Antidepressant Activity of Euparin: Involvement of Monoaminergic Neurotransmitters and SAT1/NMDAR2B/BDNF Signal Pathway

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Depression is the most significant risk factor for suicide, yet the causes are complex and disease mechanism remains unclear. The incidence and disability rate of depression are very high and the efficacy of some traditional antidepressants is not completely satisfactory. Recently, some studies have found that benzofurans have anti-oxidation and anti-monoamine oxidase properties, which are related to depression. Euparin is a monomer compound of benzofuran, previous work by our team found that it improves the behavior of depressed mice. However, additional antidepressant effects and mechanisms of Euparin have not been reported. In this study, the Chronic Unpredictable Mild Stress (CUMS) model of mice was used to further investigate the effect and mechanism of Euparin on depression. Results showed that Euparin (8, 16 and 32 mg/kg) reduced depression-like behavior in mice compared with the model group. Meanwhile, all doses of Euparin were found to increase the contents of monoamine neurotransmitter and decrease monoamine oxidase and reactive oxygen species (ROS) levels in brain of depression mice. Additionally, Euparin restored CUMS-induced decrease of Spermidine/Spermine N1-Acetyltransferase 1 (SAT1), N-methyl-D-aspartate receptor subtype 2B (NMDAR2B) and brain derived neurotrophic factor (BDNF) expression. These findings demonstrate that Euparin has antidepressant properties, and its mechanism involves the SAT1/NMDAR2B/BDNF signaling pathway.

Key words Euparin; depression; oxidative stress; brain derived neurotrophic factor; monoaminergic neurotransmitter; signal pathway

INTRODUCTION

Depression is a complex psychiatric disorder associated with disability and reduced QOL.1) The mechanisms underlying depression remains poorly understood despite it being a common clinical diagnosis. Gerontism, chronic diseases, and high pressure living environment caused by rapid society development, may be related to the occurrence and development of depression.2,3) Depression will be the second most disabling condition worldwide in 2020 as reported by the WHO.4) The catastrophic and fatal disease will place substantial health and economic burdens on patients, families and medical systems.5,6) More troubling is the fact that, in the current clinical antidepressant first-line drug treatment, only about half of the patients are reactive and have lagging therapeutic response and drug resistance.7,8) Therefore, it is important to continue to discover and research new antidepressants and explore the pathogenesis of depression.

Although the pathogenesis and mechanism of depression are complex and unknown, the dysfunction of hypothalamic–pituitary–adrenal (HPA) axis and the increase of reactive oxygen species (ROS) caused by adverse stress have attracted attention. Indeed, there is growing evidence that stress can induce hyperfunction of the HPA axis and increase ROS levels in depression, and ROS usually weakens the negative feedback system of glucocorticoids (GCs), resulting in a vicious cycle.8,9) Lim team found that the expression of brain derived neurotrophic factor (BDNF) in brain tissue of stress-hormone treated mice was decreased, while ROS and monoamine oxidase (MAO) were up-regulated. After drug treatment, the depressive symptoms of mice were improved by preventing MAO activation induced by ROS.10) Over-stress causes HPA axis imbalance to promote ROS increase, and MAO activation may cause damage of nerve cells and abnormal levels of monoamines in the synaptic cleft, which may lead to mood disorders and behavioral changes. If a drug can reverse this pathological cross-linking reaction, it could be further researched for its potential as a new antidepressant drug.

At present, the discovery and research of new anti-depressant drugs from natural products are receiving more and more attention. Such as Flavonoids, Ginsenoside Rgl and Hypericum perforatum extracts have been reported to have antidepressant effects, and can affect certain molecular targets or signal pathways related to depression to improve animal depression symptoms.11–13) These have aroused our interest and attention to natural products. Recently, some studies have found that benzofuran compounds have anti-inflammatory, antioxidant effects and can inhibit Histamine H3 receptor (regulatory neurotransmitter and monoamine oxidase).14–16) In addition, other studies have shown that Verazodone nicotinate, a marketed drug that uses benzofuran as a pharmacophore, can treat major depression.17) These findings suggest that Benzofurans may have potential clinical effects on depression and other age-related neurodegenerative diseases.18) Euparin is a...
monomeric compound of Benzofuran, which is able to against ROS. The chemical structure of Euparin is shown in Fig. 1, which can be extracted from plants such as Eupatorium buniifolium Hook and Petasites hybridus. In our previous research work, we have isolated Euparin from the rhizome of Ligularia stenocephala Matsum, and found that it has a role in improving the behaviour of depressed mice. Therefore, we think it is necessary to further study the antidepressant effect of Euparin and its molecular mechanism.

MATERIALS AND METHODS

Animals and Drugs One hundred and twenty male C57BL/6J mice, specific-pathogen-free (SPF) grade (24 ± 1 °C, 45 ± 10% humidity), 18–22 g, 5–6 weeks old, purchased from Hualan Biological Engineering Inc., Xinxiang, China (License No.scxk-Henan-2010-0001), the 12h light and dark cycle (lights on at 7:30 a.m.) every day, housed in the animal facilities of Xinxiang Medical University. The animals were provided with food and water. All animal experiments were conducted in strict accordance with international ethical guidelines and guidelines of the National Institutes of Health for the protection and use of laboratory animals, and were approved by the Animal Ethics Committee of Xinxiang Medical College. Fluoxetine (FLU) was provided by Changzhou Siyao Pharmaceutical Co., Ltd. (China), Euparin (purity: 98%; molecular weight: 216.23), was purchased from Wuhan Qiongge Biotechnology Co., Ltd. (China).

Experimental Groups and Animal Handling Process Mice were numbered and labelled, and the animals were randomly divided into 2 groups, which 20 animals were in the control group, and the remaining animals were in the model group. The mice depression models were successfully prepared, and these model animals were randomly divided into Chronic Unpredictable Mild Stress (CUMS) (model group), CUMS + FLU (positive control group), Euparin 8, 16 and 32 mg/kg group, 20 animals in each group. The mice in control and model groups were given 5% dimethylsulfoxide (DMSO) solution (DMSO: double distilled water = 5:95), and the mice in CUMS + FLU group were given 30 mg/kg FLU; the mice in each dose group of Euparin were given the corresponding dose of Euparin. All of these agents were administered by the gavage with in a volume of 0.1 mL/10 g/d. The drug was administered twice a day at 9 a.m. and at 4 p.m., respectively. Animals need to be continuously administered for 14 d (the 27th to 40th days of the experiment). The animal was weighted in each group once a week. The open field experiment in mice was tested on the first day after 24 h of the last administrated drug of the mice, and the training of animal and the sucrose preference experiment was detected on the second to fifth days. The flow chart of experimental process and animal grouping were shown in Fig. 2A. After the sugar water preference test was completed, the animals were anesthetized with 4% chloral hydrate (0.1 mL/10 g), and then the animals were sacrificed by spinal dislocation. The brain tissue of mice was removed, and the frontal cortex and hippocampus were quickly separated in some animals. Twenty animals in each group were subjected to behavioral testing, 6 animals in each group were used for HPLC detection (1 to 2 animals in each group were used for each pre-experiment), and 6 animals in each group were used for MAO and ROS levels (the left frontal cortex and hippocampus of the mice were used for MAO detection, and the whole right brain tissue was used for ROS level detection), 4 animals in each group were used for quantitative (q)PCR or Western blot detection.

The Depression Model of Mouse The depression model of mice was induced by CUMS according to the description by previous study. The methods of stress stimulation mainly include the following 9 types. (1) Fasting for 24 h; (2) no water for 24 h; (3) binding for 2 h; (4) clip tail hanging for 30 min; (5) wet paddling (humidity to 80–90%) for 24 h; (6) 45° tilting squirrel cage for 24 h; (7) ice water swimming for 6 min; (8) light and dark upside down (8:00 a.m.–8:00 p.m.); (9) empty cage for 12 h. After 1 week of adaptive feeding of the mice, mice were exposed to CUMS stress stimulation for 21 d in this protocol. Two stressors were randomly selected every day, the same stimulus cannot be used continuously, and the same stimulus cannot be used more than 3 times, so that the mice cannot predict the next day’s stimuli. Animals in the control group were fed normally without any stimulation. Behavioural indicators of experimental tests such as open field and sugar water preference were mainly used to judge whether the model is successful. The CUMS protocol is shown in Table 1.

Open Field Test (OFT) Method On the 22nd day of the experiment, the animal’s OFT experiment was performed. The experiment needs to be carried out in a quiet environment. The black box was a box with no cover (80 × 80 × 45 cm), and the bottom surface was divided into 16 squares of 20 × 20 cm. The mice were placed in the center of the box and were allowed to move freely for 2 min to adapt to the new environment. The number of mobiles crossed and rearing frequency were observed over 5 min. After each measurement, the box was wiped clean with a low concentration of alcohol to prevent residual odor from interfering with the measurement of the next mouse. After the drug has been administered for 14 d, the OFT in mice were measured again at the 41th day of the experiment.

Sucrose Preference Test (SPT) Method On the 23rd day of the experiment, SPT of animals was performed. Three days before (the 23rd–25th day of the experiment) the SPT experiment, the mice were trained to adapt to sugary drinking water according to literature method. The animal SPT was then carried out: each mouse was individually raised and given two bottles of liquid at the same time: one bottle of 1% sucrose solution and one bottle of pure water, with 100 mL per bottle, and the position of the bottle was changed every 4 h. After 12 h (the 26th day of the experiment), the volume of the two bottles of liquid was measured separately, and the mouse sugar water preference index was calculated. Animal sugar water preference index formula: sugar water preference
Fig. 2. Effects of Euparin on the Body Weight and Depressive-Like Behaviors in Depression Mice Induced by CUMS

Mice were exposed to CUMS for 3 weeks and administered with 5% DMSO solution (vehicle), fluoxetine (FLU, 30 mg/kg) or Euparin (8, 16, 32 mg/kg) for 2 weeks, respectively. After animals were administrated, the body weight and behavior of mice were detected, respectively. The results of mouse behavior and weight body in the figure are the results of the last measurement. (A) The flow chart of experimental process and animal grouping. (B-D) Euparin increased the weight body, the mobile scores and sucrose preference value of depressed mice. DMSO: dimethylsulfoxide; CUMS: Chronic Unpredictable Mild Stress. Twenty mice in each group were used for this experiment, and data are expressed as mean ± standard deviation (S.D.). *p < 0.05 or **p < 0.01 model vs. control group; #p < 0.05 or ##p < 0.01 drug management vs. model group; ^p < 0.05 or ^^p < 0.01 Euparin vs. fluoxetine. One-way ANOVA followed by LSD method.

Table 1. The Protocol of Chronic Unpredictable Mild Stress

| Stressors                                      | Control | Model | FLU (mg/kg) | Euparin (mg/kg) |
|------------------------------------------------|---------|-------|-------------|-----------------|
| Mon (1) Fasting for 24h                        |         |       |             |                 |
| Tue (2) No water for 24h                       |         |       |             |                 |
| Wed (5) Wet padding for 24h                    |         |       |             |                 |
| Thu (6) 45° Tilting squirrel cage for 24h      |         |       |             |                 |
| Fri (9) Empty cage for 12h                     |         |       |             |                 |
| Sat (2) No water for 24h                       |         |       |             |                 |
| Sun (8) Light and dark upside down              |         |       |             |                 |
| (3) Binding for 2h                             |         |       |             |                 |
| (4) Clip tail hanging for 30min                |         |       |             |                 |
| (7) Ice water swimming for 6min                |         |       |             |                 |
| (8) Light and dark upside down                  |         |       |             |                 |
| (5) Wet padding for 24h                        |         |       |             |                 |
| (3) Binding for 2h                             |         |       |             |                 |
| (4) Clip tail hanging for 30min                |         |       |             |                 |
index = sugar water consumption/(sugar water consumption + pure water consumption) × 100%. After the drug has been administered for 14 d, the SPT in animals needs to be tested again at the 42nd–45th day of the experiment.

**The Measurement of HPLC Fluorescence Method**  Six mice from each group were sacrificed by euthanized, and the frontal cortex and hippocampus are rapidly separated. Then 0.1 mol/L perchlorate solution that contains 0.14 mol/L sodium metabisulfite sodium metabisulfite was added to the frontal cortex and hippocampus separately, and homogenized by conventional methods. The homogenate was centrifuged for 30 min (13000 × g) at 4 °C, and the supernatant was taken out and filtered through a 0.45 µm hydrophilic membrane. Five microliters of the solution was injected into the HPLC column (column: 5 µm, 250 × 4.6 mm MI corsorb-mv 100-5c18; fluorescence excitation wavelength: 280 nm, emission wavelength: 360 nm; injection rate: 1 mL/min; column temperature: 26 °C). The mobile phase consisted of 40% methanol and 60% phosphate buffer (pH 3.3), and the buffer consisted of 0.02 mol/L NaH2PO4 and 0.005 mol/L sodium l-octanesulfonate. The mobile phase was thoroughly mixed and filtered with 0.45 µm filter membrane and degassed for 30 min. A standard solution of serotonin (5-HT), dopamine (DA) and noradrenaline (NE) was prepared in the same manner, and the standard equation of the standard solution was calculated from the peak area of the chromatogram to determine the linear range; and the concentration of the sample to be tested was calculated according to the obtained equation.

**The MAO Activity**  The mice were euthanized, and the frontal cortex and hippocampus were quickly separated to prepare a homogenate. Samples were processed according to the instructions in the kit purchased through Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and absorbance was measured using a multifunctional enzyme label (Molecular Devices, U.S.A.). The total MAO (including MAO-A and MAO-B) content was calculated according to the formula.

**ROS Detection**  The production of ROS in brain tissues was detected by ROS fluorescent probe—Dihydroethidium (DHE). DHE is oxidized by intracellular ROS to form ethylene oxide. Ethylene oxide binds to chromosome DNA, and produces red fluorescence. Ten micrometers tissue slices were made with the cryostat. The tissue samples were then fixed in cold acetone for 10 min, and immersed in phosphate buffer saline (PBS) twice. In a dark room, 10 µM DHE solutions was added to the tissue, and the wet box was incubated at 37°C for 60 min, and then counterstained with 4'-6-diamidino-2-phenylindole (DAPI), soaked in PBS and photographed with an immunofluorescence microscope.

**qPCR**  The frontal cortex and hippocampus of mouse brain tissue were stripped, and total RNA was extracted by Trizol reagent (Life Technologies, Carlsbad, CA, U.S.A.). After reverse transcription of cDNA, the expression level of the gene was analyzed by qPCR. β-Actin was used as an internal reference control. The qPCR cycle conditions were as follows: 95 °C for 30 s; 45 amplification cycles at 95 °C for 5 s, 55 °C for 30 s and 72 °C for 30 s. The serial number of the primer was as follows: spermine N1-acetyltransferase 1 (SAT1) (5'-AAG CAG GTG TGC CAT GAA GT-3'; 3'-GGA CAG ATCCG AGC ACC CTC-5'), N-methyl-d-aspartate receptor type 2B (NMDAR2B) (5'-CGA TGG CGA CTC TAAGAT CC-3'; 3'-TGT GTA GCC GTA GCC AGTC A-5'), BDNF (5'-GGA CTG AGA AGC TGA AT-3'; 3'-GTC CTC ATC CAG CAG CTTCT T-5') and β-actin (5'-CCT CTA TGC CAA CAC AGT GC-3'; 3'-GTA CTC CTT CTT GCT GAT TCC-5'). It was verified by melting curve analysis whether all primers produced a single product. For quantitative analysis, relative expression was calculated using 2^(-ΔΔCt) method.

**Western Blotting**  The total protein in the brain tissue was extracted by RIPA buffer (Beyotime, China), and the protein concentration was measured by using the bicinchoninic acid protein assay kit (Beyotime). The protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis, and was transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was then incubated in 5% skim milk powder for 2 h at room temperature, followed by primary antibody (Abcam, Cambridge, MA, U.S.A.) incubation at 4 °C overnight. The primary antibodies include anti-β-actin (1:3000), anti-SAT1 (1:500), anti-NMDAR2B (1:1000) and anti-BDNF (1:3000) antibodies. Then the secondary antibody was added at 1:2000 and incubated for 2 h at room temperature. The ultraviolet photometry (UVP) gel imager was used to image and the gray value was determined using Image J image analysis software. The signal gray level was standardized with β-actin.

**Statistical Analysis**  Statistical analysis of the data was performed using SPSS 19.0 version software. All measurement results were expressed as mean ± standard deviation (S.D.). One-way ANOVA was used for comparison among groups, and LSD method was used for comparison between multiple samples. Probability values less than (p < 0.05) were considered statistically significant.

**RESULTS**

**Euparin Alleviated the CUMS-Induced Depressive-Like Behaviours and Increased the Weight of Mice**  The effect of drugs on animal depression and treatment can be reflected by behavioural indicators. In the behavioural tests of depression, OFT was mainly used to evaluate the ability of animals to explore independently in the new environment, while SPT was used to observe the anhedonia caused by depression.23) We first determined whether treatment with Euparin could alleviate CUMS-induced depression in mice. It can be seen from Fig. 2, after Euparin (8, 16, 32 mg/kg) and fluoxetine were administrated in depression mice, the mobile scores, number of rearing and sucrose consumption of the mice significantly increased compare with model mice (p < 0.05 or p < 0.01). Moreover, the therapeutic effect of high-dose Euparin was not significantly different from that of the positive control drug fluoxetine (p > 0.05). In addition, consider that the occurrence of depression may affect the weight of the animal. Further, the body weight of animals in each group was observed. Figure 2B result found that the body weight of the mice in the model group was significantly lower than that of the control group, while the body weight of mice in the Euparin and fluoxetine treatment groups was significantly increased compared with the model group (p < 0.05 or p < 0.01). The effect of each dose of Euparin on increasing the body weight of mice in the model group was similar to that of fluoxetine (p > 0.05). These results suggest that Euparin treatment can alleviate depressive symptoms in depressed mice, and can increase its weight, thereby improving the animal’s depressive state.
Euparin Reverses the CUMS-Induced Reduction of 5-HT, DA and NE Level of Brain Tissue in Mice

The content of neurotransmitters 5-HT, DA and NE in the brain was determined by HPLC fluorescence method. Figure 3A is a standard HPLC chromatogram of 5-HT, DA and NE. The HPLC chromatogram of one of the samples in the frontal cortex and hippocampus is shown in Figs. 3B and C, respectively. A series of standard solutions of known concentration were prepared by concentration gradient method. The peak area of each neurotransmitter standard was obtained by HPLC fluorescence detection. Linear regression analysis was performed to establish a standard curve. The equation is as follows: 5-HT ($y = 0.1782x + 0.0279$, correlation coefficient ($r$) = 0.9983); DA ($y = 0.3452x - 0.0244$, $r$ = 0.9980) and NE ($y = 0.1991x + 0.0025$, $r$ = 0.9990). The peak area and content of 5-HT, DA and NE showed a significant linear relationship. The 5-HT, DA and NE contents of each sample were calculated by the above equation, as shown in Figs. 3D and E. Compared with the control group, the levels of 5-HT, DA and NE in the model group were significantly decreased in the frontal cortex and hippocampus ($p < 0.05$ or $p < 0.01$), but after 14 d of treatment with Euparin and fluoxetine, this decline was.
significantly reversed ($p < 0.05$ or $p < 0.01$), suggesting that Euparin can increase the levels of 5-HT, DA and NE in the brain of depressed mice and the effect of high-dose Euparin is equal to that of fluoxetine ($p > 0.05$).

**Euparin Decrease the CUMS-Induced Increased of MAO and ROS Activity of Brain Tissue in Mice** 5-HT, DA and NE belong to monoamine neurotransmitters. Euparin can increase the content of 5-HT, DA and NE in the brain of depressed mice, suggesting that it may affect the metabolism of monoamine neurotransmitters. The physiological role of MAO is related to the metabolism of monoamine neurotransmitters in the nervous system. Therefore, we evaluated the effect of Euparin on MAO activity in the frontal cortex and hippocampus of mouse. As shown in Fig. 4, the content of MAO in the frontal cortex and hippocampus of the model group increased by 1.3 and 1.9 times, compared with the normal group ($p < 0.01$). After treatment with Euparin, the content of MAO in the frontal cortex and hippocampus decreased significantly compared with the model group ($p < 0.01$). The high-dose Euparin increased MAO activity compared with the fluoxetine group in the frontal cortex ($p < 0.05$) and all doses of Euparin were better than fluoxetine group in the hippocampus ($p < 0.05$ or $p < 0.01$). These results indicate

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**Fig. 4. Effects of Euparin on MAO Activity and ROS Content of the Brain Tissue in Mice**

(A) The MAO levels of the frontal cortex in mice. (B) The MAO levels of the hippocampus in mice. (C) The ROS Level of brain tissue in mice. (D) The immunofluorescence images of DHE in mice brain tissue ($\times 200$). Red denotes DHE, the stronger the red fluorescence; blue denotes DAPI, it is mainly used for the nuclear staining. 6 animals in each group were used for MAO and ROS levels. (The left frontal cortex and hippocampus of the mice were used for MAO detection, and the whole right brain tissue was used for ROS level detection.) Data are expressed as mean ± S.D. *$p < 0.05$ or **$p < 0.01$ model vs. control group; †$p < 0.05$ or ††$p < 0.01$ drug management vs. model group; ✱$p < 0.05$ or ✱✱$p < 0.01$ Euparin vs. fluoxetine. One-way ANOVA followed by LSD method. (Color figure can be accessed in the online version.)
that the effect of Euparin anti-depressive may be related to the decrease of ROS levels.

**Effect of Euparin on the Expression of SAT1, NMDAR2B and BDNF mRNA in Different Brain Regions of Mice** We probed the possible cellular and molecular mechanisms that might be responsible for the behavior changes in these depressive mice exposed to Euparin. Therefore, we tested SAT1, NMDAR2B and BDNF mRNA expression to explore possible antidepressant mechanisms of Euparin. Since the above experimental results indicate that the 32 mg/kg of Euparin has a better antidepressant effect, we only examined the effect of this dosage on the expression levels of these genes. By analysing the results of qPCR, it can be seen (Fig. 5), that the expression levels of SAT1, NMDAR2B and BDNF mRNA in the frontal cortex and hippocampus of the model group were significantly lower than those in the control group ($p < 0.01$). However, these gene were reversed by the Euparin treatment ($p < 0.05$ or $p < 0.01$), and the effect of increasing NMDAR2B in Euparin were stronger than that of fluoxetine ($p < 0.05$ or $p < 0.01$). The above results indicate that part of Euparin’s antidepressant effect is exerted through the SAT1/NMDAR2B/BDNF signaling pathway. The protein expression levels of those genes were consistent with their gene expression.

**DISCUSSION**

The main findings from this study can be summarized as follows: (1) Euparin can improve the depressive symptoms of mice by increasing the mobile scores, number of rearing and sucrose intake, and increase the weight of depressed mice; (2) Euparin increased the contents of 5-HT, DA and NE in the frontal cortex and hippocampus of mice; (3) Euparin decreased the activity of total MAO and the level of ROS in the frontal cortex and hippocampus of mice; (4) Euparin increased the expression of SAT1, NMDAR2B and BDNF mRNA and protein in the frontal cortex and hippocampus of depressed mice.

Previous study have shown that a prolonged state of stress is positively associated with the onset of depression, which are involved in HPA axis dysfunction in brain.\(^{26}\) CUMS has been acknowledged as a valid animal model that can emulate the dysfunction of HPA axis, and it has been widely used to simulate the pathophysiological mechanisms of depression and to determine the efficacy of antidepressants.\(^{27,28}\) Our
results showed that the contents of 5-HT, DA and NE in the frontal cortex and hippocampus of CUMS mice were decreased, while the levels of MAO and ROS were increased, all of the results were reversed by the Euparin treatment. At the same time, Euparin improved the behavioral indicators of depressed mice and increased the weight of depressed mice.

MAO plays an important role in central nervous system and peripheral nervous system by regulating the level of monoamine neurotransmitters. MAO inhibitors can increase the concentration of neurotransmitters in the brain by preventing the decomposition of MAO on neurotransmitters, thereby producing antidepressant activity. However, Euparin is a phenylpropanfuran drug that can non-selectively inhibit MAO, and MAO inhibitors may affect blood pressure. As reported by other research, the use of MAO inhibitors may induce hypertension, but if the dosage is small or combined medicine, the blood pressure may rise within a certain range, but overall it can remain basically normal. Studies have reported that MAO inhibitors may cause a decrease of blood pressure. This response is likely related to an increase in the concentration of NE in the brainstem and the reduction of peripheral sympathetic nerve output through a negative feedback mechanism. In our experiment, the doses of Euparin were small, and the animals always behave normally, which may have little effect on blood pressure. However, if Euparin is considered as a future antidepressant drug for further research and development, its impact on blood pressure needs to be considered. In addition, depression is also related to the increase in the level of ROS. ROS is important parameters to measure and predict the status of depression and to determine the efficacy of antidepressants. But above all, our results suggested that Euparin has good antidepressant effect.

The changes of neural plasticity caused by stress and other negative stimuli play an important role in the occurrence and development of depression. Antidepressant drug treatment has also been found to exert its antidepressant effect by regulating neuroplasticity. The hippocampus is the most commonly studied brain area in depression research. The hippocampus contains high levels of GCs receptors and glutamate, which regulates the HPA axis, so making it more vulnerable to stress and inhibition. Increased levels of glucocorticoids and sustained stress caused by hyper-function of the HPA axis can affect the structure, memory acquisition and emotional regulation of the hippocampus. This may affect neuroplasticity of the hippocampus, such as hippocampal synaptic plasticity and impaired neurogenesis, a significant reduction in hippocampal volume and hippocampal neuronal apoptosis. The prefrontal cortex, as an important nerve centre for thinking and behaviour in the brain, is also associated with depression. Its ventral medial prefrontal cortex (VmPFC) is involved in the

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**Fig. 6. Effects of Euparin Treatment on the SAT1, NMDAR2B and BDNF Protein Levels in the Frontal Cortex and Hippocampus**

(A) Representative western blots of frontal cortex SAT1, NMDAR2B and BDNF expression. (B) Representative western blots of hippocampus SAT1, NMDAR2B and BDNF expression. (C) The quantitative analysis of SAT1, NMDAR2B and BDNF protein levels in the frontal cortex. (D) The quantitative analysis of SAT1, NMDAR2B and BDNF protein levels in the hippocampus. The β-actin blotting was used to ensure equal loading. Four mice in each group were used for Western blot. *p < 0.05 or **p < 0.01 model vs. control group; ^p < 0.05 or ^^p < 0.01 drug management vs. model group; ^p < 0.05 or ^^p < 0.01 Euparin vs. fluoxetine. One-way ANOVA followed by LSD method.
regulation of emotions, including the generation of negative emotions, while the dorsolateral sectors (dlPFC) can regulate cognitive functions, such as intention formation, goal-oriented behaviour and attention control.\textsuperscript{50} Many studies have found that the volume of the prefrontal cortex of animals decreases during depression, which may be caused by the destruction and atrophy of neurons and neuroglia during depression.\textsuperscript{37,41} Therefore, the prefrontal cortex and hippocampus are important target organs for depression. Our research mainly uses the frontal cortex and hippocampus to study the antidepressant effect and mechanism of Eupain.

Our results showed that the expression of SAT1, NMDAR2B and BDNF mRNA and protein in the frontal cortex and hippocampus cortex and hippocampus of CUMS mouse model was significantly decreased, while Euparin treatment reversed this effect, and its effect intensity was comparable to that of fluoxetine. SAT 1 is the rate-limiting enzyme of polyamine catabolism, and its expression, in brain tissue of patients with depression, is reduced.\textsuperscript{42} Studies have reported that SAT1 can increase the activity of NMDAR ion channel in hippocampal pyramidal neurons and frontal cortex.\textsuperscript{43,44} The combination of NMDA and NMDAR results in the opening of non-selective cation channels, causing calcium influx.\textsuperscript{45} Intracellular calcium can act as a second messenger to trigger phosphorylation of cAMP response element binding protein (CREB), which activates transcription of BDNF by binding to a key Ca\textsuperscript{2+} response element within the BDNF gene.\textsuperscript{46} BDNF binds to the tropomyosin-related kinase B (TrkB) receptor and exerts an antidepressant-like effect through an autocrine and/or paracrine loop.\textsuperscript{47} In addition, when NMDA is combined with NMDAR, the influx of Ca\textsuperscript{2+} can also increase the TrkB levels on the cell surface, thereby enhancing the biological effects caused by BDNF.\textsuperscript{48} Our result suggested that Euparin may partially exert antidepressant effects through SAT1/NMDAR2B/BDNF pathway of the MAO-ROS correlation. This is consistent with the results of others.\textsuperscript{49}

NMDAR is the ionotropic receptor of Glutamate receptors, which is widely grouped in the central nervous system and plays an important role in neural plasticity and mood regulation.\textsuperscript{50} NMDAR consists of three subunits: NMDAR1, NMDAR2A and NMDAR2B,\textsuperscript{48} in which the density of NMDAR2B subunit on the membrane of hippocampus neurons in depression-like mice is significantly lower than in control mice.\textsuperscript{51} This is consistent with our experimental results; the expression of NMDAR2B was decreased in the frontal cortex and hippocampus of depressed mice induced by CUMS, and Euparin may have an antidepressant effect by increasing NMDAR2B receptor. However, Maeng and Zarate have found that the expression of NMDAR2B in the hippocampus of depression model rats, prepared by chronic mild stress, is significantly increased.\textsuperscript{52} These differences in studies are likely due to the variations in the experimental animals and depression models.

In conclusion, our experiments found that Euparin has a good antidepressant effect, which may involve its regulation on SAT1/NMDAR2B/BDNF signaling pathway of MAO-ROS correlation. In addition, the high-dose of Euparin was superior to fluoxetine in increased 5-HT, DA contents and reduced MAO activity, other effects of Euparin were basically comparable to those of fluoxetine. This suggests that Euparin has the potential continue research to provide experimental and theoretical basis for the development of new types of antidepressant drugs.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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