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Ormosia henryi Prain Leaf Extract Alleviates Cognitive Impairment in Chronic Unpredictable Mild Stress Mice*

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Abstract Cognitive deficits were one of the core symptoms of depression, which has become a major challenge in the treatment of depression. *Ormosia henryi Prain* (OHP) is a green perennial tree widely distributed in southern China. Although OHP was demonstrated to function in antidepression, its effect on cognitive deficits still remains to be elucidated. The aim of the present study was to investigate the effect of OHP on cognitive deficits caused by chronic stress. A mice model of chronic unpredictable mild stress (CUMS) was employed to evaluate the cognitive improvement effect of OHP. Cognitive behaviors were assessed with novel object recognition and Morris water maze test. The levels of GFAP and Aβ protein in the hippocampus were analyzed by immunohistochemistry. The results showed that OHP significantly improved the short-term memory which presented as the increased discrimination index, and also alleviated the deficits of spatial learning and memory manifested as a decrease in the escape latency to reach the platform and an increase in the number of platform crossing. OHP could significantly increase the levels of 5-HT and DA、NE in hippocampal tissue. Moreover, it increased the levels of GFAP and reduced Aβ-positive cells in the hippocampal CA1 region of stressed mice and could significantly decreased the expression of NLRP3, TNF-α, and IL-1β in hippocampus. This study demonstrates for the first time that OHP significantly improves the learning and memory abilities of chronic stress mice which may be related to the amelioration of hippocampal inflammation involved in NLRP3, TNF-α, and IL-1β, and up-regulation of GAFP and down-regulation Aβ in the hippocampus, indicating that OHP may be a new potential drug for the treatment of cognitive disorders caused by chronic stress.

Key words *Ormosia henryi Prain* leaf extract, chronic unpredictable mild stress, NLRP3, GAFP, Aβ
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Depression is a chronic and serious mental illness that afflicts nearly 350 million people worldwide, it is predicted to be the second leading cause of premature death and disability in 2020[1-2]. Depression is highly heterogeneous, characterized by black mood, irritability, anxiety or insomnia. Fortunately, part of these symptoms can be effectively alleviated by current antidepressants such as the tricyclic antidepressants, the selective serotonin, and noradrenalin reuptake inhibitors[3]. However, the

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antidepressant drugs were limited to a certain extent due to the delayed response to therapeutic effects and the presence of adverse reactions[4-5]. Even worse, cognitive deficits as another core symptom is widely identified in patients with depression[6]. A recent systematic review and meta-analysis revealed that significant deficits in the domains of cognitive functioning was showed in individuals remitted from major depressive disorder, and recurrent episodes can aggravate the deterioration of cognitive function[7]. Therefore, the development of new drugs to suppress cognitive deficits in depressed patients has become an urgent issue.

Phytotherapy is generally considered to have good efficacy and low adverse reactions, and has been used to prevent and treat various diseases including depression[8]. *Ormosia henryi Prain* (OHP) belongs to Red bean (leguminosae) which is a green perennial tree widely distributed in southern China. It is an excellent garden tree and an important herbal medicine used in folk medicine for the treatment of dysphagia, pain and inflammation[9]. Evidence from traditional folk medicine shows that OHP exhibits refreshing and anti-depressive effects[10]. Recent studies have observed that the ethanol extract of OHP leaf containing flavone C-glycosides exerts antidepressant effects with an increase in expression of brain-derived neurotrophic factor[11]. The essential oil from OHP has also been shown to inhibit depression-like behaviors in depressed mouse model through olfactory pathways[12]. These findings indicate that OHP can alleviate depressive behavior. However, the potential neuroprotective effect of OHP against the chronic unpredictable mild stress-induced cognitive deficits has not been reported. In the present study, we aimed to investigate the effects of OHP on cognitive behavior and its pathology in stressed mice.

1 Materials and methods

1.1 Plant material and reagents

OHP was provided from Guiyang University of Traditional Chinese Medicine, China. The purity was 95%. The preparation process was as follows: OHP leaf were extracted by steam distillation method to obtain extract solution, which was extracted with 75% ethanol for three times to concentrate the liquid and get solid. Donepezil HCl was provided by Eisai Co., Ltd., China.

1.2 Animals

Male mice (n=70, 18-22 g) were supplied by Hunan SJA Laboratory Animal Co. Ltd (Qulified No. SCXK 2016-0001, Changsha, China). The animals were acclimatized for 3 days and housed in environmentally controlled temperature 22℃ −26℃ with 12 h light and dark cycles. All the experimental protocols were prepared according to the National Institute of Health Guidelines for Laboratory Animals (NIH Publications NO. 80−23, revised 1996). and approved by the Ethical Committee of Guiyang university of traditional Chinese medicine (Study# 2019032).

1.3 Establishment of chronic unpredictable mild stress model （CUMS）

70 male ICR mice, weighing 18−22 g, except for the normal control group, the remaining mice were subjected to chronic unpredictable stress, including fasting (12 h), water-free (12 h), forced swimming (10 min) , Strobe (12 h), noise (30 min), restraint (12 h) (placed in a 50 ml centrifuge tube, the tube diameter is 3 cm, the length is about 10 cm, the tube wall has 6 to 7 vents with diameter 0.5 mm), tilt Cages (12 h), wet cages (12 h), day and night are upside down, *etc*. During the restraint period, animals are fasted and water-free.

1.4 Grouping and administration

Animals were randomly divided into 5 groups (model control group, fluoxetine group, low, medium and high doses of Palmetto extract, normal control group and model control group) according to the results of sugar and water preference test taking into account the weight of sugar and water after 28 consecutive days of modeling, respectively. Distilled water was orally administered daily, and the remaining groups were given the corresponding medicinal solution at a dose of 20 ml/kg for 35 consecutive days. After the administration, the mice continued to undergo chronic unpredictable stress. Mice were tested for new object recognition and Water maze.

1.5 Behavioral assessment

1.5.1 Novel object recognition test（NORT）

The NORT was preformed to evaluate recognition memory based on the spontaneous preference of rodents for novel objects[13]. The experiment box is a black closed box with a volume of 60 cm × 40 cm × 80 cm. Inside the box, there are

Table 1 Time and length of stressors used in the CUMS procedure

Time	Building model methods	Period of time
Monday	Forced swimming 10 min+ Stroboscopic 12 h+	8:50 am-9:00 am forced swimming
	Noise 30 min	9:00 am-9:30 am noise
		9:00 am-9:00 pm stroboscopic
Tuesday	Fasting 12 h+ day and night upside down	8:30 am-8:30 pm fasting
Wednesday	CUMS 12 h+	8:30 am-8:30 pm Solied cage
	Solied cage 12 h	9:30 pm-9:30 am CUMS
Thursday	Forced swimming 10 min+ Stroboscopic 12 h+	8:50 am-9:00 am forced swimming
	Noise 30 min	9:00 am-9:30 am noise
		9:00 am-9:00 pm day and night upside down
Friday	Fasting12 h+	8:30 am-8:30 pm fasting
	Stroboscopic12 h+	9:00 am-9:30 am noise
	Noise 30 min	9:00 am stroboscopic
Saturday	CUMS 12 h+	9:30 am-9:30 pm CUMS
	Cage tilt 12 h	9:30 pm-9:30 am Cage tilt
Sunday	Fasting 12 h+	8:30 am-8:30 pm fasting
	Cage tilt 12 h+	9:00 pm-9:00 am Cage tilt
	Stroboscopic	

two rows of LED strips on the upper side. The lighting light is the background light of the experiment. A camera mounted on the top can observe and record all the activities of the animals during the experimental testing. The experiment was composed of the adaptation period, the familiarization period and the test period. The experiment lasted for 3 days. On the 1st day of the adaptation period, the rats were put into the corresponding experimental box according to the animal number to familiarize themselves with the environment for 10 min. On the second day of the familiarization period, two identical objects were placed on the diagonal of the experimental box, with a distance of 8 cm from the back wall of the box. After an interval of 30 min, the test period entered. During the test period, the familiar object in the lower right corner was replaced with a novel object of similar appearance and similar size, and the activity track and exploration time of the mice were recorded within five minutes.

Data recording method: relative discrimination index method (DI) was used to record the exploration time of experimental animals. Calculation method: DI = (exploration time of new objects - exploration time of familiar objects)/ (exploration time of new objects + exploration time of moving familiar objects) ×100%.

1.5.2 Morris water maze test（MWM）

The Morris water maze was widely used to study spatial memory and learning[14]. Water maze detection: positioning and navigation training for each group of animals. Fill the pool with tap water. The water temperature is stable at 25℃, making the water level slightly higher than the platform by about 1.5 cm. Pour black ink into a pool of tap water to make the platform invisible. After placing the animal on the platform for 10 s, select three entry points and familiarize yourself with them for 90 s. 4 consecutive days of training, the fifth day of space exploration, detection of animal movement across the platform times.

1.6 Neurotransmitters in hippocampal tissue in CUMS mice

After weighing the hippocampal tissue and adding a certain amount of normal saline, a tissue homogenizer was used to homogenize then centrifuged at 12 000 r/min for 10 min at 4℃. The level5-HT、DA and NE of hippocampal tissue was detected by multifunctional enzyme marker according to the Elisa kit.

1.7 Histology

Histological evaluation was conducted to observe neurons morphology, glial cell, and inflammation. Mice was sacrificed brain dissected out

and isolated hippocampal tissue fixed in 4% paraformaldehyde. After dehydration, hippocampal was cut into six coronal 2 mm-thick slices and embedded. For histology, 5 μm thick section was cut, stained and observed by using light microscopy.

1.8 Immunohistochemistry

After the behavioral test, hippocampal tissue was taken from each group of mice for immunohistochemical analysis of the expression of GAFP, Tau and Aβ protein. After subjecting to dewax and hydrate, the sections were placed in 3% H_2O_2 solution to inactivate endogenous peroxidase. After washing, the sections were incubated with primary antibodies: anti-GFAP antibody (1∶300, ab7260, Abcam), recombinant anti-Tau (phospho T231) antibody [EPR2488] (1∶200, ab151559, Abcam), anti-$A\beta_{1\text{-}42}$ antibody (1∶100, ab10148, Abcam) overnight at 4℃, respectively. The sections were incubated sequentially for 1 h with each of the corresponding secondary antibody at 37℃. Dyeing was performed using 3, 3'-diaminobenzidine tetrahydrochloride hydrate. Sections were counter-stained with hematoxylin, then dehydrated with alcohol and fixed with neutral glue, and finally observed under a microscope. Five randomly selected fields were captured from each section and three sections were studied per animal.

1.9 Western blot analysis

Hippocampus isolated from the brain of rats was homogenized in RIPA lysis buffer (Solarbio Science & Technology Co., China) containing protease and phosphatase inhibitor (Thermo Scientific)cocktail. After homogenization, the cocktail was centrifuged 12 000 r/min 10 min to remove debris at 4℃. The protein concentra-tion of the individual sample was determined by using bicinchoninic acid protein assay kit (Solarbio Science & Technology Co., China). The prepared homogenate was used to estimate the protein expression levels of NLRP3, TNF-α, IL-1β. Proteins were electrophoresed by using 12% SDS-PAGE, transferred to PVDF membrane, and protein expression was determined by using antibodies for NLRP3, TNF-α,IL-1β, and actin (1∶1 000; Abcam), respectively, as described earlier.

1.10 Statistical analysis

SPSS 16.0 program (SPSS Inc. Chicago, IL, USA) was employed for statistical analysis. All data are presented as $\bar{x} \pm s$. Statistical significance between groups was by using one-way ANOVA, followed by post hoc LSD tests for multiple comparisons. A value of $P<0.05$ was considered as significant.

1.11 The experimental procedure

The experimental procedure is shown in Figure 1.

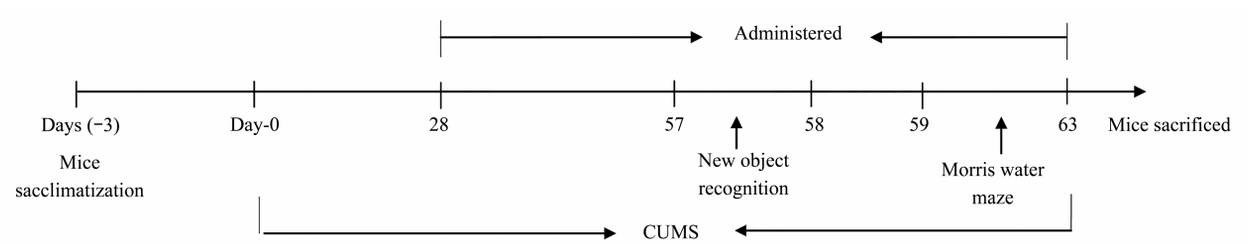

Fig.1 The experimental procedure

2 Results

2.1 OHP alleviated the deficits of recognition memory in CUMS mice

Novel object recognition test was performed to evaluate the effect of OHP on short-term memory. The results were shown in Figure 2. The mouse discrimination index of the model group was significantly decreased compared with the normal control group ($P<0.01$); however, OHP extract at 50, 100 and 150 mg/kg and positive control drug Donepezil (3.0 mg/kg) significantly increased the discrimination index compared with model group ($P<0.01$ or $P<0.05$; Figure 2). These findings suggested that OHP improves short-term memory in a dose-dependent manner.

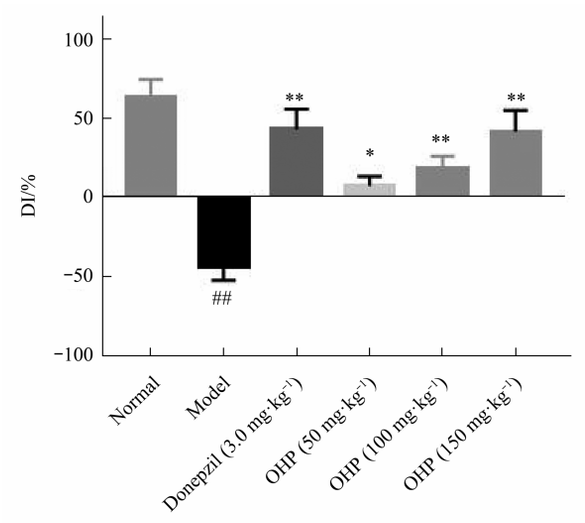

Fig. 2　Effect of OHP on novel object recognition test

Data are expressed as $\bar{x} \pm s$, n=10, $^{\#\#}P$<0.01 compared with the normal group, $^{*}P$<0.05, $^{**}P$<0.01 compared with the model group.

2.2　OHP ameliorated the deficits of spatial learning and memory in CUMS mice

Morris water maze test was conducted to evaluate the effect of OHP on spatial learning and memory. Analysis of the results of escape latency to reach the fixed hidden platform showed that the escape latency of the model group was significantly longer than that of the normal control group on the third and fourth days. By contrast, OHP extract at 100 and 150 mg/kg and positive control drug Donepezil (3.0 mg/kg) obviously shorten the escape latency to reach the platform compared with the model group (P<0.01; Figure 3a). Analysis of the movement track of mice observed that the number of platform crossing in the model group was significantly less than that in the normal control group, while OHP extract at 100 and 150 mg/kg and positive control drug Donepezil (3.0 mg/kg) can significantly increase the number of platform crossing in CUMS mice compared with the model group (P<0.01; Figure 3b). These results mean that OHP can improve spatial learning and memory in stressed mice.

Number of crossing previous platform position in probe test (b) . Data are expressed as mean ± SEM, n=10, $^{\#\#}P$<0.01 compared with the normal group, $^{**}P$<0.01 compared with the CUMS group.

2.3　Effects of OHP on neurotransmitters in hippocampal tissue in CUMS mice

As shown in Figure 4, the levels of 5-HT、DA and NE in the hippocampal tissue of the model control group were significantly lower than normal control group (P<0.01). OHP(100, 150 mg/kg) could significantly increase the level of neurotransmitter 5-HT in hippocampal tissue (P<0.05 or P<0.01). These findings suggest that OHP has a significant regulatory effect on monoamine neurotransmitters 5-HT、DA and NE in the hippocampus.

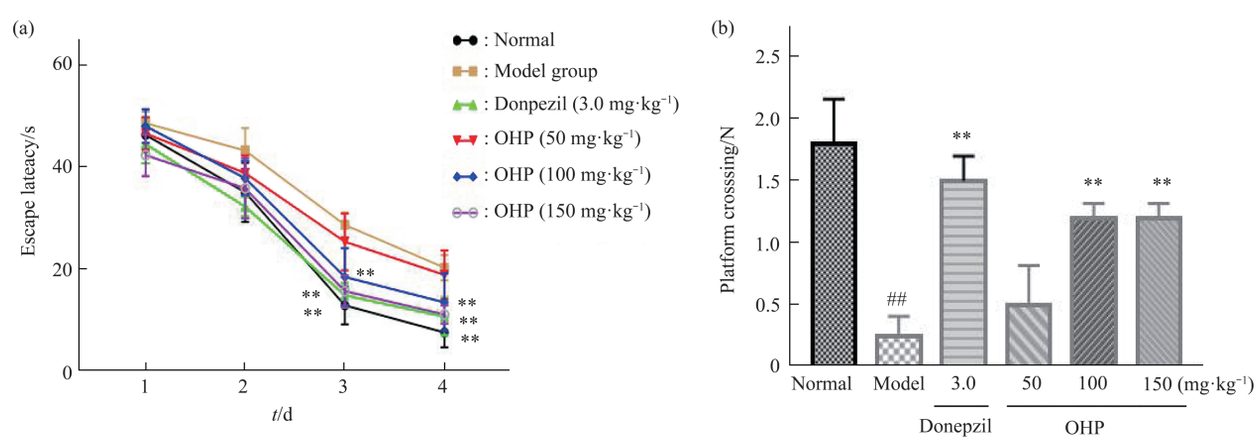

Fig. 3　Effect of OHP on the spatial memory of CUMS mice in MWM

Escape latency to find the fixed hidden platform across the 4 training days (4 trials per day, a). Number of crossing previous platform position in probe test (b) . Data are expressed as $\bar{x} \pm s$, n=10, $^{\#\#}P$<0.01 compared with the normal group, $^{**}P$<0.01 compared with the CUMS group.

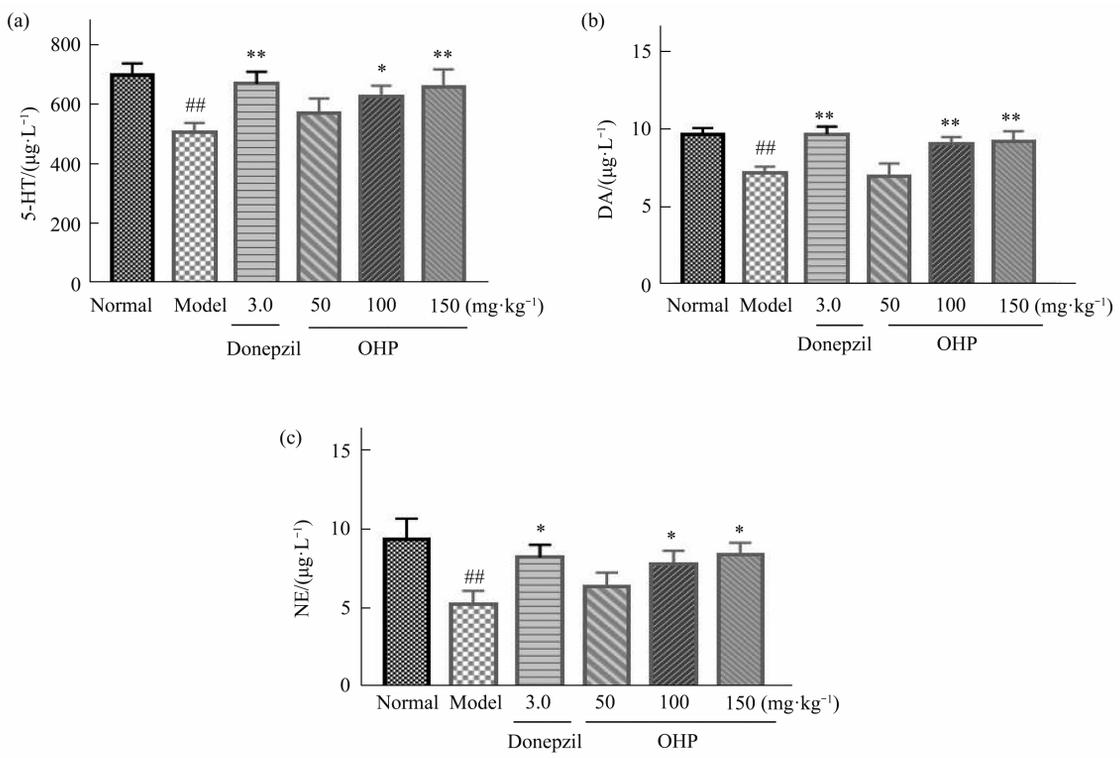

Fig. 4　Effect of OHP on neurotransmitters in hippocampal tissue

The level of 5-HT (a), DA (b), and NE (c) in the hippocampus of rats were detected by Elisa. Data are expressed as $\bar{x} \pm s$, n=10, $^{\#\#}P$<0.01 compared with the normal group, $^{*}P$<0.05, $^{**}P$<0.01 compared with the model group.

2.4　The histopathology of hippocampus in CUMS mice

As shown in Figure 5, there were no abnormalities in neuron morphology, glial cells and hippocampal pyramidal cells of rats in the normal control group. In the model group, there was a large amount of neuronal cell necrosis, glial cell proliferation, hippocampal vertebral body necrosis, interstitial edema and a large number of inflammatory cell infiltration in the hippocampal tissue. OHP (100, 150 mg/kg) performanced low, medium and a small amount of fine neuron cell necrosis, a small amount of glial cell hyperplasia, a small amount of interstitial edema, inflammatory cell infiltration.

2.5　OHP decreased the expression of GFAP and Aβ protein in the hippocampus of CUMS mice

The expression of GFAP, Aβ and Tau protein were analyzed by immunohistochemistry to evaluate the effect of OHP on the changes of memory-related molecules in the hippocampus of mice. Positive responses of GFAP and Aβ protein to their antibodies were exhibited in the hippocampus with dark brown color. The staining intensity and area of the image represent the levels of protein expression. GFAP and Aβ were distributed in the CA1 region of the hippocampus. As shown in Figure 4, the number of GFAP and Aβ -positive cells were elevated in the model group compared with the normal control group. Interestingly, OHP extract at 100 and 150 mg/kg could effectively reverse the decrease of these proteins caused by CUMS. Although the effect of positive control drug Donepezil on the Aβ -positive cells was obvious, it could increase the expression of GFAP protein. In addition, the levels of Tau protein in the CA1 region of the hippocampus was also detected. Compared with the normal control group, the expression of Tau protein was significantly changed in the model group. 150 mg/kg of OHP could markedly reduce the expression of Tau protein in the CA1 region of the hippocampus in CUMS mice, although the effect of 50 mg/kg and 100 mg/kg of OHP on this protein is not obvious (Figure 6). These results reveal that the memory improvement of OHP may be related to the down-regulation of GFAP and Aβ protein in the

hippocampus.

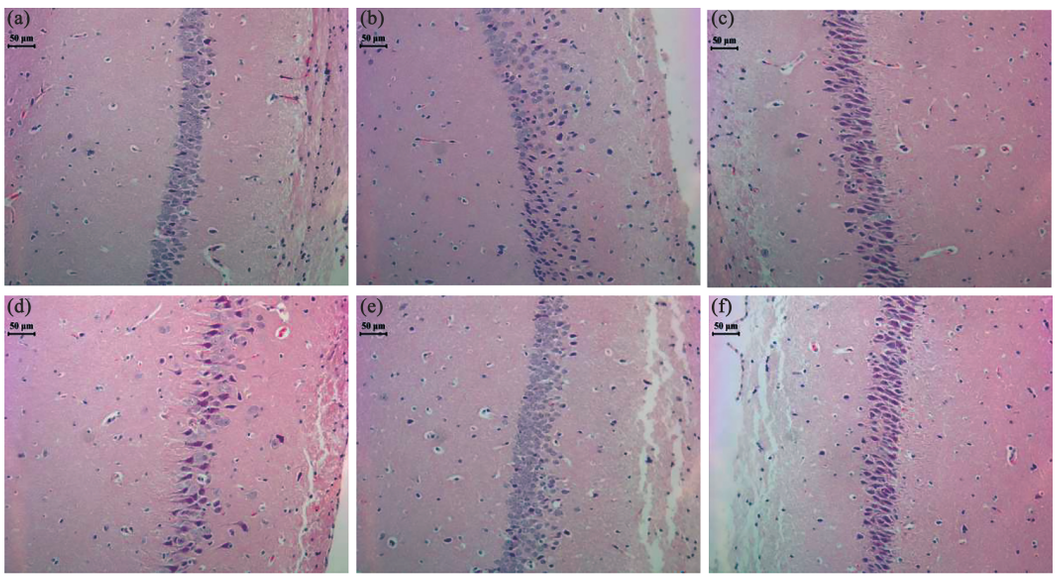

Fig. 5　The histopathological examination of brain tissue of hippocampus mice was conducted by OHP

The histopathological examination of brain tissue of hippocampus in the Con, Model, Don, and OHP-treated groups (as indicated in panels a−f), respectively.

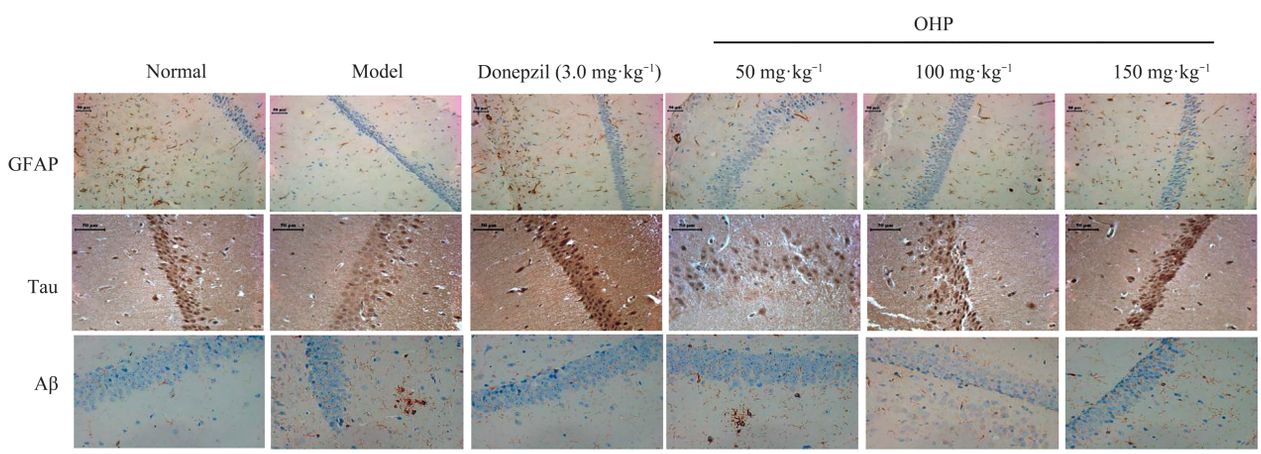

Fig. 6　Effects of OHP on the expression of GAFP，Tau and Aβ in the hippocampus of the chronic unpredictable mild stress mice

Panels indicated as follows: the expression of GAFP in the Con, Model, Don, and OHP-treated groups; that of Tau in the Con, Model, Don, and OHP-treated groups; and that of Aβ in the Con, Model,Don, and OHP -treated groups.

2.6　Effects of OHP on the expression of NLRP3，TNF−α，IL−1β in hippocampus of mice

As shown in Figure 7, Hippocampal activated of NLRP3, TNF-α, IL-1β were significantly decreased as compared to the model rats (P<0.01).; Treatment with OHP(100, 150 mg/kg) significantly increased the expression of NLRP3, TNF-α, IL-1β in hippocampus (P<0.05 or P<0.01).

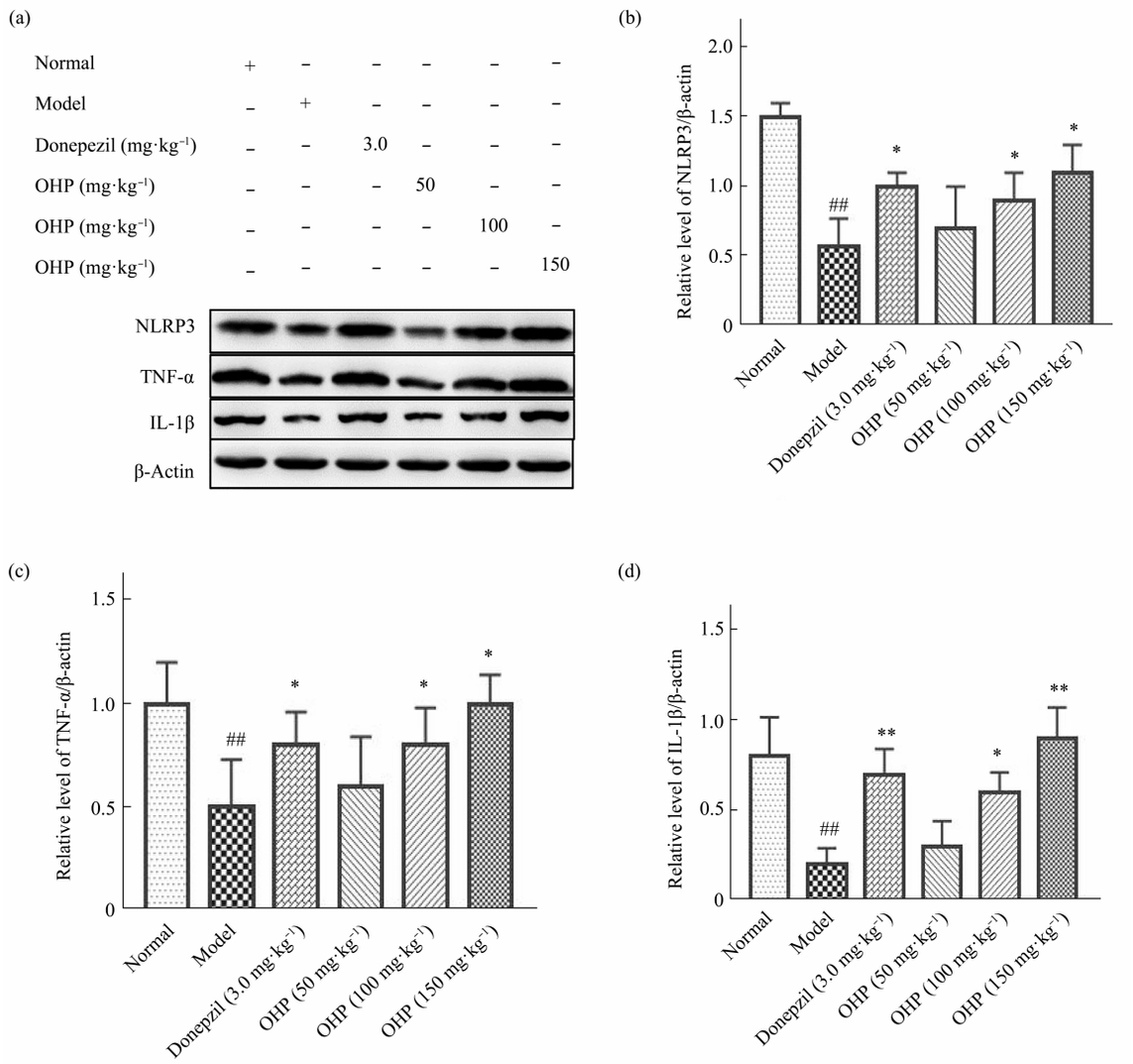

Fig. 7　Protein expression of NLRP3，TNF−α，IL−1β in hippocampus of chronically stressed mice

(a) Western Blots were analyzed and plotted in densities for NLRP3 (b), TNF-α. (c) and IL-1β (d). Values represented as $\bar{x} \pm s$, $^{\#\#}P$<0.01 compared with the normal group, $^{*}P$<0.05, $^{**}P$<0.01, compared with the model group.

3　Discussion

Natural products such as traditional Chinese medicine have long been widely used in the prevention and treatment of central nervous system diseases due to their rich sources, diverse structures and novel activities[15] . In the present study, we demonstrated that OHP could dramatically mitigate the different forms of cognitive deficits including recognition memory and spatial memory in the CUMS mice. Furthermore, the changes of GFAP and Aβ protein induced by chronic stress were counteracted after OHP treatment. These findings indicated that the beneficial effects of OHP on memory impairment may be related to the down-regulation of GFAP and Aβ protein. With the acceleration of the social process and the pace of life, people are facing a sharp increase in stress. Although the appropriate pressure promotes the progress of human society, sustained stress can destroy the human body's ability to cope with stress and bring a detrimental effect on people's health[16-17]. The brain is an organ that is sensitive to stress. Under the condition that the stress intensity exceeds the tolerance of the body, the stress can cause the occurrence of neuropsychiatric diseases[18-19]. Epidemiological data show that stress is an important risk factor for depression and Alzheimer's

disease[20-21]. Cognitive impairment often occurs in depressed patients and is considered to be the main symptom of depression in addition to mood disorders. Chronic unpredictable mild stress is a widely accepted depression model that simulates depressive symptoms such as lack of pleasure and loss of interest[22]. Numerous animal behavioral research had shown that cognitive deficits had also been observed in CUMS mice[23]. In the present study, CUMS mice were used to evaluate the effect of OHP on cognitive deficits. The data from NOR test showed that the discrimination index of CUMS mice was decreased significantly at the 8th week, suggesting that the recognition memory was impaired in CUMS mice. In addition, the MWM test was also conducted and found that CUMS mice had a longer escape latency to reach the platform and a lower number of platform crossing than normal mice, which are consistent with previous studies[24], means that the spatial learning and memory was impaired in CUMS mice. These findings indicated that CUMS mice successfully reproduced the stress-induced cognitive impairment. OHP leaf ethanol extract has been reported to exert an antidepressant effect, which is characterized by increasing sucrose preference index and prolonging tail suspension time in CUMS mice[11]. However, as a valuable traditional Chinese medicine, its effect on cognitive memory has not been reported so far. Interestingly, this study found for the first time that OHP treatment can significantly inhibit CUMS-induced the above cognitive behavior changes, which indicates that OHP can not only inhibit emotional disorders but also alleviate cognitive impairment.

Central neurotransmitter plays an important role in regulating emotion, learning and memory. Chronic stress could affect the content of monoamine neurotransmitters, resulting in decreased levels of 5-HT, DA and NE in the hippocampal tissues of stressed mice, as well as anxiety, depression and cognitive dysfunction[25-26]. 5-HT and DA are important neurotransmitters involved in cognitive function, and the regulation of 5-HT, DA and its receptors may alleviate depression-related cognitive deficits. The level of 5-HT, DA and NE of Chronic unpredictable stimuli in the hippocampus of mice were lower compared with normal mice, and the decrease were reversed significantly by administration of OHP.

The hippocampus is an important part of the limbic system, which is involved in emotion and memory. Chronic binding stress caused damage to hippocampal neurons in mice[27-28]. OHP could significantly reduce hippocampal neuron damage caused by chronic binding stress. Astrocytes are the most abundant type of glial cells. They play a key role in the central nervous system, such as maintaining and supporting the brain microenvironment, secreting neurotrophic factors to promote neurogenesis, and buffering neurotransmitters to regulate neuronal plasticity[29]. As a marker of astrocytes, the Glial fibrillary acid protein (GFAP) is commonly used to study the number and function of astrocytes in various regions of the brain[10]. Several previous clinical studies pointed out that the levels of GFAP protein and the GFAP-positive astrocytes were reduced in cerebral former cortex and hippocampus from patients with depression[30]. Similarly, a large body of evidence supported that GFAP expression is decreased in the hippocampus of the CUMS animal model[31-32]. Here we also observed a significant decrease in the number of GFAP-positive cells in the hippocampus of CUMS mice. However, a study on the astrocytic plasticity in an animal model of recurrent depression indicates that stress re-exposure can increase the number of astrocytes[26]. This indicates that the inconsistent number of GFAP-positive cells in the hippocampus may be caused by different modeling conditions. To explore the potential mechanism of OHP in alleviating CUMS-induced cognitive impairment, this study evaluated the effect of OHP on astrocytes. Fortunately, CUMS induced-these changes were offset with OHP treatment. A previous study confirmed that OHP leaf ethanol extract could significantly increase the expression of BDNF protein the hippocampus of CUMS[11]. BDNF could repaired astrocytic plasticity in the hippocampus from depressed rats[32]. These evidences support our results suggesting that the cognitive improvement of OHP may be related to the regulation of GFAP.

The amyloid-β plaque deposition and hyperphosphorylated Tau-mediated neurofibrillary tangles are the major pathological hallmarks of Alzheimer's disease[33]. As an important risk factor causing depression and dementia, stress can aggravate Aβ deposits by promoting the secretion of glucocorticoids[34]. Proved that chronic stress significantly increased phospho-tau (Ser 356, Thr 231) protein levels in hippocampus and frontal cortex of rats[35]. In the present study, we found that Aβ was

obviously elevated in the CA1 region of the hippocampus in CUMS mice, which was consistent with the previous literature[36]. Strikingly, CUMS-induced an increase in Aβ was significantly inhibited after OHP treatment. These results may partly explain why OHP reduces cognitive impairment caused by chronic stress. Our study observed that CUMS did not cause a significant increase in Tau compared to the normal control group. Since the levels of phospho-tau were more evident in rats re-exposed to CUMS Green [35], we believe that the modeling in this study may be too light to induce a significant increase in Tau. In addition, 50 mg/kg of OHP could markedly reduce the expression of Tau protein in the CA1 region of the hippocampus in CUMS mice, although the effect of 100 mg/kg of OHP on this protein is not obvious. Therefore, our findings indicate that OHP's ability to improve memory may be mainly related to decreased Aβ expression in the hippocampus.

Inflammation in the brain was triggered by activated microglia and reactive astrocytes. Glial cells were known to play a role in both neuroprotection and neurodegeneration [36]. A large number of clinical and preclinical studies have shown that neuroinflammatory response is one of the main pathophysiological factors leading to cognitive impairment[37]. NLRP3 Inflammasome was a multi-protein complex, Changes in pro-inflammatory cytokines such as TNF-α and IL-1β would be induced during stress[38-39]. In the CUMS animal models, the activation of NLRP3 inflammatome (protein expression) was increased, leading to the upregulation of various pro-inflammatory cytokines[38]. Our results were consistent with the above studies. After OHP administration, NLRP3, TNF- α and IL-1β protein expression could be significantly down-regulated. The results showed that OHP could improve the cognitive impairment caused by CUMS mice by improving hippocampal inflammatory markers.

This study demonstrates for the first time that the inhibition of OHP on cognitive impairment caused by chronic stress was related to the decreased expression of NLRP3, TNF- α, IL-1β and Aβ and GFAP in the hippocampus. The following limitations in this study require further study. The active ingredients of OHP for improving cognition and antidepressant are unclear. Since the antidepressant effect of OHP leaf ethanol extract, enriched in flavone C-glycosides and the anti-oxidant activity of ormosinol, a polyprenylated isoflavanone from *Ormosia henryi* have been reported[11], whether these two chemical components have the effect of improving learning and memory is worth exploring. Secondly, the method to evaluate the impact of OHP on cognitive ability is too single, which needs to be further confirmed by a variety of cognitive impairment models, such as the model of scopolamine or Aβ -Induced Learning and Memory Deficits, senescence accelerated mouse, APP/PS1 transgenic animal model of Alzheimer's disease. Third, this study only preliminary explored the effect of OHP on NLRP3, TNF-α and IL-1β, GFAP, Aβ and Tau. The detailed mechanism of OHP against cognitive impairment still needs further research using inhibitors or gene interference techniques.

4 Conclusion

In conclusion, *Ormosia henryi Prain* (OHP) significantly alleviates learning and memory disorder caused by chronic stress, which might be related to decreased inflammation and increased levels of neurotransmitters in the hippocampus and reduced the expression of Aβ and GFAP in the hippocampus of CUMS mice. Therefore, OHP might be considered as a potential drug for alleviating the memory disorder by chronic stress.

References

[1] Smith K. Mental health: a world of depression. Nature, 2014, **515**(7526): 181

[2] Matthews K, Robbins T W. Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. Neurosci Biobehav Rev, 2003, **27**(1-2): 45-55

[3] Yan T, He B, Wan S, *et al*. Antidepressant-like effects and cognitive enhancement of Schisandra chinensis in chronic unpredictable mild stress mice and its related mechanism. Sci Rep, 2017, **7**(1): 6903

[4] Shang X, Shang Y, Fu J, *et al*. Nicotine significantly improves chronic stress-induced impairments of cognition and synaptic plasticity in mice. Mol Neurobiol, 2017, **54**(6):4644-4658

[5] Sun X, Wang M, Wang Y, *et al*. Melatonin produces a rapid onset and prolonged efficacy in reducing depression-like behaviors in adult rats exposed to chronic unpredictable mild stress. Neurosci Lett, 2017, **642**:129-135

[6] Yu T, Guo M, Garza J, *et al*. Cognitive and neural correlates of depression-like behaviour in socially defeated mice: an animal model of depression with cognitive dysfunction. Int J Neuropsychopharmacol, 2011, **14**(3):303-317

[7] Semkovska M, Quinlivan L, O'Grady T, *et al*. Cognitive function

following a major depressive episode: a systematic review and meta-analysis. Lancet Psychiatry, 2019, **6**(10):851-861

[8] Kandilarov I K, Zlatanova H I, Georgieva-Kotetarova M T, *et al*. Antidepressant effect and recognition memory improvement of two novel plant extract combinations - antistress I and anti-stress II on rats subjected to a model of mild chronic stress. Folia Med (Plovdiv), 2018, **60**(1): 110-116

[9] Feng S, Hao J, Xu Z, *et al*. Polyprenylated isoflavanone and isoflavonoids from Ormosia henryi and their cytotoxicity and anti-oxidation activity. Fitoterapia, 2012, **83**(1):161-165

[10] 王国强 . 全国中草药汇编: 下册[M]. 北京: 人民卫生出版社, 2005: 579
Wang G Q. People's Medical Publishing House, 2005: 579

[11] Lu Y, Zhu S, He Y, *et al*. Phytochemical profile and antidepressant effect of ormosia henryi prain leaf ethanol extract. Int J Mol Sci, 2019, **20**(14): 3396

[12] 陈军, 徐金勇, 徐蓉, 等 . 花梨木精油通过嗅觉通路改善小鼠抑郁样行为及其神经递质 . 江苏医药, 2012, **38**(6):657-659+616
Chen J, Xu J Y, Xu R, *et al*. Jiangsu Medical Journal, 2012, **38**(6): 657-659+616

[13] Kinnavane L, Albasser M M, Aggleton J P. Advances in the behavioural testing and network imaging of rodent recognition memory. Behav Brain Res, 2015, **285**:67-78

[14] Pehrson A L, Sanchez C. Serotonergic modulation of glutamate neurotransmission as astrategy for treating depression and cognitive dysfunction. CNS Spectr, 2014, **19**(2): 121-133

[15] Zhang J, He Y, Jiang X, *et al*. Nature brings new avenues to the therapy of central nervous system diseases-An overview of possible treatments derived from natural products. Sci China Life Sci, 2019, **62**(10):1332-1367

[16] Xu Z, Lay B, Oexle N, *et al*. Involuntary psychiatric hospitalisation, stigma stress and recovery: a 2-year study. Epidemiol Psychiatr Sci, 2019, **28**(4):458-465

[17] Shimazu A, Schaufeli W B, Kubota K, *et al*. Is too much work engagement detrimental? Linear or curvilinear effects on mental health and job performance. Plos One, 2018, **13**(12): e0208684

[18] Juster R P, McEwen B S, Lupien S J. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev, 2010, **35**(1): 2-16

[19] Gulbins A, Grassme H, Hoehn R, *et al*. Regulation of neuronal stem cell proliferation in the hippocampus by endothelial ceramide. Cell Physiol Biochem, 2016, **39**(2): 790-801

[20] Quervain D J, Roozendaal B, Nitsch R M, *et al*. Acute cortisone administration impairs retrieval of long-term declarative memory in humans. Nat Neurosci, 2000, **3**(4): 313-314

[21] Han B, Wang J H, Geng Y, *et al*. Chronic stress contributes to cognitive dysfunction and hippocampal metabolic abnormalities in APP/PS1 mice. Cell Physiol Biochem, 2017, **41**(5): 1766-1776

[22] Evans J, Sun Y, McGregor A, *et al*. Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. Neuropharmacology, 2012, **63**(8): 1315-1326

[23] Liu S C, Hu W Y, Zhang W Y, *et al*. Paeoniflorin attenuates impairment of spatial learning and hippocampal long-term potentiation in mice subjected to chronic unpredictable mild stress. Psychopharmacology (Berl), 2019, **236**(9):2823-2834

[24] Xi G, Hui J, Zhang Z, *et al*. Learning and memory alterations are associated with hippocampal N-acetylaspartate in a rat model of depression as measured by 1H-MRS. Plos One, 2011, **6**(12): e28686

[25] Akter S, Sasaki H, Uddin K R, *et al*. Anxiolytic effects of gamma-oryzanol in chronically-stressed mice are related to monoamine levels in the brain. Life Sci, 2019, **216**: 119-128

[26] Cowen P, Sherwood A C. The role of serotonin in cognitive function: evidence from recent studies and implications for understanding depression. J Psychopharmacol, 2013, **27**(7): 575-583

[27] Miller B R, Hen R. The current state of the neurogenic theory of depression and anxiety. Curr Opin Neurobiol, 2014, **30**:51-58

[28] Miguel-Hidalgo J, Whittom A, Villarreal A. Apoptosis-related proteins and proliferation markers in the orbitofrontal cortex in major depressive disorder. J Affect Disord, 2014, **158**: 62-70

[29] Ding X F, Liu Y, Yan Z Y, *et al*. Involvement of normalized glial fibrillary acidic protein expression in the hippocampi in antidepressant-like effects of Xiaoyaosan on chronically stressed mice. Evid Based Complement Alternat Med, 2017:1960584

[30] Cobb J A, O'Neill K, Milner J, *et al*. Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience, 2016, **316**: 209-220

[31] Zhang X H, Huang S J, Wang Y Y, *et al*. Effects of kaixin jieyu decoction on behavior and glial fibrillary acidic protein expression in cerebral hippocampus of a rat vascular depression model. Chin J Integr Med, 2015, **21**(3): 223-228

[32] Ayuob N N. Evaluation of the antidepressant-like effect of musk in an animal model of depression: how it works. Anat Sci Int, 2017, **92**(4): 539-553

[33] Yang C, Guo X, Wang G H, *et al*. Changes in tau phosphorylation levels in the hippocampus and frontal cortex following chronic stress. Braz J Med Biol Res, 2014, **47**(3): 237-244

[34] Lemere C A. Immunotherapy for Alzheimer's disease: hoops and hurdles. Mol Neurodegener, 2013, **8**: 36

[35] Green K N, Billings L M, Roozendaal B, *et al*. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. J Neurosci, 2006, **26**(35): 9047-9056

[36] Wei F, Song J, Zhang C, *et al*. Chronic stress impairs the aquaporin-4-mediated glymphatic transport through glucocorticoid signaling. Psychopharmacology(Berl), 2019, **236**(4): 1367-1384

[37] Alcocer-Gómez E, de Miguel M, Casas-Barquero N, *et al*.NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. Brain Behav Immun, 2014, **36**: 111-117

[38] Cao C, Su M, Zhou F. Mangiferin inhibits hippocampal NLRP3 inflammasome and exerts antidepressant effects in a chronic mild stress mice model. Behav Pharmacol, 2017, **28**(5): 356-364

[39] Sang X S, Chen Y H, Chen W, *et al*. Celastrol specifically inhibits the activation of NLRP3 inflammasome. Sci China Life Sci, 2018, **61**(3): 355-357

花楸木提取物对慢性不可预知应激小鼠认知损害的影响*

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摘要　认知缺陷是抑郁症的核心症状之一，已成为抑郁症治疗面临的的一大挑战. 花楸木为我国南方广泛分布的一种绿色乔木，具有抗抑郁作用. 然而，花楸木对认知损害的作用尚未见报道. 本研究旨在探讨花楸木提取物对慢性应激引起的认知障碍的影响，通过建立小鼠慢性不可预测轻度应激（CUMS）模型，评价花楸木改善认知功能的作用. 认知行为采用新物识别和Morris水迷宫测试，免疫组织化学分析海马组织中GFAP和Aβ蛋白的水平，Western Blotting分析海马组织NLRP3、TNF-α和IL-1β蛋白表达. 结果显示，花楸木提取物能明显增加小鼠的物体识别辨别指数，明显减少定位航行小鼠平台潜伏期，增加平台穿越次数. 花楸木提取物能显著提高海马组织中5-HT、DA、NE的水平；同时提高应激小鼠海马CA1区GFAP水平，降低Aβ沉积，减少海马区NLRP3、TNF-α和IL-1β的表达. 以上数据表明，花楸木提取物能明显改善CUMS小鼠学习记忆障碍，其作用机制与上调GAFP、减少Aβ沉积、缓解炎性有关.

关键词　花楸木提取物，慢性不预知应激，NLRP3，GAFP，Aβ

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