Agarwood essential oil inhalation exerts antianxiety and antidepressant effects via the regulation of Glu/GABA system homeostasis

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Abstract. Depression and anxiety are common diseases that endanger the physical and mental health of individuals. Agarwood incense inhalation has been used as a traditional Chinese medicine for relaxation and to improve sleep for centuries. In a previous study by the authors it was demonstrated that agarwood essential oil (AEO) injection exerted anxiolytic and antidepressant effects. Therefore the present study further investigated the anxiolytic and antidepressant effects of AEO inhalation on anxiolytic mice induced by M-chlorophenylpiperazine and depressive mice induced by chronic unpredictable mild stress. The results demonstrated that AEO exerted a significant anxiolytic effect, whereby autonomous movements were inhibited during the light dark exploration test and open field test. Furthermore, the tail suspension test and the forced swimming test demonstrated that AEO also exerted an antidepressant effect, whereby the immobility times were decreased. Moreover, AEO was determined to increase the levels of 5-hydroxytryptamine, γ-aminobutyric acid (GABA) A receptor ($GABA_A$) and glutamate (Glu) in anxiolytic mice and inhibit the levels of $GABA_A$ and Glu in depressive mice. Further investigations into how AEO affected the Glu/GABA system demonstrated that AEO markedly increased the protein expression levels of GABA transaminase (GABAT), glutamate metabotropic receptor 5 (GRM5), glutamate ionotropic receptor AMPA type subunit 1 (GluR1) and vesicular glutamate transporter 1 (VGluT1). Furthermore, AEO reduced the expression levels of GABAT, glutamate ionotropic receptor NMDA type subunit 2B and GRM5, and enhanced the expression levels of GluR1 and VGluT1. These results demonstrated that AEO potentially possesses antianxiety and antidepressant properties. The present study determined that the mechanism was related to the regulation of Glu/GABA neurotransmitter system homeostasis.

Introduction

Depression and anxiety are complex neurological and psychological diseases that are rated as two of the most severe health problems by the World Health Organization (1). These diseases have a high incidence and low diagnosis and treatment rates; however, anxiety and depression seriously affect the health of individuals (2). The clinical symptoms of anxiety and depression disorders include depressed mood, irritability, impaired concentration, poor appetite and insomnia (3). Moreover, in addition to these aforementioned symptoms, anxiety and depression can induce nervous dread, hypervigilance, an increased heart rate and blood pressure and can result in suicide (4-6).

At present, the pathological mechanisms of anxiety and depression are unclear and complex. An increasing number of studies have reported that their pathogenesis is related to the autonomic nervous system, hypothalamic-pituitary-adrenal (HPA) axis, neural circuits and the immune system (7). Several neurotransmitters, including dopamine (DA), 5-hydroxytryptamine (5-HT), glutamate (Glu), and γ-aminobutyric acid (GABA), serve an important role in nervous system regulation (8). 5-HT reuptake inhibitors can reduce the treatment

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Abbreviations: AEO, agarwood essential oil; mCPP, *meta*-chlorophenylpiperazine; CUMS, chronic unpredictable mild stress; LDET, light-dark exploration test; OFT, open field test; TST, tail suspension test; FST, forced swimming test; $GABA_A$, γ-aminobutyric acid A receptor; Glu, glutamate; 5-HT, 5-hydroxytryptamine; CNS, central nervous system; GRIN2B, glutamate ionotropic receptor NMDA type subunit 2B; GRM5, glutamate metabotropic receptor 5; DA, dopamine; NE, norepinephrine

Key words: AEO, inhalation administration, antianxiety, antidepression, Glu/GABA system homeostasis

of depression by reducing central 5-HT production, which damages nerve cells and leads to depression (9). The excitatory Glu-inhibitory GABA neurotransmitter secretion balance is critical for transmission of neural signals. Anxiety-like responses are accompanied by abnormal synaptic transmission, which results in an imbalance of excitatory/inhibitory (E/I) neurotransmission (10). GABA and its receptors serve an important role in regulating anxiety and depression (11). Moreover, the various receptors of GABA and Glu are potential therapeutic targets for anxiety and depression (12,13).

Agarwood is an important spice that has been widely used in incense, religion and aromatherapy for centuries (14-16). As a traditional Chinese medicine, agarwood is considered to serve a major role in pain relief, arresting vomiting, relieving asthma and it also has been reported to exert sedative effects (17). Agarwood has also previously been used to treat digestive, neurodegenerative and sedative diseases (18). Modern pharmacological studies have reported that agarwood essential oil (AEO) (19,20), extracts (21) and the sesquiterpene and chromone components (22,23) exert favorable sedative and hypnotic, anti-anxiety/depression, neuroinflammatory and neuroprotective effects. In traditional Chinese medicine, agarwood incense is used for its availability and the incense is also the main product of the agarwood market. Generally, AEO is the main active constituent of agarwood and serves numerous pharmacological functions, especially in neural regulation. Moreover, AEO vapor is able to sedate mice (24). Agarofuran, a constituent of AEO, has been revealed to exert anxiolytic and antidepressant activity (25). Furan compounds have been isolated from agarwood and synthesized via structural modification of bugufuran. These have further been developed and declared as class 1.1 anti-anxiety novel therapeutics, for which a phase III clinical trial has been launched (26). Diterpenoids of agarwood have also been demonstrated to exert antidepressant activity via the inhibition of the synaptic reuptake of serotonin and norepinephrine (NE) (27). The antianxiety, antidepressant and other neuroregulatory effects of AEO are currently the focus of research and AEO is expected to be developed into innovative novel neuroregulatory drugs. Furthermore, in previous studies by the authors it was demonstrated that an AEO injection could promote sleep and provide antianxiety and antidepressant effects (19,20). However, its pharmacological components and mechanism of action remain unclear.

Therefore, in the present study AEO incense inhalation was used to study the antianxiety and antidepressant effects of AEO and investigate its possible underlying mechanism in regulating neurotransmitters. The effects of AEO on *meta*-chlorophenylpiperazine (mCPP)-induced anxiety and chronic unpredictable mild stress (CUMS)-induced depression-like behaviors were assessed using animal behavioral tests.

Materials and methods

Instruments and reagents. A video analysis system for spontaneous activity (model: JLBehv-LAM-4) was purchased from Shanghai Jiliang Software Technology Co., Ltd. The incense box (50x50x40 cm) was formed of plexiglass with a 20x20x20 cm^3 hollow cylinder inside with incense inserts in the middle. The centrifuge and microplate reader were purchased from Thermo Fisher Scientific, Inc. (model: Multiskan Go, serial no. 1510-04123). The neurotransmitter and its receptor kits glutamate (Glu; cat. no. SRA-EMS-20177), 5-hydroxytryptamine (5-HT; cat. no. SRA-ESM-00901) and γ-aminobutyric acid receptor A (GABAA; cat. no. SRA-ES-31001; all from DXY.cn; Hangzhou Lianke Meixun Biomedical Technology Co., Ltd.) were purchased from Beijing Bosheng Jingwei Biotechnology Co., Ltd. The primary antibodies GABA transaminase (GABAT; cat. no. AG1008), glutamate metabotropic receptor 5 (GRM5; cat. no. AF1744), glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B) (cat. no. AF7029), and glutamate ionotropic receptor AMPA type subunit 1 (GluR1; cat. no. AF2473) were purchased from Beyotime Institute of Biotechnology. Vesicular glutamate transporter 1 (VGluT1; cat. no. bs-11167R) was purchased from BIOSS. The secondary antibodies HRP goat anti-rabbit (cat. no. A0208) and HRP goat anti-mouse (cat. no. A0216) were also obtained from Beyotime Institute of Biotechnology. Diazepam (cat. no. DZ-225336; www.biaowu.com) was purchased from Shangcheng Beina Chuanglian Biological Technology Co., Ltd. mCPP (cat. no. R031956) and paroxetine (a positive drug on antidepressant experiment) (cat. no. 200317) were purchased from the Beijing Lianshi Yunshang Network Technology Co., Ltd.

Materials. The agarwood raw material was artificially propagated agarwood (28) and tested according to the standards of the Chinese Pharmacopoeia by the Agarwood Identification Center of Hainan Branch, Institute of Medicinal Plant, Chinese Academy of Medical Sciences (Haikou, China). Voucher specimens (no. JC2016112) were deposited at the Agarwood Identification Center of our institute. AEO was prepared via steam distillation.

Animals. A total of 96 adult male KM mice (weight, 18-20 g) were purchased from the Hainan Provincial Institute of Medicine [cat. no. SCXK (Qiong) 2020-0007]. All animals were kept in a specific pathogen-free (SPF) animal facility at a temperature of 23±2˚C, a humidity of 60±5% and a 12-h light/dark cycle. Animals had free access to food and water. The animal experiments were performed in the SPF animal room of Hainan Institute of Materia Medica Co., Ltd. (Haikou, China) and followed the guidelines for the care and use of laboratory animals of this institute under the approval and supervision of the Animal Ethics Committee of the Hainan Institute of Materia Medica Co., Ltd. (approval no. 2022HL014).

Anxiolytic effects of AEO inhalation. The anxiolytic effects and the underlying mechanisms of AEO inhalation in an anxious mouse induced by mCPP were explored. The animals were divided into the following six groups (8 mice/group): i) Control group; ii) mCPP (8 mg/kg) model group; iii) diazepam (2.5 mg/kg) group; and iv) to vi) the AEO (2, 4 or 8 μl) inhalation groups. With the exception of the control group, the mice were administered with mCPP intraperitoneal injections for 2 days to create the anxiety animal model. The control group was given an equal volume of saline. The diazepam group was injected intraperitoneally for 7 days with 2.5 mg/kg (10 ml/kg) diazepam. The AEO groups were administered with AEO

via inhalation for 7 days. After 7 days, animal behavior was assessed via the open field test (OFT) and the light-dark exploration test (LDET). Following the behavioral tests, the mice were sacrificed via cervical dislocation and blood samples and brain tissues were rapidly collected and preserved in liquid nitrogen. The blood samples were centrifugated at 314 x g for 15 min at 4˚C and stored at -20˚C.

Antidepressant effects of AEO inhalation. The antidepressant effects of AEO were assessed in depressed mice induced via CUMS. The animals were divided into the following six groups (8 mice/group): i) Control group; ii) model group; iii) paroxetine (10 mg/kg) group, and iv) to vi) the AEO inhalation (2, 4 and 8 μl) groups. With the exception of the control group, mice were stimulated using CUMS molding boxes, which included plantar stimulation for 1 min, light/dark stimulation for 2 min, fasting for 24 h, water deprivation for 24 h, sleep deprivation for 24 h and restraint stress for 3 h/day. For random unpredictability, half of the mice were selected per day and were treated with continuous stimulation for 28 days. The paroxetine and AEO were administered from day 22 to day 28 for 7 days. The inactive time of tail suspension test (TST) and the forced swimming test (FST) were used to assess depression in the mice. After the behavior was assessed, the mice were sacrificed via cervical dislocation and the blood samples and the brain tissues of the mice were rapidly collected and preserved in liquid nitrogen. The blood samples were centrifugated at 314 x g for 15 min at 4˚C and the supernatant was stored at -20˚C.

LDET. The LDET was used to observe the exploratory behavior of mice when they were added to a new environment. As the rodents were innately averse to brightly lit areas, they developed anxiety and the animal activity was disordered. The apparatus contained two parts, a light box and a dark box, which were used to detect the movement of the animals. The apparatus was connected to a computer to record the time spent, distance moved and number of transitions in each part during a 6-min session. For this experiment, the mice were administered diazepam as aforementioned, to detect the anxiolytic effects of AEO. In the present study, the data from the last 4 min were selected for analysis.

OFT. The OFT evaluated the general exploratory behaviors of mice. For this experiment, the mice were administered diazepam as aforementioned, to detect the anxiolytic effects of AEO. Briefly, 1 h after the last treatment was administered, OFT real-time detection analysis was performed. The mice were placed into the open field along the barrel wall for 10 min of observation and the system automatically recorded the spontaneous activities of the animals, such as movement distance and movement time. The time spent and distance moved in the central area were collected to reveal the anxiolytic effect of AEO.

TST. The TST was performed as previously stated with certain modifications. In brief, 1 h after the last treatment was administered, the mice were fixed in place with adhesive tape and hung upside down. The tension change signal of the mice struggling was transmitted to the computer via a sensor, as well as to the signal conditioning unit and transmission circuit. The computer online detection system for the suspended tail automatically recorded the accumulated immobility time and movement time of mice within 6 min. It also detected the immobility time of the suspended tail in the last 4 min.

FST. The FST was performed according the Porsolt swim test method. In brief, 1 h following the last treatment administration, forced swimming real-time detection and analysis were performed. After setting the experimental parameters, geometry and illumination calibration, the mice were placed in a thermostatic swimming apparatus (height, 20 cm; diameter, 18 cm; water depth, 12 cm; water temperature, 23-25˚C). The system automatically recorded the activity status of the mice over 6 min and assessed the accumulated immobility time of the mice within the last 4 min.

ELISA. In this experiment, the mice were administered with diazepam and paroxetine as aforementioned, to detect the anxiolytic and antidepressant effects of AEO, respectively. Next, 1 h after the last administration, the mice were sacrificed via cervical dislocation and blood was collected. The brain tissue was also collected. The hippocampus was removed and frozen in liquid nitrogen. Subsequently, the tissue was weighed and added to nine volumes of normal saline before being fully homogenized in an ice bath and centrifuged at 314 x g for 15 min at 4˚C. The supernatant was collected and stored at -20˚C. The precipitate was used for protein extraction. The levels of 5-HT, Glu and $GABA_A$ in the supernatant were determined using the aforementioned ELISA kits, according to the manufacturer's protocols.

Western blotting. The precipitate of hippocampal tissues was added to RIPA (cat. no. BL504A; Biosharp; Beijing Lianshi Yunshang Network Technology Co., Ltd.) buffer supplemented with protease and phosphatase inhibitors. The homogenate was centrifuged at 1,364 x g, for 10 min at 4˚C. The protein concentration was determined using a BCA Protein Assay Kit. According to the molecular weight of the target protein, the protein samples (10 μg) were separated using 10% gel electrophoresis. Subsequently, the separated proteins were transferred to a PVDF membrane and blocked with 5% skimmed milk powder for 1 h at room temperature. The membranes were then incubated with the following primary antibodies; GABAT, GRIN2B, GRM5, GluR, VGluT1 (all at 1:1,000) and β-actin (1:2,000; cat. no. AF0003; Beyotime Institute of Biotechnology) at 4˚C overnight. Following the primary incubation, the membranes were incubated with secondary antibodies (1:2,000) for 2 h at room temperature. The membrane was treated with an ECL kit (cat. no. BL520A; Biosharp; Beijing Lianshi Yunshang Network Technology Co., Ltd.) and was observed using a gel imager. The grayscale of the protein bands were scanned and quantitative analysis was performed using the Gel-Pro Analyzer 4.0 (Media Cybernetics, Inc.). Subsequently, histograms were produced.

Statistical analysis. SPSS 17.0 software (SPSS, Inc.) was used for data analysis. Experimental data are presented as the mean ± SD and experiments were perfromed in triplicate. All the data were first analyzed for normal distribution and variance homogeneity. The statistical comparisons between more

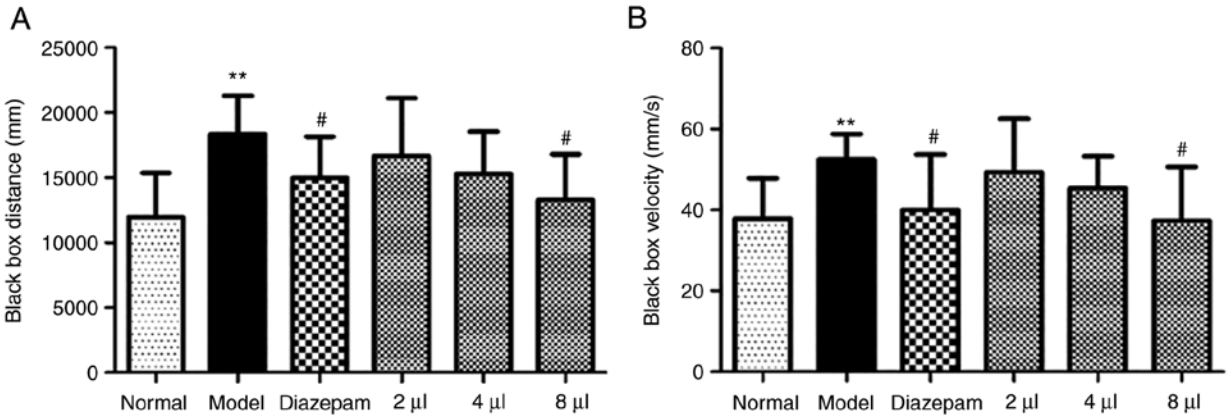

Figure 1. Effects of agarwood essential oil inhalation determined using a light-dark exploration test. (A) Black box distance and (B) black box velocity. All values are expressed as the means ± SD (n=8). **P<0.01 vs. the normal group; $^{\#}$P<0.05 vs. the model group.

than three groups were performed using one-way ANOVA followed by Tukey's post hoc tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of AEO on the LDET in mCPP-induced anxious mice. The distance and velocity of the animals were markedly enhanced in the model group compared with the control group in the dark box (P<0.01), which suggested that the anxiety model was successful. However, AEO significantly shortened the distance travelled in a dose-dependent manner (P<0.05 in the 8-μl group) (Fig. 1A) and decreased the velocity (P<0.05 in the 8-μl group) (Fig. 1B). This indicated that AEO inhalation potentially exerted an antianxiety effect. These results indicated that the effect of the high dose of AEO inhalation was the same as that of the diazepam.

Effects of AEO on the OFT in mCPP-induced anxious mice. The distance and velocity were significantly enhanced in the model mice (P<0.01) compared with the control mice, which demonstrated that the mice exhibited symptoms of anxiety. The results further demonstrated that AEO markedly shortened these distance increases in a dose-dependent manner (Fig. 2A). Furthermore, following treatment with AEO the velocity of the animals was decreased (Fig. 2B) and increases were observed in the central area route (Fig. 2C), central area velocity (Fig. 2D), stick-a-wall distance (Fig. 2E) and rest time (Fig. 2F) (P<0.05). These data indicated that AEO inhalation potentially resulted in improved antianxiety effects. Moreover, the effect of the high dose of AEO inhalation was the same as that of the positive drug.

Effects of AEO on the TST in CUMS-induced depressed mice. The immobility time of TST was prolonged in the model mice, which indicated that the mice exhibited depression-like symptoms. AEO treatment markedly shortened the immobility time (Fig. 3; P<0.05), which indicated that AEO treatment potentially exerted an improved antidepressant effect.

Effects of AEO on the FST in CUMS-induced depressant mice. The immobility time in the FST was significantly prolonged in the model mice (P<0.05), which indicated that the animals were exhibiting depression-like symptoms. AEO significantly reduced the immobility time (Fig. 4) (P<0.05), which indicated that AEO administration potentially exerted an antidepressant effect.

Effects of AEO on the 5-HT, $GABA_A$ and Glu levels in mCPP-induced anxious mice. The levels of 5-HT, $GABA_A$ and Glu were assessed in mCPP-induced anxious mice and the results revealed that the level of 5-HT was significantly reduced (P<0.001), the level of $GABA_A$ was also reduced (P<0.01) and the level of Glu was enhanced (P<0.05) in the model mice. AEO treatment significantly increased the levels of 5-HT and $GABA_A$ and decreased the levels of Glu in a dose-dependent manner (Fig. 5A-C; P<0.05 or P<0.01). These results indicated that AEO potentially serves a role in regulating neurotransmitter levels.

Effects of AEO on the 5-HT, $GABA_A$ and Glu levels in CUMS-induced depressant mice. The levels of 5-HT, $GABA_A$ and Glu were assessed in CUMS-induced depressant mice and the results revealed that the levels of 5-HT were significantly reduced (P<0.001) and the levels of $GABA_A$ and Glu were enhanced (P<0.05) in model mice. AEO significantly increased the levels of 5-HT and decreased the levels of $GABA_A$ and Glu (Fig. 6A-C; P<0.05 or P<0.01). Furthermore, the effect of AEO treatment was the same as that of treatment with paroxetine.

Effects of AEO on protein expression levels in mCPP-induced anxious mice. The protein expression levels of GABAT, GRM5, GluR1 and VGluT1 were assessed in mCPP-induced anxious mice and the results revealed that AEO inhalation significantly upregulated the protein expression levels of GABAT, GRM5, GluR1 and VGluT1 (P<0.05, P<0.01 and P<0.001). Furthermore, the results demonstrated that AEO treatment potentially served a role in relieving anxiety via regulating the protein expression levels and transport of GABA and Glu, and therefore the balance of the Glu/GABA system (Fig. 7).

Effects of AEO on protein expression levels in CUMS-induced depressant mice. The protein expression levels of GABAT, GRIN2B, GRM5, GluR1 and VGluT1 were assessed in

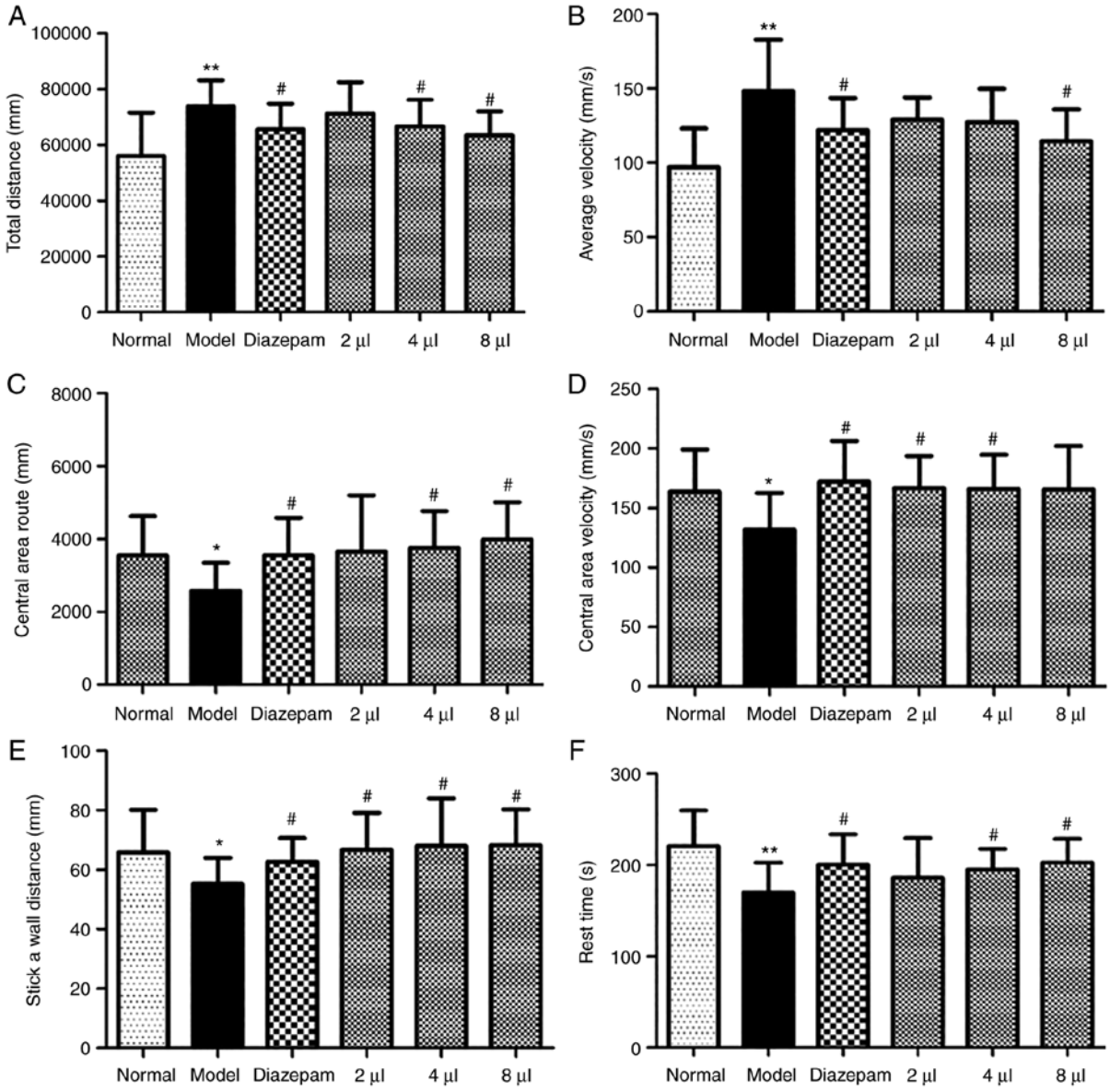

Figure 2. Effects of agarwood essential oil inhalation determined using an open field test. (A) Total distance, (B) average velocity, (C) central area route, (D) central area velocity, (E) stick-a-wall distance and (F) rest time were assessed. All values are expressed as the means ± SD (n=8). *P<0.05 and **P<0.01 vs. the normal group; $^{\#}$P<0.05 vs. the model group.

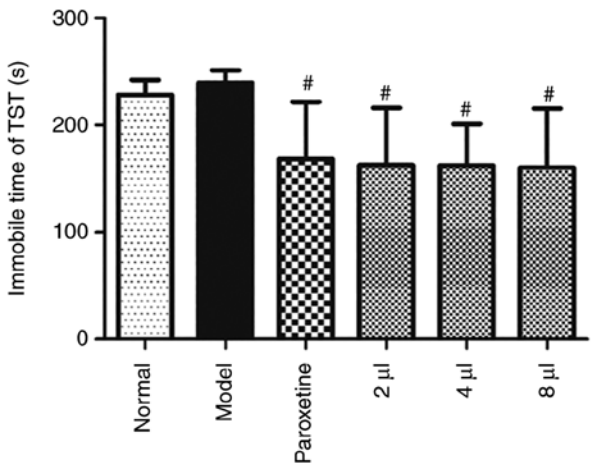

Figure 3. Effects of agarwood essential oil inhalation determined using a tail suspension test. All values are expressed as the means ± SD (n=8). P>0.05 vs. the normal group; $^{\#}$P<0.01 vs. the model group. TST, tail suspension test.

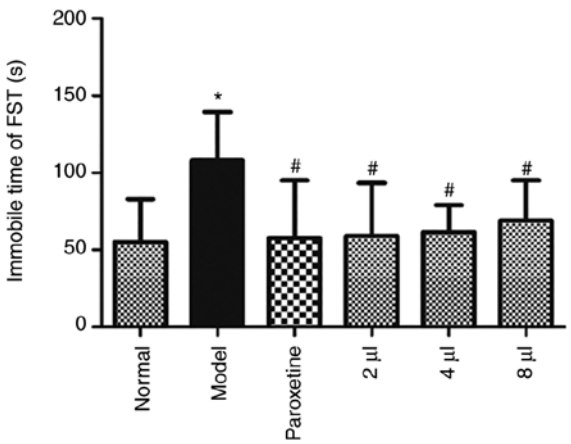

Figure 4. Effects of agarwood essential oil inhalation determined using a forced swimming test. All values are expressed as the means ± SD (n=8). *P<0.05 vs. the normal group; $^{\#}$P<0.05 vs. the model group. FST, forced swimming test.

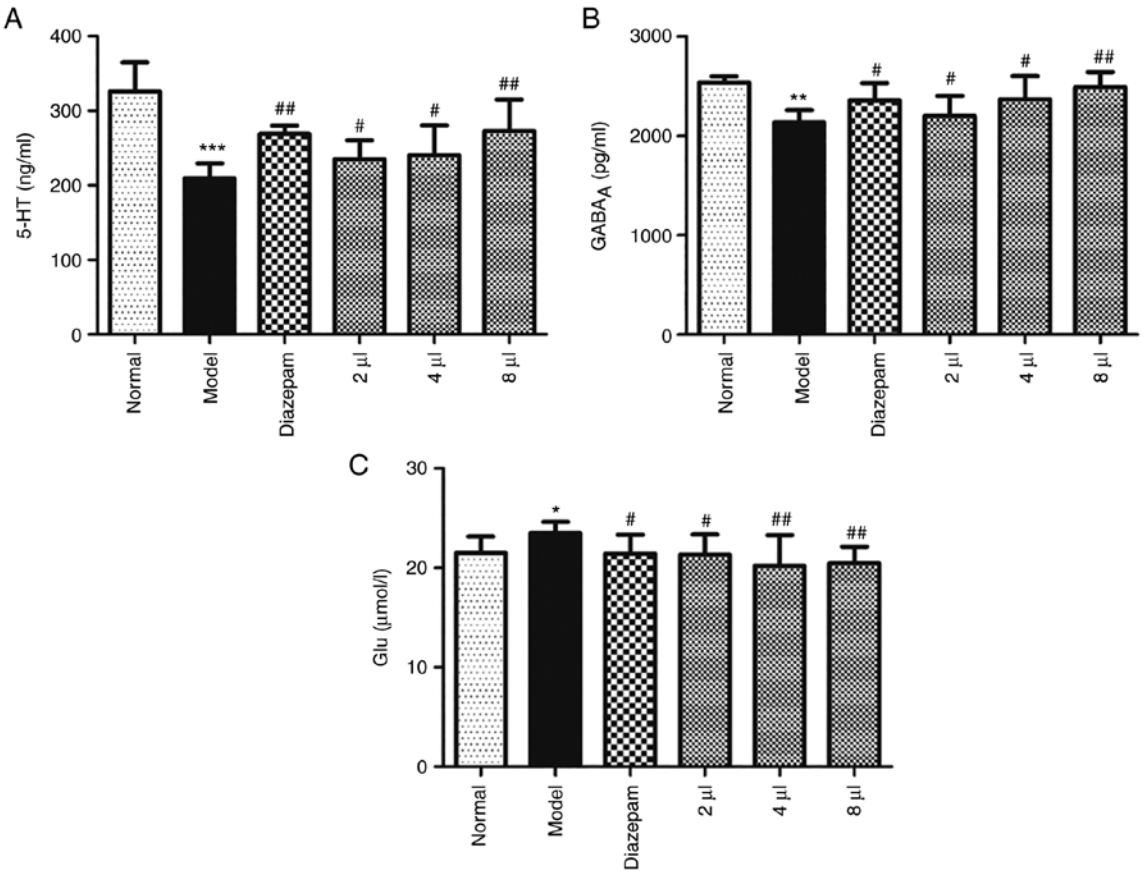

Figure 5. Effects of agarwood essential oil inhalation on the levels of 5-HT, $GABA_A$ and Glu in M-chlorophenylpiperazine-induced anxious mice. The levels of (A) 5-HT, (B) $GABA_A$ and (C) Glu. All values are expressed as the means ± SD (n=8). *P<0.05, **P<0.01 and ***P<0.001 vs. the normal group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the model group. 5-HT, 5-hydroxytryptamine; $GABA_A$, γ-aminobutyric acid A receptor; Glu, glutamate.

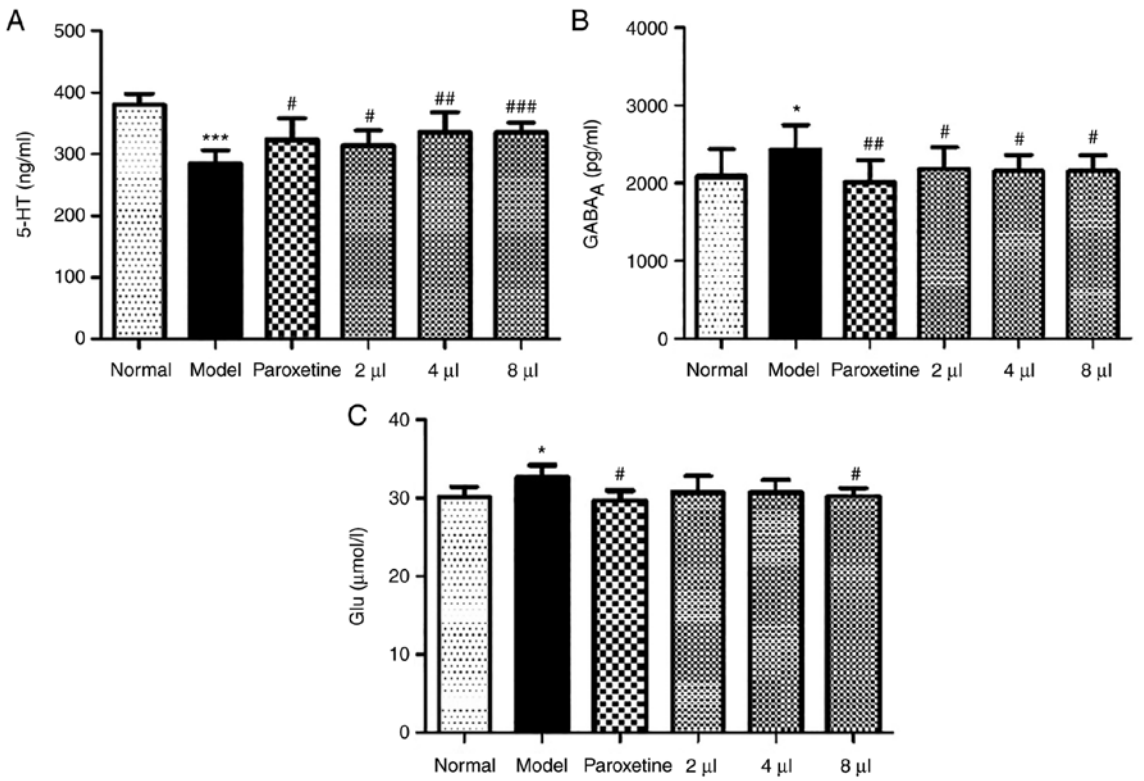

Figure 6. Effects of agarwood essential oil inhalation on the levels of 5-HT, $GABA_A$ and Glu in chronic unpredictable mild stress-induced depressant mice. The levels of (A) 5-HT, (B) $GABA_A$ and (C) Glu. All values are expressed as the means ± SD (n=8). *P<0.05 and ***P<0.001 vs. the normal group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the model group. 5-HT, 5-hydroxytryptamine; $GABA_A$, γ-aminobutyric acid A receptor; Glu, glutamate.

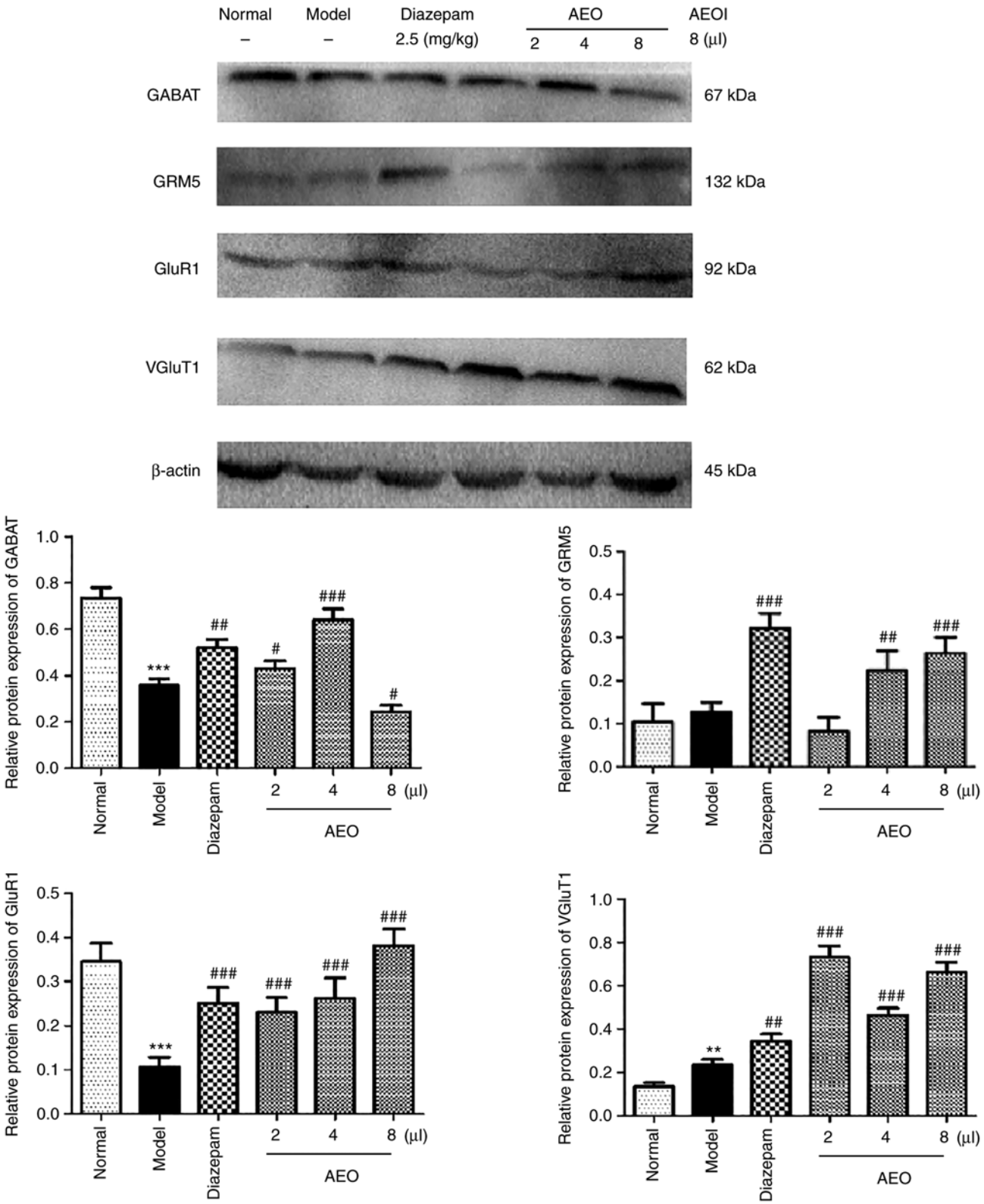

Figure 7. Effects of inhalation of agarwood essential oil on the protein levels of GABAT, GRM5, GluR1 and VGluT1 in M-chlorophenylpiperazine-induced anxious mice. The protein levels of GABAT, GRM5, GluR1 and VGluT1 were assessed using western blotting. All values are expressed as the means ± SD (n=3). **P<0.01 and ***P<0.001 vs. the normal group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. the model group. GABAT, GABA transaminase; GRM5, Glu metabotropic receptor 5; GluR1, Glu ionotropic receptor AMPA type subunit 1; VGluT1, vesicular Glu transporter 1; AEOI, agarwood essential oil inhalation.

CUMS-induced depressant mice and the results revealed that AEO inhalation markedly decreased the protein expression levels of GABAT, GRIN2B and GRM5, and increased the protein expression levels of GluR1 and VGluT1. The results demonstrated that AEO treatment potentially served an anti-depressant role via the regulation of the protein expression levels of GABA and Glu and the balance of the Glu/GABA system (Fig. 8).

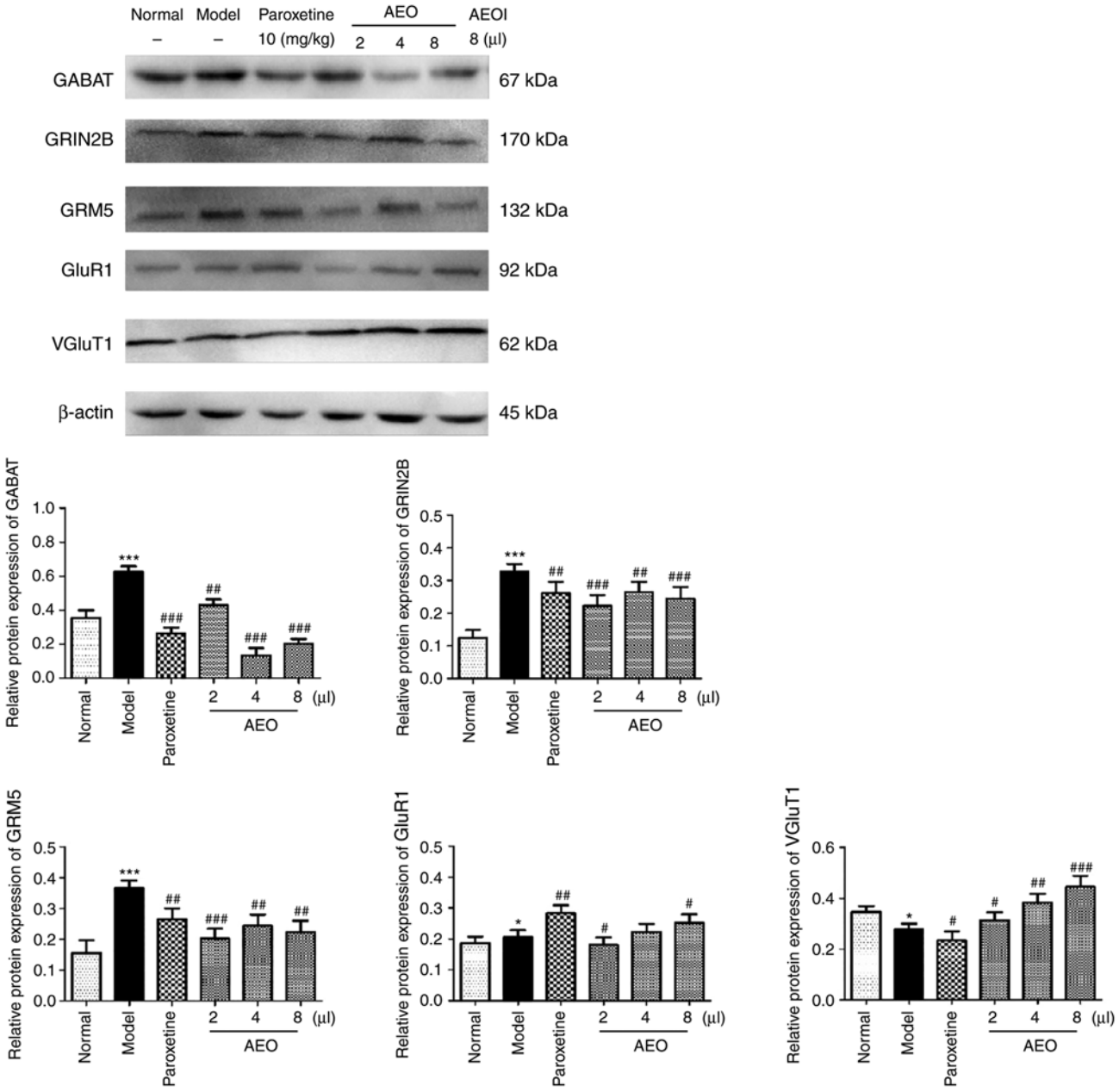

Figure 8. Effects of inhalation of agarwood essential oil on the protein levels of GABAT, GRIN2B, GRM5, GluR1 and VGluT1 in chronic unpredictable mild stress-induced depressant mice. The protein levels of GABAT, GRM5, GluR1 and VGluT1 were assessed using western blotting. All values are expressed as the means ± SD (n=3). *P<0.05 and ***P<0.001 vs. the normal group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. the model group. GABAT, GABA transaminase; GRIN2B, glutamate receptor N2B subunit; GRM5, Glu metabotropic receptor 5; GluR1, Glu ionotropic receptor AMPA type subunit 1; VGluT1, vesicular Glu transporter 1; AEOI, agarwood essential oil inhalation.

Discussion

The present study suggested that AEO displays anxiolytic and antidepressant effects via balancing the E/I Glu and GABA neurotransmissions in anxious and depressant mice. Behavioral evaluation further confirmed that AEO not only significantly inhibited the anxiety of mice as assessed by LDET and OFT, but also exerted a significant antidepressant effect by decreasing the immobility time of mice as determined using TST and FST. In summary, the results indicated that AEO may represent a potential treatment of anxiety and depression by regulating the homeostasis of the Glu-GABA system.

Spontaneous locomotor activity tests, including the LDET and OFT, are used to generate general parameters to study the effects of a drug (29). A decrease in locomotor activity indicates that the drug exerts an inhibitory effect on the central nervous system (CNS) (30). The results of a previous study by the authors demonstrated that AEO exerted significant antianxiety and antidepression effects via inhibition of the HPA axis (19). It was also determined that AEO exerted sleep-promoting effects via increasing $GABA_A$ receptor function (20), indicating that AEO exerted antidepressant or anxiolytic effects. In the present study, it was confirmed that AEO exerted anxiolytic and antidepressant effects by inhibiting the locomotor activity as assessed using LDET and OFT, and prolonging the immobility time as

determined using TST and FST, which indicated that AEO may prevent and treat neurological disorder diseases by regulating both E/I bidirectional effects.

The abnormal secretion of neurotransmitters is important in the pathogenesis of anxiety, depression and comorbid disorders (31). The GABA counterbalance with Glu serves an important role in anxiety and depression (32). Glu is the main excitatory neurotransmitter in the CNS and is a precursor of GABA, whose role in the pathogenesis of anxiety and depression remains unclear. Previous studies have reported that increases in Glu levels are closely associated with anxiety in the clinic, which suggests that glutamatergic neurons serve an important role in the etiology of anxiety and depression (33,34). In the present study, AEO increased the levels of 5-HT and $GABA_A$ and decreased the levels of Glu in anxiety-induced mice, and decreased the levels Glu and $GABA_A$ in depression-induced mice. These results suggested that AEO potentially affected the two most important amino acids with regard to anxiety, depression and other neurological disorders, which was consistent with a previous study (35).

The hippocampus may play a critical role in the pathophysiology and treatment of anxiety and depression. Its functions correspond to those altered in anxiety and depression (36-38). The hippocampus, involved in the regulation of multiple neurotransmitter systems, including GABA, Glu, 5-HT, DA and NE, is a common target of antianxiety and antidepressant treatments (20,39-41). The balance of E/I synaptic transmission is an important factor in regulating normal physiological functions of the CNS and in regulating neurological disorders. Alterations of Glu, GABA and their corresponding receptors could reflect the balance of mental processing. Enhanced excitatory transmission and reduced inhibitory transmission was revealed to result in anxiety-like behaviors (42,43). In addition, increased and decreased GABA may result in depression-like behaviors (44). In previous studies it was reported that the level of vesicular glutamate transporter 1 (VGluT1) was decreased in the CUMS model, and ketamine injection alleviated this abnormality (45,46). The *N*-methyl-D-aspartate receptor actively induced anxiety through its antagonists (47). Concurrently, the $GABA_A$ receptor, as the main inhibitory neurotransmitter of the CNS, mainly modulates the behavioral responses deriving from stressful conditions (48). Neurotransmitters and their receptors play different roles in various neurological disorders. In the present study, the expression levels of GABA and Glu receptors and transporters including GABAT, GRIN2B, GRM5, GluR1 and VGluT1 were assessed. AEO inhalation increased the expression of GABAT and GRM5 in anxiety-induced mice, but decreased the levels of GABAT, GRIN2B and GRM5 in depression-induced mice. Concurrently, AEO also increased the levels of GluR1 and VGluT1 in anxiety- and depression-induced mice. These results suggested that AEO played an antianxiety and antidepressant role by regulating excitatory Glu and inhibitory GABA (E/I) neurotransmitter secretion balance.

In conclusion, the present study demonstrated that AEO incense exerted potential antianxiety and antidepressant effects via the regulation of their receptors and via the GABA/Glu system. These effects were similar to those of treatment with diazepam and paroxetine. The inhalation of AEO resulted in two-way effects in regulating the balance of the GABA/Glu system, which suggested that AEO could serve as a potential therapeutic candidate aiding in the treatment of anxiety, depression and CNS diseases. Furthermore, the present study may also provide a theoretical basis for the development and utilization of agarwood. However, the specific underlying molecular mechanism of AEO needs to be further explored.

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Availability of data and materials

The datasets used during the present study are available from the first author or corresponding author upon reasonable request.

Authors' contributions

CW and JW designed the study. CW, BG and YW performed the experiments. YL and DC extracted the AEO, and assisted with the experiments. CW contributed to the preparation of the manuscript. CW and JW revised the manuscript. CW and BG analyzed the data and confirm the authenticity of all the raw data. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The animal care and experimental protocol was approved by the Institutional Animal Care and Use Ethics Committee of Hainan Institute of Materia Medica Co., Ltd., Haikou, China (approval no. 2021HL014).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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