# Investigation of the antidepressant effects of exopolysaccharides obtained from *Marasmius androsaceus* fermentation in a mouse model

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**Abstract.** *Marasmius androsaceus*, a well-known medical fungus, possesses antihypertensive, analgesic and antioxidant effects. Exopolysaccharide (EPS), produced by microorganism secretion, exerts various types of biological activities. The present study aimed to investigate the antidepressant-like effect of the EPS produced during *Marasmius androsaceus* submerge fermentation (MEPS). Based on the assessment of acute toxicity and behavior, a forced swimming test (FST), tail suspension test (TST), 5-hydroxytryptophan-induced head-twitch assessment and reserpine-induced hypothermia assessment were performed. The administration of MEPS for 7 days enhanced mouse locomotor and balance ability in the mice. Similar to the results following treatment with fluoxetine, which was used as positive control drug, MEPS significantly decreased the duration of immobility in the FST and TST, increased head twitches in the 5-HTP-induced head-twitch test and enhanced rectal temperature in resperpine-induced hypothermia. MEPS altered the abnormal concentrations of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, dopamine and norepinephrine in the hypothalamus in the resperine-induced mouse model. Additionally, an increase in the expression of tyrosine hydroxylase and a reduction in the level of dopamine transporter in the hypothalamus were noted following 7 days of MEPS administration. Taken together, the EPS produced during MEPS exhibited antidepressant-like effects, which may be associated with its regulation on the dopaminergic system. The results of the present study provide experimental evidence supporting the clinical use of MEPS as an effective agent against depression.

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## Introduction

Patients suffering from depression present with dysfunctions in brain morphology and activity (1,2). Due to its relatively high lifetime prevalence and substantial associated disability, depression is considered a worldwide problem in humans (1,2). According to statistics, depression affects almost 15-25% of the world's population, and is expected to become the second largest global burden by 2020 (3). According to the monoamine hypothesis, depression is interrelated with the metabolic turnover of dopamine (DA) in the hippocampus, and/or serotonin (5-hydroxytryptamine; 5-HT) in the prefrontal cortex and striatum in rat brains (4). In addition, serotonin transporter, dopamine transporter (DAT) and tyrosine hydroxylase (TH) are important in antidepressants (5). The four types of antidepressant drugs, selective serotonin re-uptake inhibitors (SSRIs), tricyclic antidepressants, serotonin-noradrenergic re-uptake inhibitors and monoamine oxidase inhibitors, are commonly used in clinical treatment (6,7). However, each of these drugs exhibit various adverse effects, with high relapse rates and a long onset of therapeutic action (8). Due to the limited using of existing antidepressant drugs, the development of novel alternative therapies is in high demand (9).

Herbal medicine have become a valuable reservoir for novel drugs due their limited side effects (9). The use of Traditional Chinese medicine has been confirmed to be an effective alterative in treating depression. *Acanthopanax senticosus* possesses antidepressant-like effects and has beneficial effects in patients with depression (10). Studies have suggested that *Areca catechu* fruit extract exerts antidepressant activities in an animal model (11). Wuling capsule, produced by Wuling submerged fermentation mycelium, has also been used as an antidepressant agent for clinical use in China (12,13). *Marasmius androsaceus*, a well-known medical fungus, possesses antihypertensive, analgesic and antioxidant properties (14,15). In China, 'An-Luo Tong', which is produced from the fermented mycelium of *Marasmius androsaceus,* has been

used as a painkiller for ~10 years (16). However, the regulatory effects of *Marasmius androsaceus* by-products following its submerged fermentation on depressant-like effects in mice remain to be elucidated.

Exopolysaccharide (EPS) is produced from microorganism secretion and can be readily separated from bacteria. Compared with plant polysaccharides, EPS has the advantage of efficient production duration, convenient extraction and the absence of geographical restrictions (17). Investigations on the various types of biological activity of EPS have revealed antioxidant effects (18) and enhanced immunity (19). The EPS produced by *Cordycept militaris* has an antihyperglycemic effect in diabetic mice induced by streptozotocoin injection (20), and EPS separated from *Morchella conica* markedly prolongs the life-span of fruit flies (21).

Therefore, the present study hypothesized that The EPS produced during *Marasmius androsaceus* submerge fermentation may possess antidepressant effects. To test this hypothesis, the present study aimed to investigate the associated biological activities of *Marasmius androsaceus* EPS (MEPS) using a mouse model. The effects of MEPS were analyzed by performing a mouse forced swimming test (FST) and tail suspension test (TST), and changes in the concentrations of monoamine neurotransmitters, including DA, norepinephrine (NE), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus were detected. To further analyze its underlying mechanism, the expression levels of DAT and TH in the hypothalamus were detected using western blot analysis. The resulting data may provide experimental evidence supporting the clinical use of *Marasmius androsaceus* exopolysaccharide as an effective agent against depression.

## Materials and methods

*Submerged fermentation of Marasmius androsaceus. Marasmius androsaceus* (CCTCC M2013175; China Center for Type Culture Collection, Wuhan, China) was cultured in a 100 liter fully-automatic fermentor (BaoXing Bioscience Company, Shanghai, China) using a defined liquid medium, containing 20 g/l sucrose, 10 g/l peptone, 10 g/l yeast extract powder, 1 g/l $MgSO_4 \bullet 7H_2O$, 1 g/l $KH_2PO_4 \bullet 3H_2O$, and 0.1 g/l vitamin $B_1$. The fermentation conditions were as follows: Initial pH 6.5; rotation speed, 300 rpm; culture duration, 6 days; culture temperature, 26˚C; inoculum volume, 5%; ventilation volume, 200 l/h; inoculum age, 4 days; loading volume, 70/100 liters. All chemical reagents used in submerged fermentation were obtained from Sigma-Aldrich (St. Louis, MO, USA).

*Sample preparation*. The fermentation products were filtered using 120 mesh sifters and centrifuged at 4,500 x g for 10 min. Using Sevag reagent (chloroform: n-butyl alcohol, 4:1), the existing proteins were extracted (22). A four-fold volume anhydrous ethanol was added for 12 h at 4˚C to precipitate the crude EPS. Following 30 min centrifugation at 4,500 x g, the precipitate was washed with anhydrous ethanol and acetone (Sigma-Aldrich) three times. Following dialyzing, the crude EPS was lyophilized using a vacuum freeze dryer (GENESIS SQ 25ES; SP Industries Inc., Warminster, PA, USA) with primary drying at 60 mT vacuum and a shelf temperature set at -25˚C for 10 h. Prior to animal administration, the *Marasmius androsaceus* EPS (MEPS) and fluoxetine hydrochloride capsules (Shanghai Zhongxi Pharmaceutical Group Co., Ltd, Shanghai, China) were dissolved in physiological saline to 1 mg/ml.

*Animals and animal care*. The experimental protocol used in the present study was approved by the ethics committee of the School of Life Sciences, Jilin University (Changchun, China). KunMing (KM) mice (6-week-old; 20-22 g; 1:1 male:female ratio) were housed in clear plastic cages and maintained in a 12 h light/dark cycle (lights on 7:00-19:00 h) at 23±1˚C, with water and food available *ad libitum*. At 8 hours prior to the experiment, the animals were deprived of food, but had free access to water. All experiments were performed in a quiet room, and each animal was assessed only once.

*Acute toxicity assessment*. The KM mice (6-week-old; 20-22 g; n=20/dosing group) were treated with MEPS at different concentrations (0.1, 1.0, 2.0, 4.0 and 6.0 g/kg) via gavage for a period of 7 days. In a separate group, the mice were treated with equal volumes of normal saline, which served as a control group. The body weights of the mice were measured prior to administration and on day 7. The animal were maintained under surveillance every day to record any adverse symptoms.

*FST*. Following MEPS treatment, an FST was used, in a similar manner to that described in a previous study with minor modifications (23). Briefly, the mice (n=10/group) were orally administered with distilled water (vehicle), MEPS (10, 50 or 250 mg/kg) or fluoxetine (10 mg/kg) for 1 week, respectively. At 30 min following the final administration, the FST was performed. The mice were placed in an open cylindrical container (30x18 cm) with a 15 cm depth, which did not permit the animal failed to touch the bottom of the cylinder. The water placed in the container was at a temperature of 24±1˚C. The duration of immobility was defined as the duration spent by the mouse floating in the water without struggling, and making only small movements necessary to maintain its head above the water. By using a stopwatch, the total duration of immobility was recorded in the final 5 min of a total duration of 6 min. A decrease in the duration of immobility was considered to be a measurement of antidepressant activity.

*TST*. The TST was performed in a quiet experimental room, according to previous report (4). The mice (n=10/group) were orally administered with either distilled water, MEPS (10, 50 or 250 mg/kg) or fluoxetine (10 mg/kg) for 1 week, respectively. At 30 min following the final administration, the TST was performed. Each mouse was suspended by its tail to a horizontal wooden bar, which was located in a yellow plastic box (40x40x40 cm), ~30 cm above the bottom. The mouse was secured to the bar by adhesive tape, which was placed 1 cm from the tip of tail so that its head was ~20 cm above the floor. The trial was performed for 6 min, during which two observers scored the latency of the first immobility episode and the total duration of immobility using a stopwatch in a

Table I. Analysis of body weights following treatment with MEPS to assess acute toxicity.

| | Body weights (g) at different concentrations of MEPS (g/kg) | | | | | |
|---|---|---|---|---|---|---|
| Day | 0 | 0.1 | 1.0 | 2.0 | 4.0 | 6.0 |
| 1 | 23.5±1.0 | 23.8±0.9 | 23.6±1.1 | 23.4±0.8 | 22.3±0.9 | 23.2±1.6 |
| 2 | 24.6±1.8 | 24.5±1.3 | 24.7±1.2 | 24.3±1.7 | 25.0±0.8 | 24.4±1.7 |
| 3 | 25.2±2.3 | 24.7±1.8 | 25.5±1.7 | 26.0±1.4 | 25.1±1.3 | 24.5±1.7 |
| 4 | 26.1±1.9 | 26.4±1.9 | 25.7±1.7 | 26.1±1.3 | 25.2±1.3 | 25.1±1.8 |
| 5 | 27.1±1.6 | 27.1±2.3 | 26.9±2.2 | 27.3±1.6 | 26.2±1.3 | 25.6±1.9 |
| 6 | 27.9±2.1 | 28.1±2.3 | 27.6±2.4 | 27.8±1.5 | 27.3±1.7 | 26.1±1.9 |
| 7 | 28.3±2.3 | 28.4±1.7 | 27.9±2.1 | 28.3±1.7 | 27.8±1.6 | 26.6±1.6 |

Body weights were monitored every day. Data are expressed as the mean ± standard error of the mean (n=20). MEPS, *Marasmius androsaceus* exopolysaccharide.

blinded-manner. The mouse was considered immobile only when it hung passively and completely motionless. Mice that climbed upwards grasping with its tail were eliminated from further analysis.

*Behavioral assessment*. With the purpose of excluding sedative or motor abnormality, the spontaneous locomotor activity was assessed (24). A clear acrylic chamber (11x11x15 cm), equipped with 12 infrared sensors for antomatic recording, was used. The mice (n=10/group) were orally administered with either distilled water, MEPS (10, 50 or 250 mg/kg) or fluoxetine (10 mg/kg) for 1 week, respectively. At 30 min following the final administration, each mouse was initially placed in the center of the testing chamber. Following a 2-min period of adaptation, behavioral data Data associated with horizontal and vertical movements were collected and recorded for 5 min.

To examine the coordination and balance of mouse movement, a rotation test was performed. The mice (n=10/group) were orally administered with either distilled water, MEPS (10, 50 or 250 mg/kg) or fluoxetine (10 mg/kg) for 1 week, respectively. At 30 min following the last administration, the mice were placed on a fatigue turning device (ZB-200; Chengdu Taimeng Software, Co., Ltd., Chengdu, China). The rotation speed was maintained at 20 rpm. Prior to assessment, each mouse was trained three times and during the subsequent assessment a stopwatch was used to record the total duration each mouse spent on the rod prior to falling.

*5-hydroxytryptophan (5-HTP)-induced head-twitch assessment*. A 5-HTP-induced head-twitch assessment (25) was used to investigate whether serotonergic mechanisms were involved in the MEPS-mediated antidepressant-like effects. The mice (n=10/group) were orally administered with either distilled water, MEPS (10, 50 or 250 mg/kg) or fluoxetine (10 mg/kg) for 1 week, respectively. At 30 min following the last administration, 5-HTP (100 mg/kg) was intraperitoneally administered to mice. Following administration, the mice were immediately placed into cages, and the cumulative number of head twitches during a 15 min period was recorded by two observers in a blinded-manner.

*Reserpine-induced hypothermia assessment*. The mice in each group, with the exception of the vehicle-treated control group, were injected intraperitoneally with 4.0 mg/kg reserpine 1 h following the 7-day MEPS (10, 50 or 250 mg/kg) and fluoxetine (10 mg/kg) treatments. After 2 h, the rectal temperatures of the mice were determined. At the end of experiment, the mice were sacrificed by administration of 20 mg/kg pentobarbital, and the hypothalamus was collected. Tissue samples (n=8) were homogenized with phosphate-buffered saline (PBS). Following centrifugation at 10,000 x g for 15 min at 4˚C, the level of monoamine neurotransmitters, including DA, NE, 5-HT and 5-HIAA, were detected using mouse ELISA kits for DA (cat. no. H170), 5-HT (cat. no. H104), NE (cat. no. H096) and 5-HIAA (cat. no. H104), from NanJing Biotechnology Co., Ltd. (NanJing, China). Briefly, 50 $\mu$l of the horseradish peroxidase (HRP)-linked target solution and 50 $\mu$l samples were added to the antibody-coated assay plate, which was incubated at room temperature for 1 h on a horizontal orbital plate shaker. The plate contents were then discarded and the wells were washed three times with 200 $\mu$l /well of 1X wash buffer. The solution was discarded, and 100 $\mu$l tetramethylbenzidine substrate was added to each well, and incubated for 10 min at room temperature. Following the addition of 100 $\mu$l STOP solution, the absorbance was measured at 450 nm within 30 min.

*Western blot analysis*. Sections of the collected hypothalamus samples were homogenized in radioimmunoprecipitation assay buffer (Sigma-Aldrich) containing 1% protease inhibitor cocktail (Sigma-Aldrich) (26). Protein concentrations were determined using the Bradford method (27), and 30 $\mu$g of the proteins were separated on an 10% SDS-PAGE gel and transferred electrophoretically onto nitrocellulose membranes (0.45 mm; Bio Basic, Inc., Markham, ON, Canada). The transferred membranes were blocked with 5% bovine serum albumin for 3 h at room temperature, followed by three washes with PBS. The membranes were then incubated with the following primary antibodies at 4˚C overnight, at a dilution of 1:500: DAT (rabbit anti-rat monoclonal; cat. no. sc-32258; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), TH (rabbit anti-rat monoclonal; cat. no. sc-14007 Santa Cruz

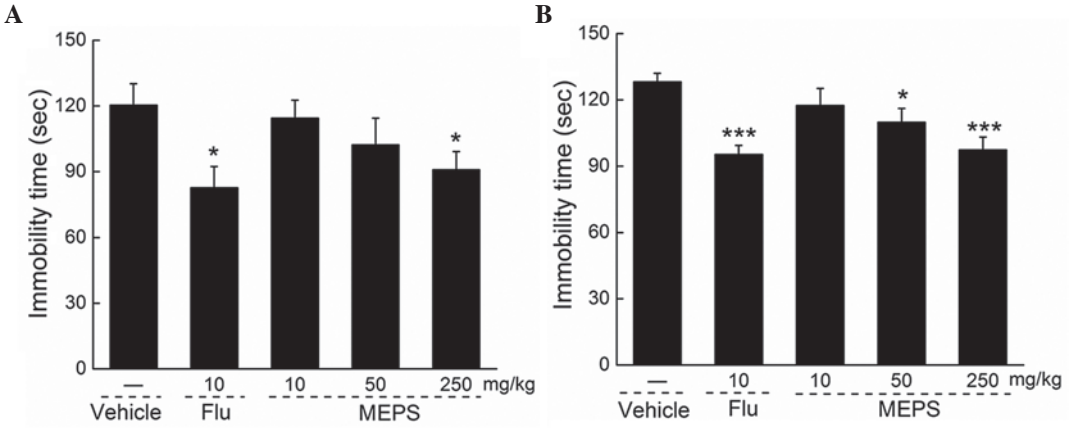


Figure 1. Effects of MEPS and Flu on the duration of immobility. The effects of MEPS (10, 50 and 250 mg/kg) or fluoxetine (10 mg/kg) on immobility duration were assessed in a (A) forced swimming test and (B) tail suspension test following treatment for 7 days. Data are expressed as the mean ± standard error of the mean (n=20) and were analyzed using one-way analysis of variance followed by Dunn's test. $^{*}$P<0.05 and $^{***}$P<0.001, vs. vehicle group. MEPS, *Marasmius* exopolysaccharide; Flu, fluoxetine.

Biotechnology, Inc.) and GAPDH (rabbit anti-rat monoclonal; cat. no. ABS16 (Merck Millipore, Darmstadt, Germany), followed by treatment with horseradish peroxidase-conjugated secondary antibodies (mouse anti-rabbit monoclonal; cat. no. sc-2357; Santa Cruz Biotechnology, Inc.) for 4 h at 4˚C. Chemiluminescence was detected using ECL detection kits (GE Healthcare Life Sciences; Chalfont, UK). The intensity of the bands were quantified by scanning densitometry using Quantity One-4.5.0 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

*Statistical analysis*. All values are expressed as the mean ± standard error of the mean One-way analysis of variance was use to detect statistical significance, followed by post-hoc multiple comparisons (Dunn's test). SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Acute toxicity assessment of MEPS*. Following 7 days of administration with MEPS (0.1, 1.0, 2.0, 4.0 and 6.0 g/kg) via gavage, none of the mice in any groups succumbed to mortality. The mice maintained a suitable state of growth, and few adverse effects were noted. In addition, no significant differences in body weight were observed among the groups (Table I). These data suggested that, at the doses selected in the present study, MEPS was deemed a safe agent for further investigation.

*Effects of MEPS on immobility duration in the FST and TST*. Compared with the vehicle-treated mice, intrastral (i.g.) MEPS (250 mg/kg) and fluoxetine (10 mg/kg) administration significantly reduced immobility duration in the FST (P<0.05; Fig. 1A). Similarly, MEPS (50 and 250 mg/kg and fluoxetine (10 mg/kg) treatment resulted in reductions in the duration of immobility in the TST (P<0.05; Fig. 1B).

*Effects of MEPS on animal behavior*. Compared with the vehicle-treated group, the administration of MEPS at all the selected doses significantly increased locomotor ability, not only horizontally (P<0.05; Fig. 2A), but also vertically (P<0.05; Fig. 2B). In addition, MEPS enhanced mouse coordination and balance, which was indicated by the increase in duration of retention on the Rota-Rod (P<0.01; Fig. 2C).

*Effects of MEPS in the 5-HTP-induced head-twitch assessment*. To investigate the possible involvement of serotonergic mechanisms in MEPS-mediated antidepressant-like effects, a 5-HTP-induced head-twitch assessment was performed. MEPS (250 mg/kg) and fluoxetine (10 mg/kg) increased the number of head twitches by ~56.27 and 108.63%, respectively, compared with the mice treated with 5-HTP (Sigma-Aldrich) alone (P<0.05; Fig. 3A). The administration of MEPS at lower doses of 10 and 50 mg/kg increased the numbers of head twitches by 21.88 and 39.92%, respectively.

*Effects of EPS on reserpine-induced hypothermia*. *T*he administration of 4.0 mg/kg reserpine significantly reduced rectal temperatures in the mouse model (P<0.001; Fig. 3B). The administration of MEPS at doses of 50 and 250 mg/kg, and of fluoxetine (10 mg/kg) significantly enhanced reserpine-reduced rectal temperature (P<0.001; Fig. 3B), with an increment magnitude of 33.7% in the 50 mg/kg MEPS group, 35.5% in the 250 mg/kg MEPS group and 35.6% in the fluoxetine group, compared with the group treated with reserpine alone.

*Effects of MEPS on neurotransmitters in reserpine-induced hypothermia*. The levels of neurotransmitters in the hypothalamic tissues were detected in the reserpine-induced hypothermia mouse model. Reserpine at dose of 4.0 mg/kg significantly reduced the levels of 5-HT, DA and NE, compared with the vehicle group. By contrast, a significant increase in the concentration of 5-HIAA was observed (P<0.05; Fig. 4). Similar to the effects of fluoxetine (10 mg/kg) treatment, the administration of MEPS at a dose of 250 mg/kg markedly elevated the levels of 5-HT and DA, and reduced the increased levels of 5-HIAA, compared with the reserpine-only group (P<0.05; Fig. 4A-C). Treatment with MEPS (250 mg/kg) for 7 days reversed the suppressive effect of reserpine on the level of NE (P<0.05; Fig. 4D).

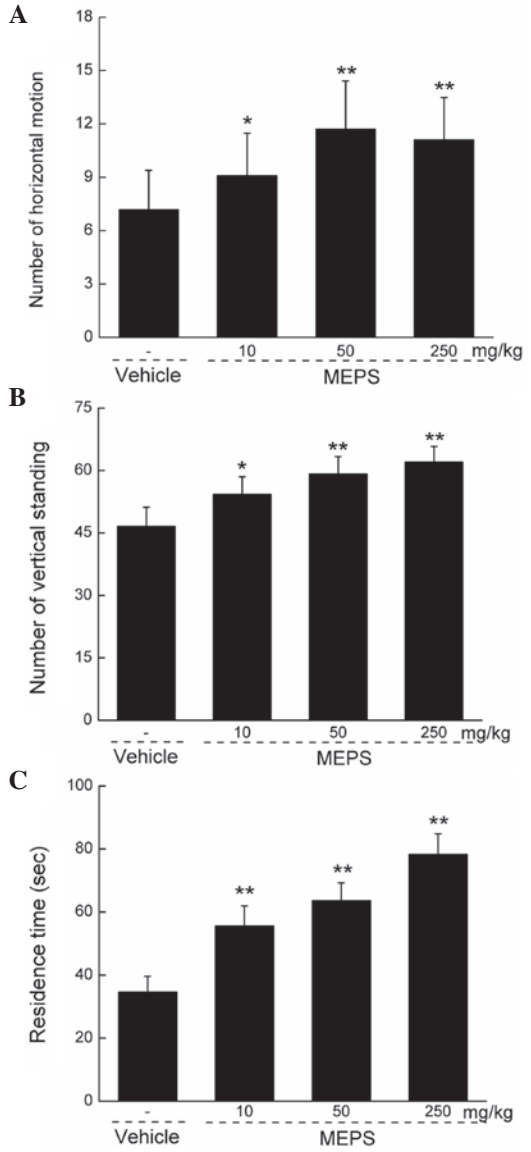


Figure 2. Effects of MEPS on animal behavior. The effects of treatment with MEPS (10, 50 and 250 mg/kg) on (A) spontaneous locomotor activity, (B) spontaneous standing and (C) residence time (duration of immobility) on a Rota rod were determined following 7 days treatment, respectively. Data are expressed as the mean ± standard error of the mean (n=10) and were analyzed using one-way analysis of variance followed by Dunn's test. $^{*}$P<0.05 and $^{**}$P<0.01, vs. vehicle group. MEPS, *Marasmius* exopolysaccharide.

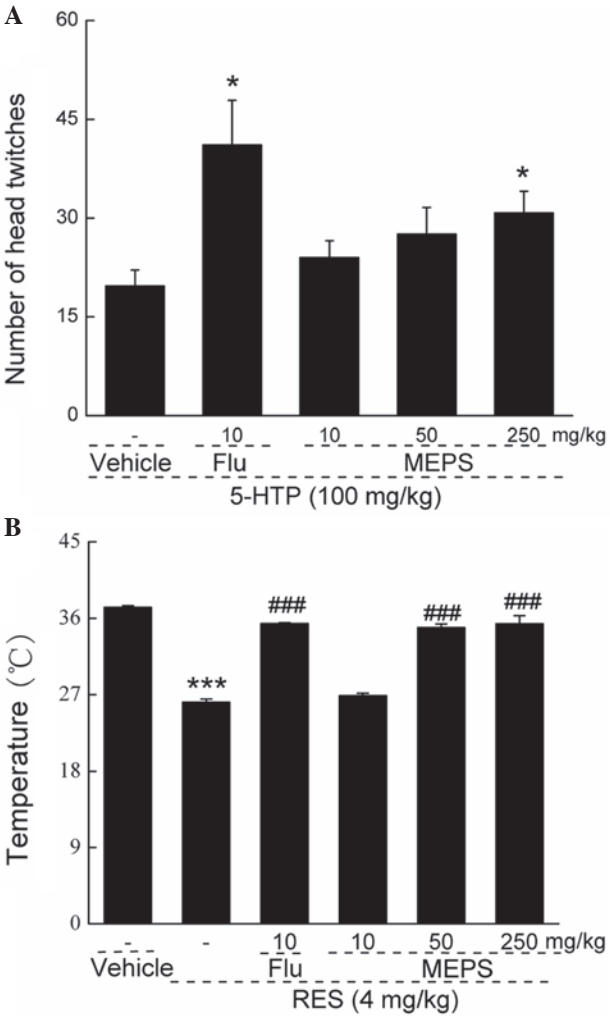


Figure 3. Effects of MEPS on RES-induced hypothermia. The administration of (A) Fluoxetine (10 mg/kg) and MEPS (250 mg/kg) for 7 days increased the number of head twitches on 5-HTP-induced head-twitch assessment. Rectal temperatures indicated that reatment with (B) fluoxetine (10 mg/kg) and EPS (50 and 250 mg/kg restored RES-induced hypothermia in the mouse model. Data are expressed as the mean ± standard error of the mean (n=10) and were analyzed using one-way analysis of variance followed by Dunn's test. $^{###}$P<0.001, vs. RES alone; $^{*}$P<0.05, and $^{***}$P<0.001, vs. vehicle. 5-HTP, MEPS, *Marasmius* exopolysaccharide; Flu, fluoxetine; RES, reserpine; 5-HT. 5-hydroxytryptamine.

*Effects of MEPS on the expression levels of DAT and TH in the hypothalamus*. The expression levels of DAT and TH in the hypothalamus in the reserpine-induced hypothermia mouse model were also determined. Reserpine (4.0 mg/kg) injection resulted in a 37.57% reduction in the expression of TH in the hypothalamus, compared with the vehicle-treated group (P<0.05; Fig. 5), however, no significant effect was detected in the level of DAT. Fluoxetine restored the abnormal expression of TH and increased the level of DAT (P<0.05; Fig. 5). Similarly, MEPS at doses between 10 and 250 mg/kg significantly suppressed the levels of DAT, between 76.90±3.88 and 50.43±5.51% (P<0.05; Fig. 5), compared with the reserpine-only group. MEPS treatment at 50 and 250 mg/kg for 7 days resulted in a 43.56 and 57.61% increase in the levels of TH, respectively (P<0.05; Fig. 5).

## Discussion

The safety of natural products has been an area of concern in the medical community and public (28). In the acute toxicity investigation performed in the present study, the highest non-toxic dose of MEPS selected for treatment of the male and female mice was >20-fold higher than its effective dose. Based on the results of the preliminary safety investigation, which revealed no significant adverse effects, the antidepressant-like effects of MEPS were assessed. Animal models of depression, including behavioral despair models (FST and TST) and drug-induced models (5-HTP-induced head-twitch assessment and antagonism of reserpine-induced hypothermia) are important in the scientific screening and evaluation of antidepressants (29,30). MEPS enhanced mouse locomotor activity following a 7-day administration period. In addition, 250 mg/kg MEPS treatment resulted in a reduction on the

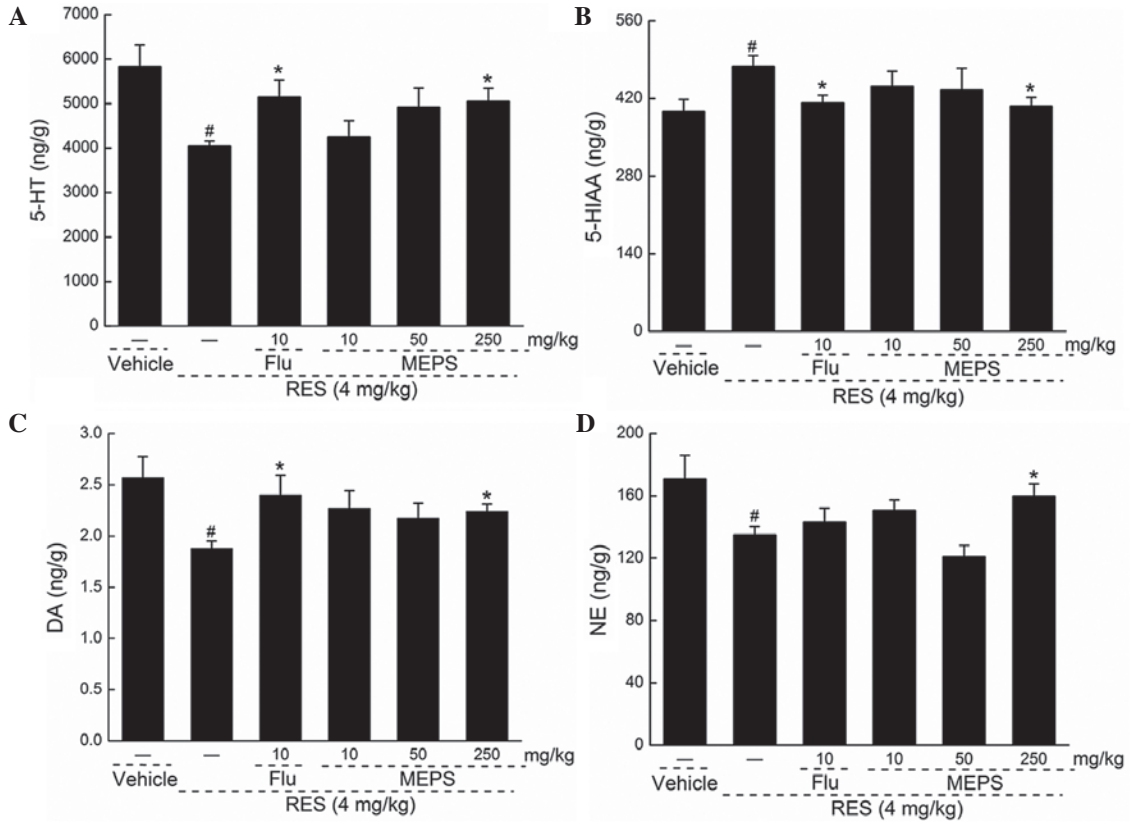


Figure 4. Effects of MEPS on neurotransmitters in reserpine-induced hypothermia. Following administration of MEPS (10, 50 and 250 mg/kg.) or fluoxetine (10 mg/kg) for 7 days, 4.0 mg/kg reserpine was injected intraperitoneally. The levels of (A) 5-HT, (B) 5-HIAA, (C) DA and (D) NE in the mouse hypothalamus were measured. Data are expressed as the mean ± standard error of the mean (n=8) and were analyzed using one-way analysis of variance. $^{\#}$P<0.05, vs. vehicle; $^{*}$P<0.05 and $^{**}$P<0.01, vs. RES alone. MEPS, *Marasmius* exopolysaccharide; Flu, fluoxetine; RES, reserpine; 5-HT, 5-hydroxytryptamine; 5-HIAA 5-hydroxyindoleacetic acid; DA, dopamine; NE, norepinephrine.

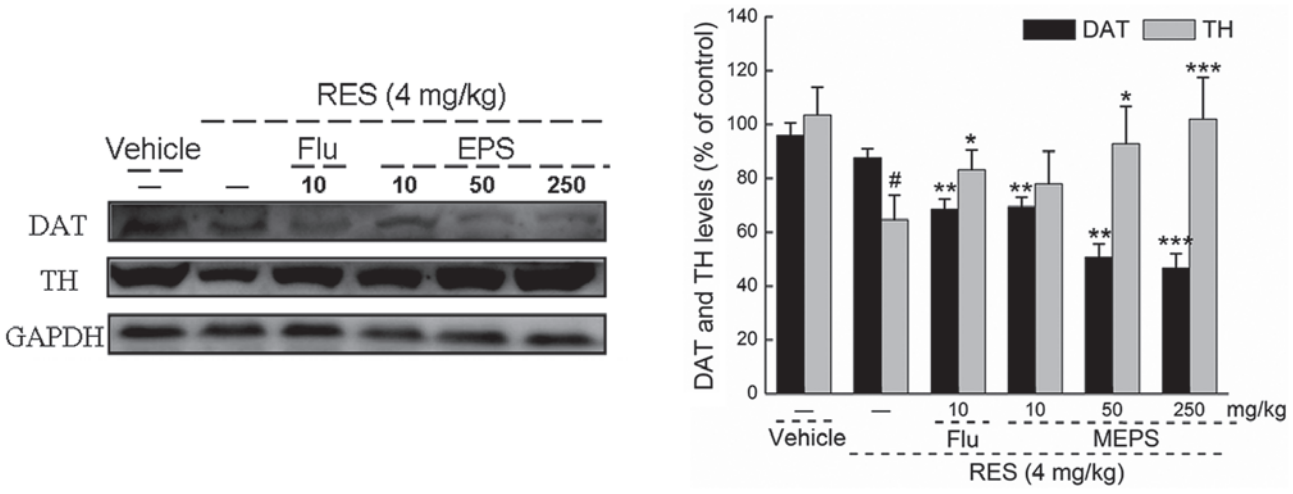


Figure 5. Effects of MEPS on the expression levels of DAT and TH in the hypothalamus. Following administration of MEPS (10, 50 and 250 mg/kg.) or fluoxetine (10 mg/kg) for 7 days, 4.0 mg/kg reserpine was injected intraperitoneally. The hypothalamus of the mice in each group were collected. Changes in the levels of TH and DAT in the hypothalamus were determined using western blot analysis. Quantifiied data were normalized by GAPDH, respectively. Data are expressed as the mean ± standard error of the mean (n=8) and were analyzed using one-way analysis of variance. $^{\#}$P<0.05, vs vehicle; $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001, vs. RES alone. MEPS, *Marasmius* exopolysaccharide; Flu, fluoxetine; RES, reserpine; DAT, dopamine transporter; TH, tyrosine hydroxylase.

duration of immobility in the FST and TST, suggesting its antidepressant activities. The 5-HTP-induced head-twitch and reserpine-induced hypothermia assessments were also used to confirm its effects.

As reported previously, SSRIs, including fluoxetine and paroxetine, can reduce immobility and increase swimming duration without affecting climbing (31). By contrast, selective NE or DA reuptake inhibitors, including desipramine or

maprotiline, reduce immobility and increase climbing, without altering swimming (32). In the FST and TST performed in the present study, no swimming or climbing durations were recorded, therefore, additional experiments are required to determine whether MEPS regulates the 5-HT system, the dopaminergic system or both.

5-HT receptor agonists induce a characteristic head-twitch response, the frequency of which is dose-dependent (33). Due to it is ease of quantification, this response provides an attractive animal model for the investigation of transmitter interactions with 5-HTergic mechanisms (34). 5-HTP is one type of amino acid, which acts as a precursor of 5-HT (35). In the 5-HTP-induced head-twitch assessment performed in the present study, MEPS (250 mg/kg) significantly increased the number of head twitches ($P<0.05$). Furthermore, similar to the effects of fluoxetine, MEPS administration enhanced the concentration of 5-HT in the hypothalamus. However, the present study did not determine the changes in the expression levels of 5-HT receptors in the mouse model. A previous clinical study suggested that platelet 5-HT content may serve as a supplementary biomarker for the function of uptake-associated mediators, including $5\text{-HT}_{1A}$ receptors, in drug-free depressed patients (36). Numerous studies involving animal models have confirmed that an increase in $5\text{-HT}_{1A}$ autoreceptor density in the dorsal raphe reduces and delays the therapeutic response to SSRIs (37,38). However, based on the results of the present study, it is difficult to conclude whether the MEPS-mediated antidepressant activity was associated with its regulation of the 5-HT system.

Reserpine is involved in the consumption of NE and 5-HT; 5-HIAA is the metabolic end product of 5-HT, and DA is the premise compound of NE (30). Based on these considerations, the levels of 5-HT, 5-HIAA, DA and NE in the hypothalamus were determined in the reserpine-induced hypothermia mouse model in the present study. Unlike fluoxetine, MEPS not only normalized the levels of 5-HT, 5-HIAA and DA; but it also reduced the hyperexpression of NE. These data suggested that the dopaminergic system may be involved in its antidepressant-like effect. To further confirm this hypothesis, the expression levels of DAT and TH in the mouse brain were analyzed. Selective noradrenaline re-uptake inhibitors and DAT inhibitors are important clinical antidepressants (39). The inhibition of DAT is considered a major mechanism underlying the therapeutic benefits of antihyperactivity medications, smoking cessation and antidepressants (40,41). In the present study, MEPS markedly suppressed the expression of DAT in the hypothalamus of the resperpine-treated group, indicating that MEPS exerted a similar effect as the DAT inhibitor. In addition, TH is reported to be an enzyme responsible for the catalysis of amino acid L-tyrosine into dihydroxyphenylalanine (42), which is the premise compound of DA, therefore, TH exerts a rate-limiting role for DA synthesis (43,44). In the present study, MEPS dose-dependently enhanced the expression of TH in the hypothalamus, which was consistent with the increase in the level of DA. Studies reporting results, which are consistent with the monoamine hypothesis have indicated that the majority of patients with depression have a deficit in brain DA and/or DA metabolites (45), and by increasing the expression levels of DA receptors or DA, the antidepressant enhances DA function (46).

Taken together, the results of the present study demonstrated that EPS produced during *Marasmius androsaceus* submerge fermentation exerts antidepressant-like effects, confirmed through use of a FWT, TST, 5-HTP-induced head-twitch assessment and reserpine-induced hypothermia assessment. MEPS regulated the concentration of neurotransmitters in the mouse hypothalamus. In addition, following MEPS administration for 7 days, the expression of DAT was suppressed; whereas the level of TH was enhanced. These results provide experimental evidence supporting the potential clinical use of MEPS as an effective agent against depression.

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