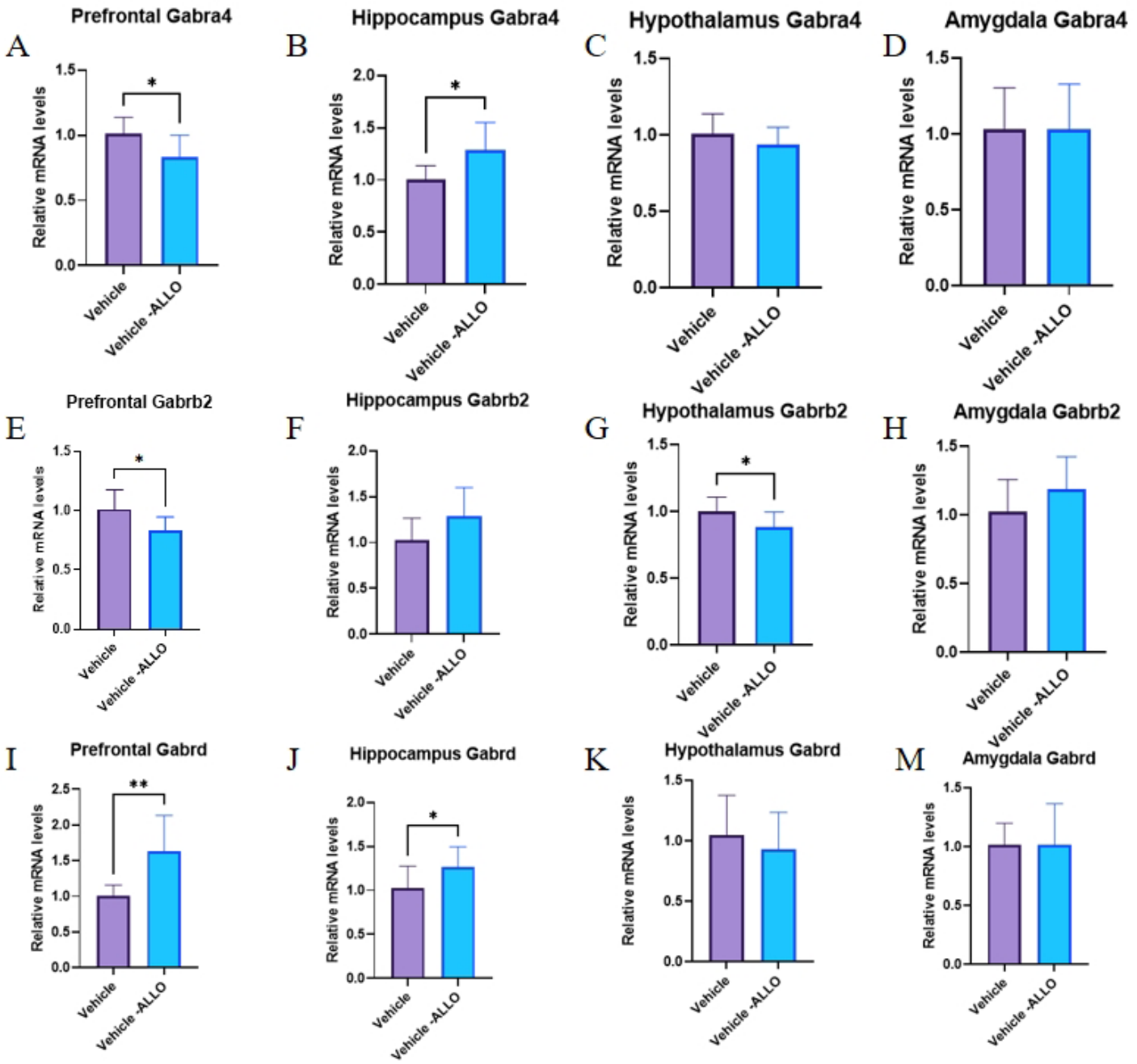

Figure 5

Effects of ALLO on GABAAR subunits mRNA expressions in the brains of PMD rats. (A-D) the mRNA expression of α4 in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; (E-H) the mRNA expression of β2 in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; (I-M) the mRNA expression of δ in the prefrontal cortex, hippocampus, hypothalamus, and amygdala of rats; *p < 0.05, **p < 0.01.

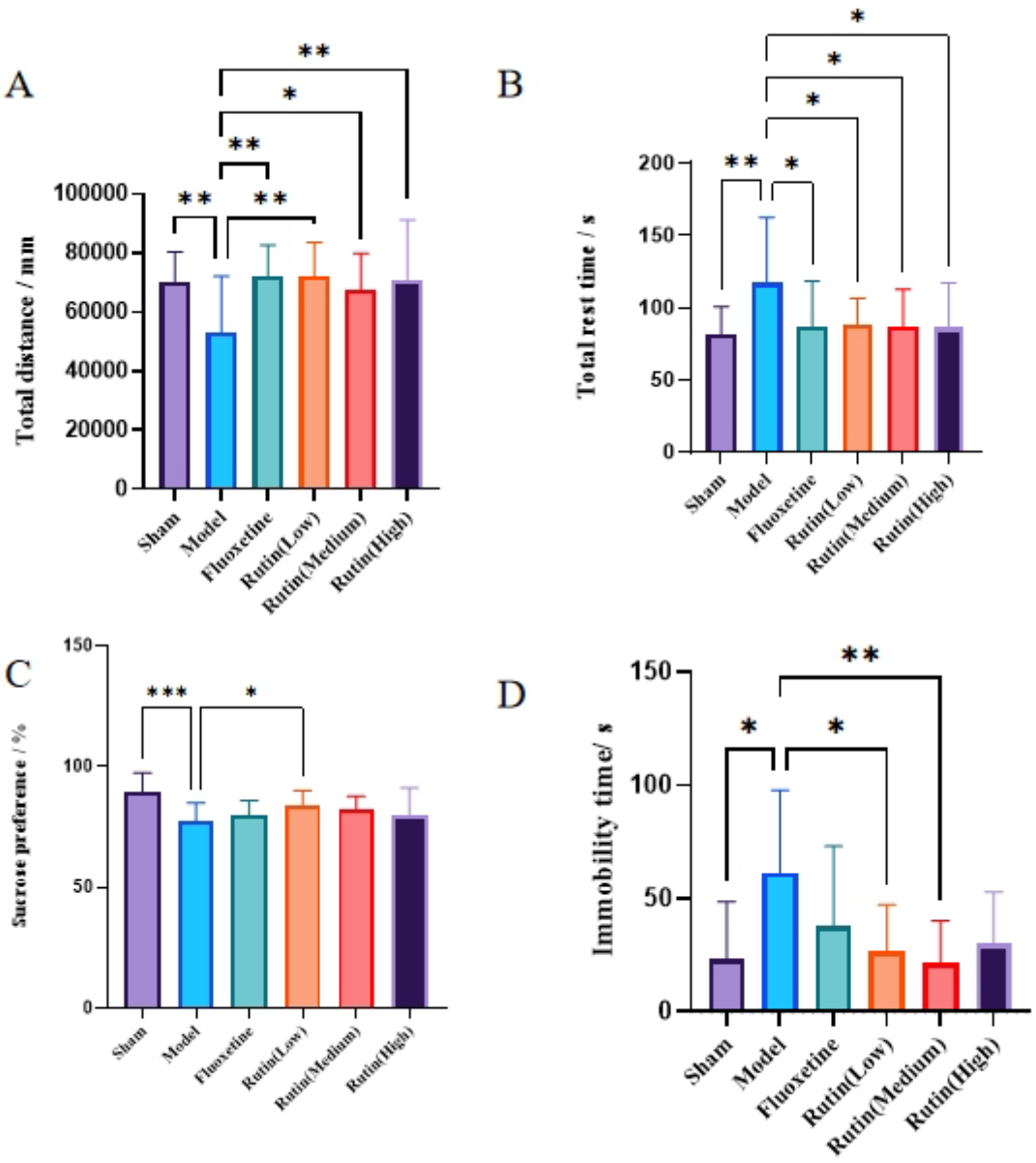

Figure 6

Effect of Rutin on behavioral assessments in PMD rats. (A,B) The total distance and total rest time of the OFT; (C) Sucrose preference in SPT; (D) Immobility time for FST; *p < 0.05, **p < 0.01,***p < 0.001.

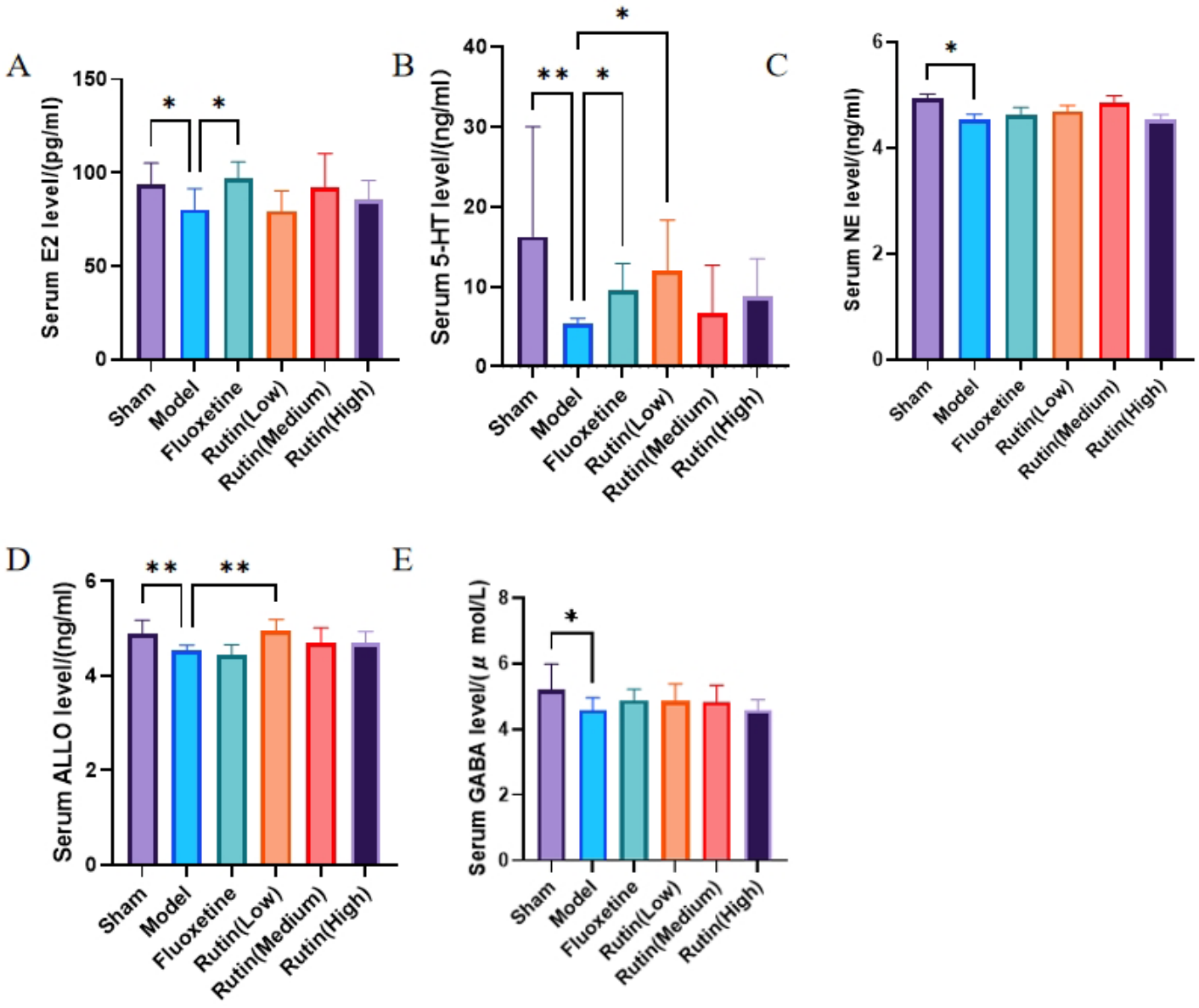

Figure 7

Effect of Rutin on ELISA in PMD rats.(A-E) Levels of E2, 5-HT, NE, ALLO, and GABA in the serum, respectively.*p < 0.05, **p < 0.01.

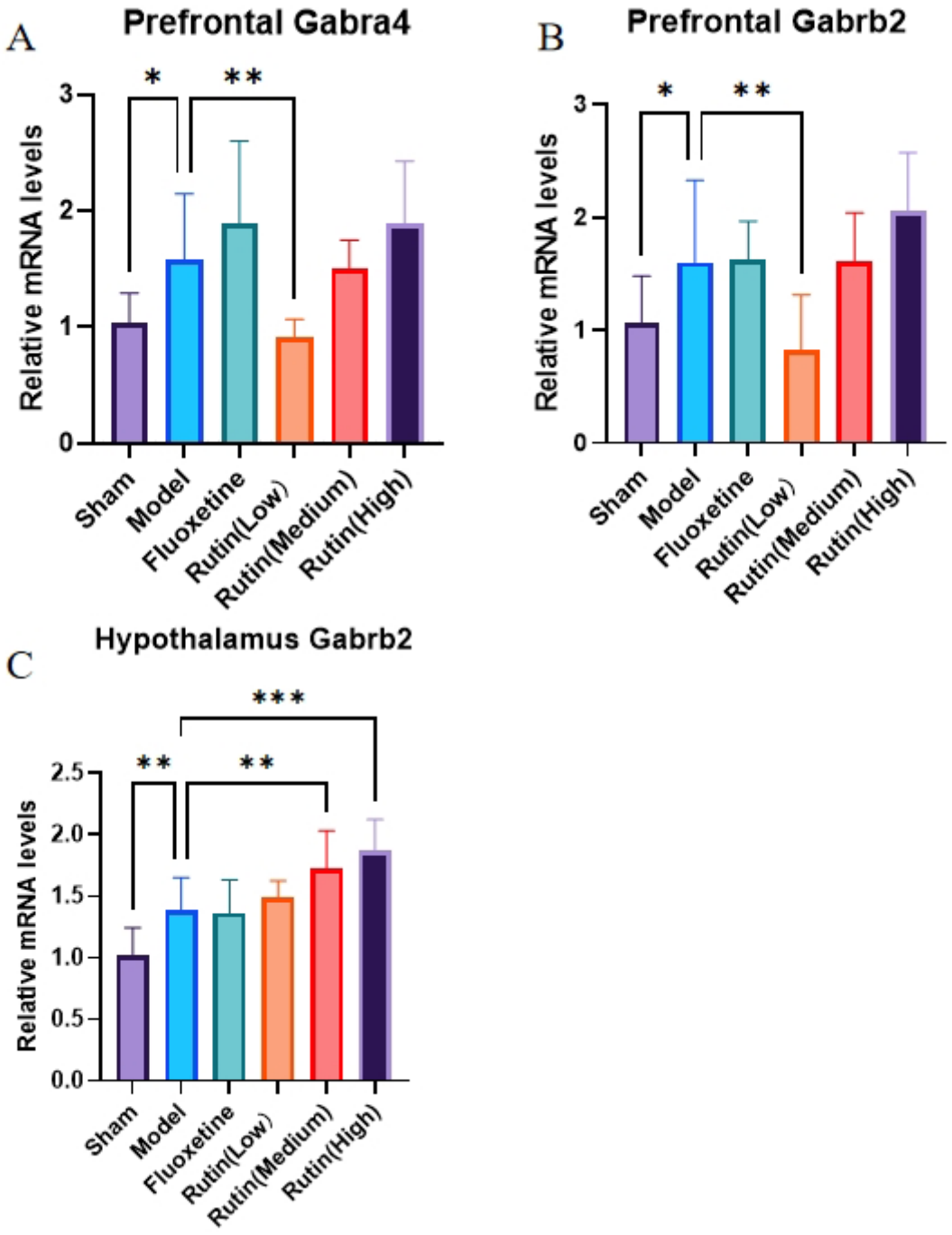

Figure 8

Effects of rutin on GABAAR subunits mRNA expressions in the brains of PMD rats. (A) the mRNA expression of α4 in the prefrontal cortex of rats; (B, C) the mRNA expression of β2 in the prefrontal cortex, and hypothalamus of rats;*p < 0.05, **p < 0.01,***p < 0.001.