

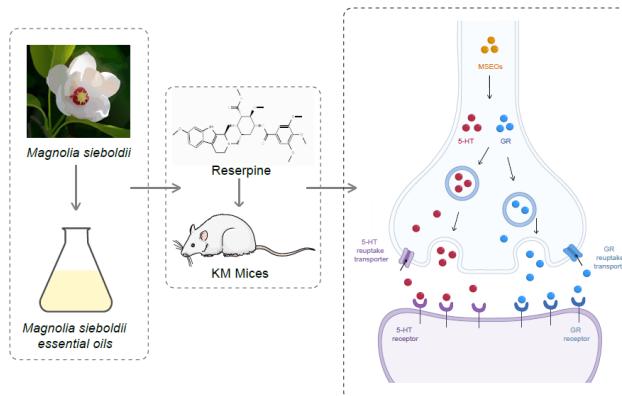
Volatile Oil from *Magnolia sieboldii* Alleviates Depression and Regulates Neurotransmitter Expression in Reserpine-Induced Depressed Mice

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Abstract: This study explores the antidepressant efficacy of *Magnolia sieboldii* essential oils (MSEOs) and their underlying mechanisms in a reserpine-induced depression model utilizing mice. The impact of varying MSEOs concentrations on depressive behaviors was evaluated through the forced swimming test. Neuronal cell counts in the cerebral cortex, hippocampus, and hypothalamus were determined using Nissl staining. The expression levels of critical proteins, including 5HT-1A, 5HT-2A, and GR, were examined via immunohistochemistry. Furthermore, RT-qPCR was employed to detect the mRNA expression levels of CRF and TrkB. The findings indicate that MSEOs, particularly at medium and high doses, significantly enhance the survival motivation of mice, increase the quantity of normal neurons in the cortex and hippocampus, and mitigate depressive symptoms. Additionally, MSEOs markedly upregulate the expression of 5HT-1A and notably increase TrkB and GR levels at medium and high doses. Compared to the control group, MSEO-treated groups demonstrate enhanced effects on neurotransmitter modulation and neuroprotection, especially at higher doses. In conclusion, MSEOs demonstrate therapeutic potential by enhancing survival motivation in mice, promoting neuronal proliferation in the brains of depressed mice, and upregulating the expression of 5HT-1A, 5HT-2A, GR, CRF, and TrkB proteins. These findings suggest MSEOs may serve as a promising candidate drug for depression treatment.



Keywords: *Magnolia sieboldii*; essential oils; serotonin; antidepressant

1 Introduction

Depression is a complex mental disorder characterized. Current treatments include pharmacotherapy, psychotherapy, and physical interventions. Pharmacotherapy primarily involves serotonin-norepinephrine reuptake inhibitors and selective serotonin reuptake inhibitors (SSRIs)^{1, 2)}, targeting serotonin pathways through receptors like 5-hydroxytryptamine 1A receptor (5HT-1A) and 5-hydroxytryptamine 2A receptor (5HT-2A)³⁾. Neurobiological markers such as glucocorticoid receptor (GR), corticotropin-releasing factor (CRF), and tropomyosin receptor kinase B (TrkB) are also critical in understanding depression^{4, 5)}. Reduced TrkB expression affects neuronal plasticity, while dysregulation of the HPA axis, reflected in altered CRF and glucocorticoid receptor (GR) levels, contributes to stress-related symptoms⁶⁾. Emerging research focuses on these biomarkers to develop more effective, personalized therapies, aiming to overcome the limitations of traditional treatments.

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Magnolia sieboldii K. Koch belongs to the family Magnoliaceae and is a deciduous shrub or small tree native to East Asia^{7, 8)}. It is primarily distributed in mountainous regions of northeastern and northern China, and in the provinces of Sichuan and Yunnan. *Magnolia sieboldii* has notable medicinal properties. Its roots, bark, and flower buds can all be used medicinally⁹⁾. The *Magnolia sieboldii* essential oil (MSEOs) contains various active ingredients. These active components demonstrate significant anti-inflammatory, antioxidant, and anti-tumor activities¹⁰⁾. Due to these bioactive components, *Magnolia sieboldii* is highly regarded in modern pharmacological research and holds considerable potential for a wide range of applications.

Previous studies have shown that the volatile oil of *Magnolia sieboldii* exhibits sleep-promoting effects in insomnia-induced mice, with its main active components and underlying mechanisms already identified. This study aims to investigate the antidepressant effects and underlying mechanisms of *Magnolia sieboldii* volatile oil in a mouse model of depression.

2 Experimental Procedures

2.1 Materials

The dried leaves of *Magnolia sieboldii* K. Koch, from the Magnoliaceae family, were obtained in a quantity of 5 kilograms from Tongrentang Company (Beijing, China). All plant samples were identified by Professor Liu Nian at Zhongkai University of Agricultural Engineering in Guangzhou, China. Some samples were preserved as voucher specimens (No. 014861) at the Institute of Natural Products and Green Chemistry, Guangdong University of Technology in Guangzhou, China. The dried leaves were crushed to a 20-mesh size and subjected to steam distillation using a reactor fitted with a mesh cover, with the distillation collection conducted over a period of 1 hour. Following distillation, the oil was allowed to separate from the water and was subsequently dried with anhydrous sodium sulfate. The volatile oil was analyzed using GC-MS (DSQ-II Ultra, Thermo Electron, USA) with a 30 m DB-5MS capillary column (0.25 μ m, Agilent, USA). The gas chromatography-mass spectrometry analysis adhered to established methodologies from prior studies. All chemicals used were of analytical grade.

$$\text{MSEOs yield (\%, w/w)} = \text{PMEOs obtained (g)/dried branches (g)} \times 100$$

2.2 GC-MS analysis

Essential oils were detected by a DSQ-II Ultra GC-MS (Thermo, USA) which was equipped with a 0.25 m long DB-5MS capillary column with 0.33- μ m film thickness (Agilent, USA). The heating procedure was set as the initial temperature of 100 °C, the maintenance time of 10 min, and then increased to 250 °C at a rate of 3 °C/min. The vaporization temperature is 250 °C. The injection volume was 1.0 μ L; the carrying current (HE) is 1 mL/min. The shunt ratio was 20:1. The bar of mass spectrometry: ionon source is electron blast; the temperature is 200 °C; the electron energy is 70 eV; the mass scanning range is m/z 30 ~ 550 amu. The GC-MS analysis followed the methodology of previous studies.

2.3 Experimental animals

Kunming male mice were purchased from Guangdong Medical Laboratory Animal Center (SCXK/20130002), and the mice were aged 5-6 weeks, weighing 20-35 g. Experimental mice were adaptively fed for a week under a temperature of 24 $\pm$ 1 °C and a 12 h light/dark cycle before experiments. This experiment is ethical, and all animal experiments have been approved by the Animal Experiment Ethics Committee.

2.4 Reserpine-induced depression model

Totally 24 male Kunming mice were stochastically divided into six groups, including MSEOs-L group, MSEOs-M group, MSEOs-H group, control group, reserpine group, and fluoxetine group, with four mice in each group. Filter paper cut into the same size, soaked in different volumes of essential oil solutions. Then filter paper was placed in four corners of the cage so that each mouse could sniff it for an hour. MSEOs-L, MSEOs-M, and MSEOs-H groups were administered with MSEOs at the doses of 25, 50, and 100 μ L every day. The fluoxetine treatment group received daily intraperitoneal injections of fluoxetine at a dose of 20 mg/kg. The weight of each mouse was recorded daily before inhalation. The cage where control group mice lived was furnished with filter paper soaked in normal saline containing 1 % Tween 80 for 1h. On the fourth day of the experiment, except for the control group mice injected with saline containing 1 % Tween 80 (PH7-8), the other group of mice was administered with reserpine (i.p) at 6 mg/kg. A Behavioral Test was carried out on the second day, namely the forced swimming test (FST). The schematic diagram illustrating the administration and testing time points in the experimental design of the FST model is shown in **Fig. 1**.

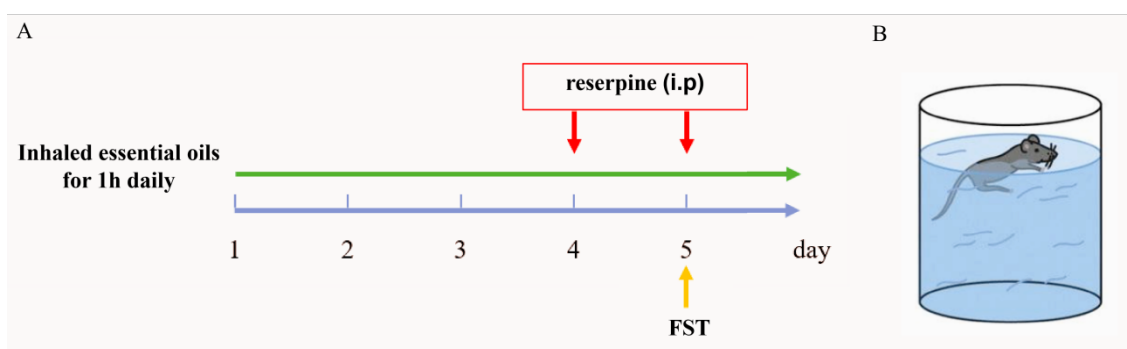


Fig. 1 (A) Experiment flowchart. (B) Experimental design forced swimming test.

2.5 Forced swimming test

Mice were forced to swim in a Glass cylindrical container (20 cm in height and 14 cm in diameter) for 6 min at 25 ± 1 °C. The glass beakers were separated by opaque partitions to prevent cross-contamination between mice. The experiment was conducted in a quiet environment with only one person responsible for video recording. Each mouse swam for 6 min, the first 2min was used for adaptation, and the last 4min was used for inactivity time. Afterwards, the mice were removed and dried. After 24 h, they were again forced to swim for 6 min. Each mouse was judged to be still when it stopped struggling and remained still in the water, or the limbs were slightly moving, and only the movements needed to keep its head above the surface were judged to be still.

2.6 Nissl staining assay

The embedded paraffin tissues were sliced at 5 μ m and soaked in xylene I for 10 min, xylene II for 10 min, 100 % ethanol for 5 min, 95 % ethanol for 5 min, 80 % ethanol for 5 min, and 75 % ethanol for 5 min. The section was stained with 0.1 % cresyl violet for 20 min and washed with distilled water. Paraffin sections were sequentially immersed in 70 % alcohol, 80 % alcohol, 95 % alcohol for 2 min, 100 % alcohol twice for 5 min, and xylene twice for 10 min. Finally, the paraffin section was sealed using resinene.

2.7 Immunohistochemistry assay

After the mice were killed, their brains were isolated on an ice pad. The remaining blood on the tissues was irrigated with saline solution and blotted with a filter paper. The brain samples were fixed in 4 % paraformaldehyde for 24 h. Brain tissue was embedded in paraffin, and sections were sliced at 4 μ m using a slicing machine (CUT 5062, Shanghai Asiagene Technology Co., Ltd, China). The slides were dried for 2 h at 60 °C. Paraffin sections were dewaxed using xylene I for 10 min, xylene II for 10 min, 100 % ethanol for 5 min, 95 % ethanol for 5 min, 80 % ethanol for 5 min, and 75 % ethanol for 5 min. Afterwards, the sections were washed in phosphate buffer saline (PBS) three times for 5 min. The slides were immersed in sodium citrate buffer (PH=6.0) at a high temperature for antigen repair. Subsequently, sections were incubated with 0.3 % hydrogen peroxide solution (H₂O₂) for 25 min at room temperature in the dark so that endogenous peroxide was blocked. After washing with PBS solution for 5 min three times, tissues were blocked using 1.5 % normal goat serum. Rabbit anti-serotonin (Incstar, Stillwater, MN; 1:15,000 dilution) was dipped and incubated at 37 °C for 2 h, followed by rabbit anti-serotonin transporter (Inc-star, 1:12,500 dilution) for 15 min, rabbit anti-tyrosine hydroxylase (Pel-Freez Biologicals; 1:5000 dilution) for 15 min. Color reaction was performed using 3, 3'-diaminobenzidine (DAB).

2.8 RT-QPCR analysis of depressed mice

After treatment of the model mice with the sample for 12 days, the hypothalamus and hippocampus were dissected from the skull and washed with saline. Following the wash, the samples were frozen with dry ice and stored at -80°C. A total of 50 mg of the cleaned hypothalamus and hippocampus was homogenized in Trizol for total RNA extraction. The total RNA was reverse-transcribed following the cDNA synthesis kit protocol. After transcription, cDNA was amplified using the primer sequences detailed in **Table 1**.

Table 1 Primer sequence for RT-qPCR.

Gene	Sequence of primer (5'→3')	
	Sense	Antisense
TrkB	AACGGAGACTACACCCTGATGG	GCAATCACCACCACGGCATA
CRF	AGCCCTTGAATTTCTTGCAGC	GCAGCGGGACTTCTGTTGAG

2.9 Statistical analysis

GraphPad Prism software (version 8.0.1, USA) was used for statistical analysis of the data. All the experimental results were presented as the mean $\pm$ standard deviation (SD). The statistical significance of the comparison results was determined via one-way analysis of variance (ANOVA) followed by Tukey multiple-comparison post hoc analysis (* $p < 0.05$, ** $p < 0.01$).

3 RESULTS

3.1 GC-MS analysis

Herein, the main volatile aromatic compounds and their main components of MSEOs were detected by GC-MS (**Table 2**). Of the essential oils, 43 effective compounds made up 92.12 %. The major compounds in MSEOs were acetic acid geranyl ester (18.96 %), α -Cubebene (8.60 %), α -cadinol (7.10 %), β -Elemene (5.19 %), and β -Farnesene (5.30 %).

Table 2 Composition and relative content of volatile components of the essential oils of *Magnolia sieboldii*.

No	Compounds ⁱ	RI ⁱⁱ	Exp. RI	Ref.	Relative content (%)
					<i>Magnolia essential oils</i>
1.	α -cubebene	1433.09	1351.00	a	8.60
2.	α -Bergamotene	1305.84	1545.00	b	5.19
3.	alpha-cadinol	1451.21	1653.00	c	5.03
4.	β -Elemene	1392.34	1396.00	d	7.10
5.	β -Farnesene	1427.29	1465.00	d	4.61
6.	Nerolidol	1567.37	1562.00	e	4.5
7.	trans-caryophyllene	1419.95	1422.00	d	3.78
8.	γ -cadinene	1523.69	1610.00	d	3.45
9.	camphene	887.20	946.00	d	3.05
10.	T-Muurolol	1643.81	1617.00	d	2.59
11.	α -Terpinene	1182.99	1182.00	f	2.12
12.	β -Bourbonene	1424.15	1392.00	d	2.11
13.	acetic acid geranyl ester	1193.06	1363.00	b	18.96
14.	α -bulnesene	1557.02	1508.00	h	1.9
15.	2-cyclopropylideneadamantane	1946.63	-	-	1.47
16.	1-(1,3a,4,5,6,7-Hexahydro-4-hydroxy-3,8-	1761.00	-	-	1.44

dimethylazulen-5-yl)ethanone					
17.	γ -Terpinene	1083.00	1055.00	d	1.38
18.	Linalool	1017.37	1098.00	c	1.31
19.	2,6-Dimethyl-2,4,6-octatriene	1039.82	1146.00	d	1.04
20.	1,8-cineole	1230.82	1048.00	d	1.04
21.	(z)-3,7-Dimethyl-1,3,6-octatriene	954.41	1051.00	d	0.98
22.	γ -Elemene	1080.81	1495.00	d	0.95
23.	Aromadendren	1422.67	-	-	0.92
24.	α -cardinene	1497.99	1515.00	b	0.83
25.	(+)-viridiflorol	1331.49	2110.00	h	0.77
26.	Epiglobulol	1845.25	1632.00	d	0.74
27.	Citronellyl acetate	1095.58	-	-	0.67
28.	Kauren-18-ol, acetate, (4beta)-	1505.54	1089.00	g	0.66
29.	terpene	1144.96	1513.00	c	0.54
30.	α -Gurjunene	2079.76	-	-	0.51
31.	α -cardinene	1537.58	-	-	0.51
32.	1,3-Cyclopentadiene,1,3-	1251.51	1567.00	h	0.41
33.	Caryophyllene oxide	1552.81	1636.00	i	0.4
34.	(+)-Spathulenol	1231.94	1582.00	j	0.62
35.	2-Octen-1-ol,3,7-dimethyl	1045.67	-	b	0.28
36.	2,3-dihydro-2,2,6-trimethylbenzaldehyde	1280.74	1173.00	b	0.27
37.	1-(benzyloxy)methyl-4-vinylbenzene	1339.24	1338.00	h	0.24
38.	α -Pinene	830.11	1372.00	b	0.24
39.	analytical standard	1716.19		b	0.23
40.	Chrysanthemylalcohol,mixtu	1527.37	-	b	0.2
41.	2-phenyl-2-ethylbutyric acid	1843.25	1594.00	k	0.18
42.	8,8-Dimethyl-9-methylene	1849.74	-	b	0.17
43.	β -ionone	2010.81	-	b	0.13
Total		92.12			

ⁱCompounds listed in the order of elution from a methyl silicone capillary column (30 m $\times$ 0.25 mm, 0.25 μ m film thickness).

ⁱⁱRetention indices (RIs) relative to those for n-alkanes (C6-C40) are in the same methyl silicone capillary column. a⁽¹¹⁾, b⁽¹²⁾, c⁽¹³⁾, d⁽¹⁰⁾, e⁽¹⁴⁾, f⁽¹⁵⁾, g⁽¹⁶⁾, h⁽¹⁷⁾, i⁽¹⁸⁾, j⁽¹⁹⁾, k⁽²⁰⁾

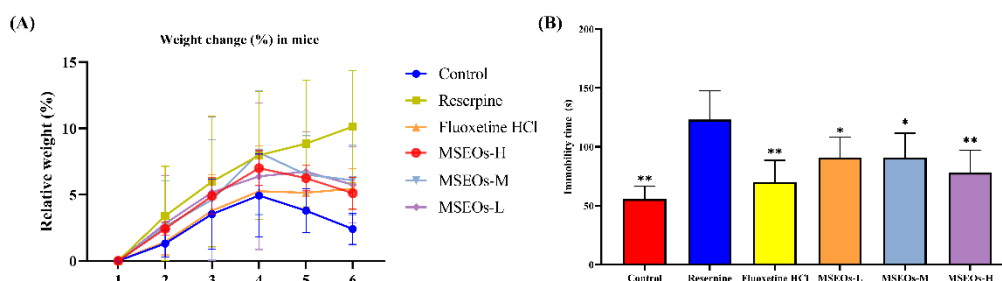


Fig. 2 (A) Body weight changes in mice. (B) FST immobility time changes in mice. Different from the reserpine group, * $p < 0.05$, ** $p < 0.01$. (n=4) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

3.2 MSEOs effects on FST immobility and weight gain in depression mice

The FST is an important animal model for assessing the effects of antidepressant drugs and researching the neurobiological basis of depression. It evaluates the impact of drugs on depressive symptoms by simulating behavioral despair. After constructing the depression mice model using reserpine, the statistical results are presented in **Fig. 2A**. From the data in **Fig. 2A**, the weight changes across different groups of mice exhibit distinct trends over time. The reserpine group demonstrates the most significant weight gain, along with a large standard error, indicating substantial fluctuation in weight gain within this group. The fluoxetine HCl group also demonstrates weight gain but with a very large standard error, indicating significant variability in weight. In contrast, the MSEOs-treated groups exhibit relatively stable weight gain across different doses, with the MSEOs-L and MSEOs-M groups showing more consistent weight gain and minimal fluctuations. The control group demonstrates the most stable weight gain, with minimal fluctuations. Therefore, the MSEOs-treated groups show improved weight management in the insomnia mice model. Although the MSEOs treatment group shows a more significant weight decrease compared to the reserpine group, its effectiveness is still slightly inferior to that of the fluoxetine HCl treatment group. However, MSEOs display a certain potential in improving weight gain associated with depression.

The data from **Fig. 2B** show that the reserpine group exhibits the longest immobility time in the FST, suggesting the most severe depression symptoms. In contrast, the immobility time in the fluoxetine HCl group is significantly shorter than that of the reserpine group, indicating that fluoxetine HCl effectively alleviates depression symptoms. Among the MSEOs-treated groups, the MSEOs-L group shows minimal reduction in immobility time. The MSEOs-M group exhibits a moderate reduction, and the MSEOs-H group shows a significant reduction, with immobility time approaching that of the control group. These results suggest that higher doses of MSEOs exhibit increased efficacy in treating depression. The control group maintains the lowest immobility time. Overall, the MSEOs-treated groups, particularly the high-dose group, show promising efficacy in relieving depression symptoms in the mouse depression model. The results of the FST more directly demonstrate the therapeutic effect of MSEOs on depression compared to the statistical results of body weight changes.

3.3 MSEOs effects on neuronal density in depression mice

The results presented in **Fig. 3** reveal significant differences in the structural organization of the cortex, hippocampus, and hypothalamus across the experimental groups. The reserpine group exhibits a reduced number of normal neurons, disorganized cellular arrangement, and substantial tissue damage, which correlate with the depressive symptoms observed in this group. In contrast, the fluoxetine HCl group demonstrates an increased number of normal neurons, suggesting that fluoxetine HCl effectively alleviates depressive symptoms and exerts neuroprotective effects. Among the MSEOs-treated groups, the MSEOs-L group exhibits minimal changes in neuronal density, the MSEOs-M group demonstrates a moderate increase in neuronal density, accompanied in contrast, the MSEOs-H group demonstrated the most significant increase in neuronal density, suggesting that higher doses of MSEOs are particularly effective in treating depression. The control group maintains stable neuronal density and intact tissue structure, with no observable changes in depressive symptoms. Overall, the MSEOs-treated groups, particularly the MSEOs-H group, demonstrate efficacy in enhancing neural structure and alleviating depressive symptoms. From the results of the Nissl staining, it can be observed that the number of neurons in the MSEOs treatment group is essentially the same as that in the fluoxetine HCl group across three regions, and both are higher than in the reserpine group. This indicates that MSEOs can effectively inhibit the reduction of neuronal numbers caused by depression.

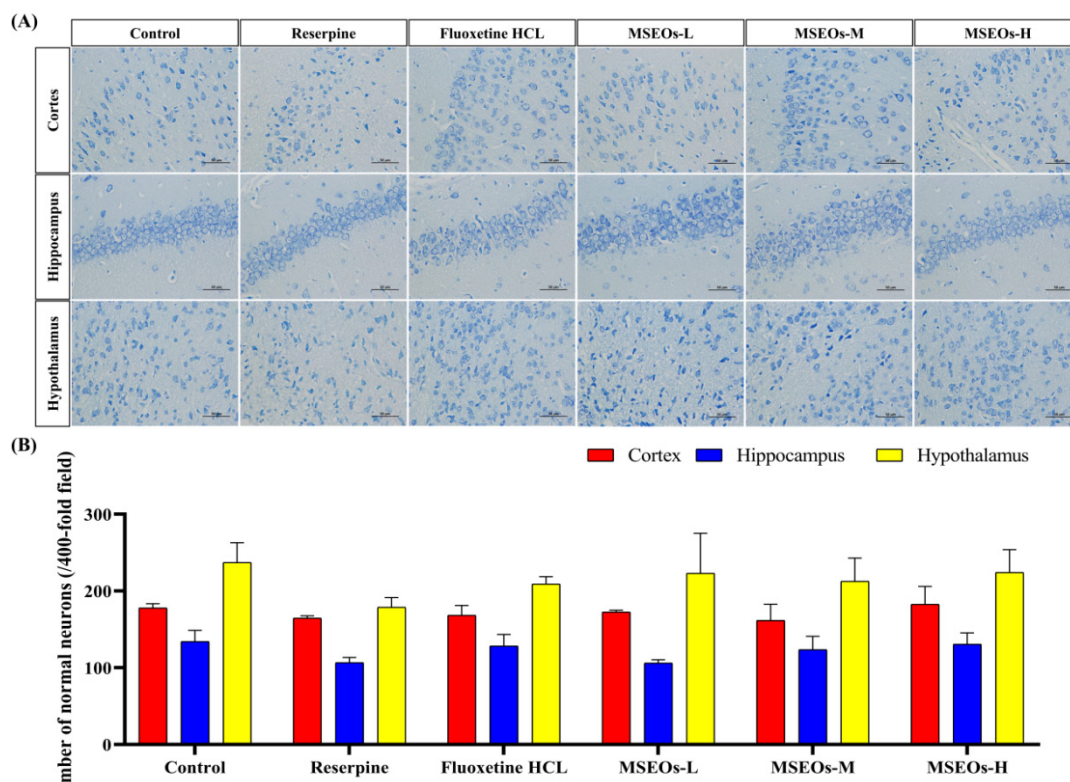


Fig. 3 Comparison of Nissl staining and the number of neurons in different brain tissues. (A) Nissl staining of different brain regions. (B) Comparison of neuron numbers. (400X magnification; n=4) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

3.4 Immunohistochemistry assay

The 5HT-1A receptor is widely distributed in neuronal regions, including the hippocampus, cerebral cortex, and hypothalamus²¹, and is closely related to the regulation of mood, anxiety, sleep, and stress responses²². In depression, 5HT-1A receptor function is often compromised, leading to impaired serotonin transmission, which exacerbates mood disturbances²³. As shown in **Fig. 4**, the Reserpine group demonstrates the lowest 5HT-1A expression, which exacerbates depressive symptoms. In contrast, the Fluoxetine HCL group shows increased 5HT-1A expression, effectively alleviating depressive symptoms. Among the MSEOs-treated groups, the MSEOs-H group shows a significant increase in 5HT-1A expression, suggesting that higher doses of MSEOs are particularly effective in treating depression. Overall, the MSEOs-treated groups, particularly the high-dose group, exhibit significant efficacy in enhancing 5HT-1A expression, alleviating depressive symptoms, and promoting neural system recovery. MSEOs may exert their antidepressant effects by increasing the release of 5HT-1A in the hippocampal region and cortex, thereby stimulating an increase in 5HT-1A receptor activity.

The 5HT-2A receptor is primarily distributed in regions such as the cerebral cortex, hippocampus²⁴, and limbic system, and is involved in regulating excitatory neurotransmission, mood, and wakefulness. Dysregulation of 5HT-2A receptors is often observed in patients with depression, where its overactivity is linked to increased excitatory neurotransmission and mood instability²⁵. This receptor is also implicated in anxiety, irritability, and insomnia, which are common symptoms of depression. Antidepressants, particularly SSRIs, and serotonin receptor antagonists help to modulate 5HT-2A receptor activity, thereby reducing neural excitability and improving mood²⁶. **Fig. 5** shows that the MSEOs groups display a dose-dependent effect, where 5HT-2A expression increases with dose. The reserpine group exhibits the lowest staining intensity. These findings suggest that 5HT-2A expression in the MSEOs groups increases with concentration, with the MSEOs-H group demonstrating the strongest effect, surpassing both the Control and Fluoxetine HCL groups. Therefore, the therapeutic mechanism of MSEOs for depression may involve promoting the substantial secretion of 5HT-2A in the cerebral cortex, hippocampus, and hypothalamic region to activate 5HT-2A receptors, thereby achieving the effect of treating depression.

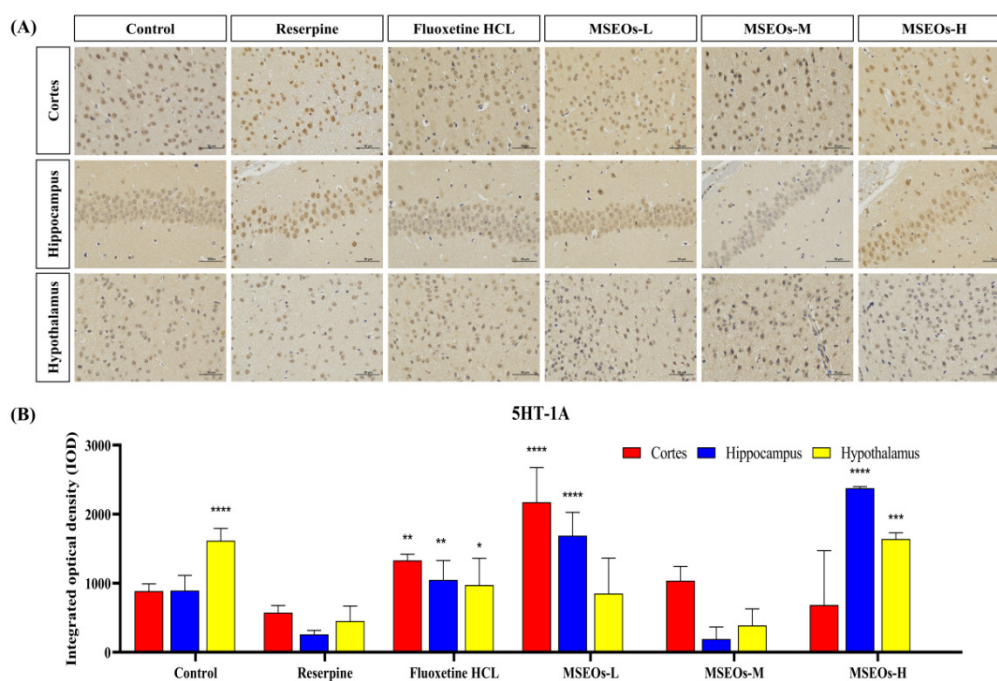


Fig. 4 Expression of 5HT-1A protein. (A) Expression of 5HT-1A proteins measured by immunofluorescence analysis. (B) Integrated optical density of different brain tissues. Different from the reserpine group, * $p < 0.05$, ** $p < 0.01$. (400X magnification; $n=4$) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

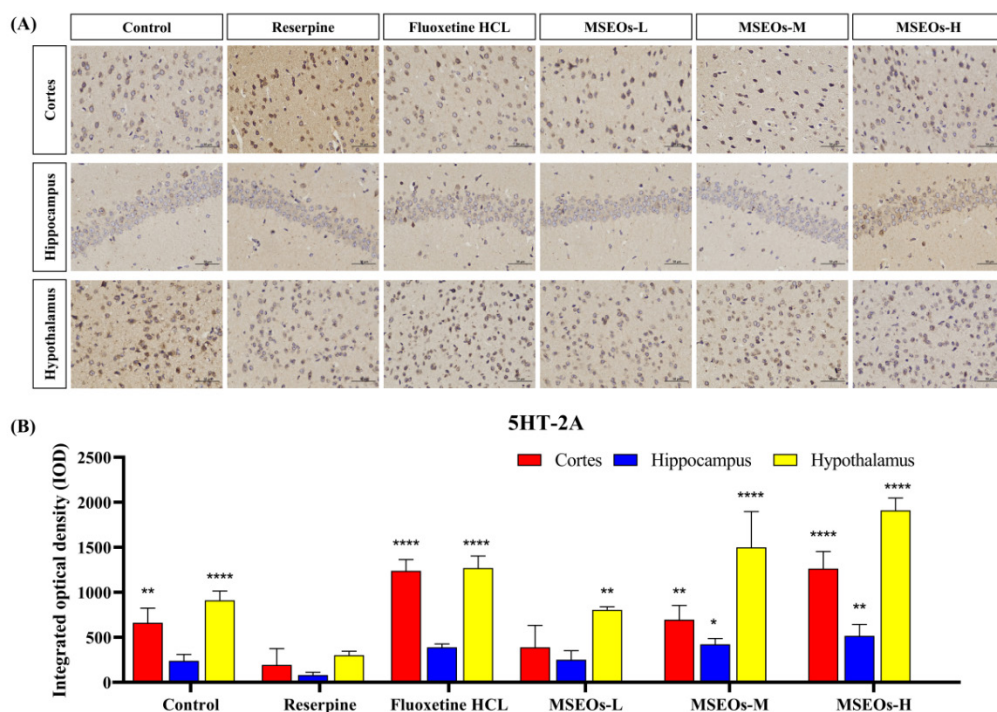


Fig. 5 Expression of 5HT-2A protein. (A) Image of 5HT-2A protein staining. (B) Integrated optical density of different brain tissues. Different from the reserpine group, * $p < 0.05$, ** $p < 0.01$. (400X magnification; $n=4$) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

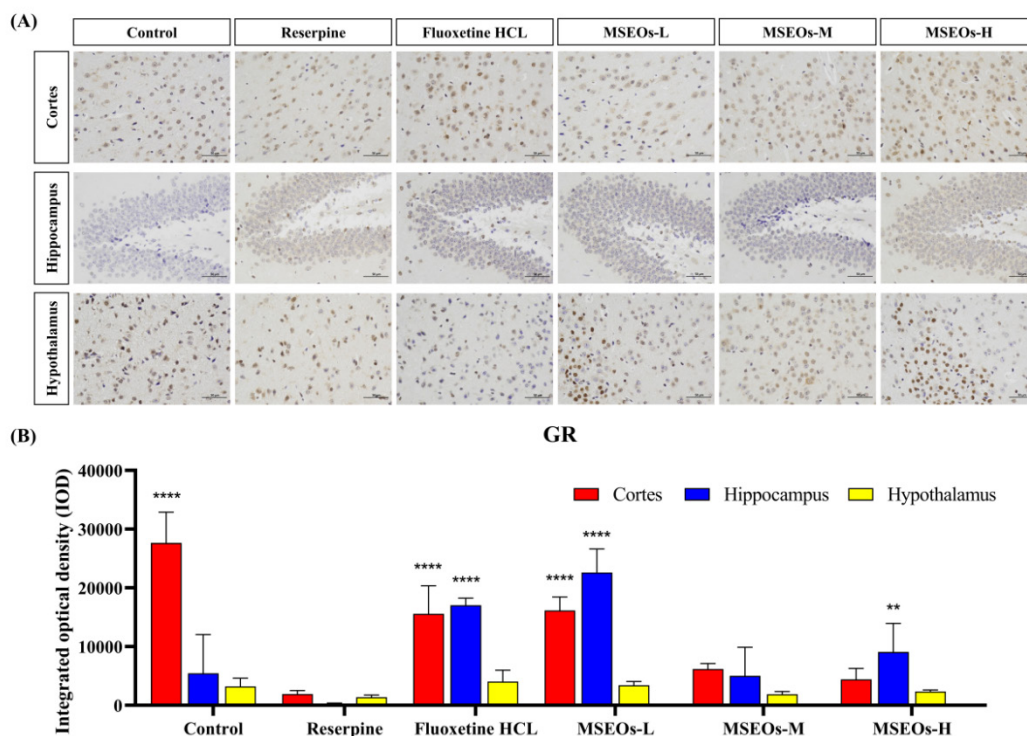


Fig. 6 Expression of the GR protein. (A) Image of GR protein staining. (B) Integrated optical density of different brain tissues. Different from the reserpine group, * $p < 0.05$, ** $p < 0.01$. (400X magnification; $n = 4$) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

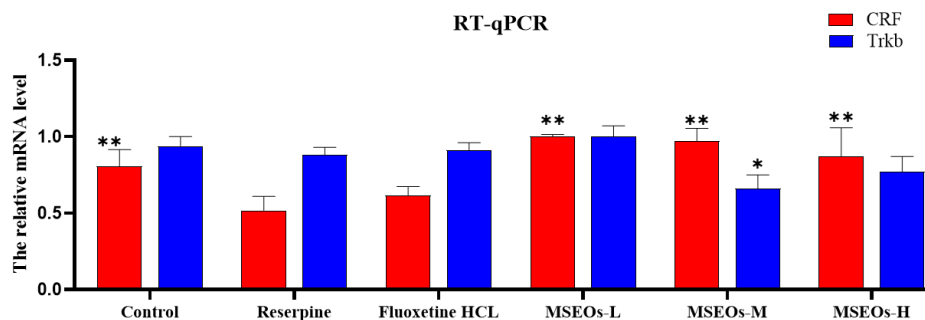


Fig. 7 Expression of the relative mRNA level by RT-qPCR. Different from the reserpine group, * $p < 0.05$, ** $p < 0.01$. ($n = 4$) MSEOs-L (25 μ L); MSEOs-M (50 μ L); MSEOs-H (100 μ L)

The GR plays a central role in the hypothalamic-pituitary-adrenal (HPA) axis, regulating stress responses²⁷. Under stress conditions, GR maintains homeostasis by modulating the release of cortisol. However, in patients with depression, the HPA axis is often dysregulated, with reduced GR sensitivity leading to excessive cortisol secretion, which exacerbates depressive symptoms and mood fluctuations²⁸. Abnormal GR function is closely linked to chronic stress responses and depression, making the restoration of GR function or the rebalancing of the HPA axis a key target in many antidepressant treatments. As shown in **Fig. 6**, the GR expression in all three regions of the MSEOs groups was significantly higher

than that in the reserpine group, indicating that MSEOs can restore GR function in depressed mice. Notably, the GR expression in the MSEO-L group was comparable to that in fluoxetine, suggesting that low-dose MSEOs have a similar effect on GR expression to the conventional antidepressant fluoxetine.

3.5 RT-QPCR analysis of depressed mice

RT-qPCR results (Fig. 7) showed no significant differences in TrkB expression across all groups, but the MSEOs-L treatment group exhibited higher TrkB expression than the reserpine group, indicating its capacity to restore depression-induced TrkB expression reduction. Compared with the Control group, the CRF mRNA level was significantly decreased in the reserpine group, indicating that the reserpine-induced depressive state suppresses CRF expression. In contrast, the CRF mRNA levels in all three MSEOs-treated groups were significantly higher than those in the reserpine group, suggesting that MSEOs can effectively reverse the inhibitory effect of reserpine on CRF expression. This may represent one of the important mechanisms by which MSEOs alleviate depressive symptoms.

4 Discussion

Depression is a common neuropsychiatric disorder characterized by immune dysregulation. This study investigated the antidepressant effects of MSEOs on a reserpine-induced mouse model of depression and explored its underlying mechanisms. The 5HT-1A receptor plays a crucial role in regulating mood, anxiety, sleep, and stress responses²³, while the 5HT-2A receptor is involved in modulating excitatory neurotransmission, mood, and arousal²⁶. MSEOs were found to significantly upregulate the expression of both 5HT-1A and 5HT-2A receptors, suggesting that they may alleviate depressive symptoms by enhancing serotonergic transmission through the upregulation of these receptors. Neuronal proliferation is a key mechanism of brain plasticity, closely associated with learning, memory, and emotional regulation²⁹. Nissl staining revealed that MSEOs increased the number of healthy neurons in the hippocampus, cortex, and hypothalamus. This indicates that MSEOs may improve cognitive function and relieve depressive symptoms by promoting neuronal proliferation. The HPA axis is the primary neuroendocrine system that regulates the stress response, and its dysfunction is closely linked to the pathogenesis of depression²⁷. MSEOs significantly upregulated the expression of GR and reversed the inhibitory effect of reserpine on CRF expression. This suggests that MSEOs may mitigate stress responses and emotional disorders in depressed patients by upregulating GR expression, thereby reducing cortisol levels. Overall, the experimental results demonstrated that the antidepressant effect of MSEOs was comparable to that of fluoxetine, suggesting its potential as an effective and safe alternative for treating depression. Fluoxetine, a commonly prescribed antidepressant, is associated with significant side effects and a delayed onset of action. In contrast, MSEOs, as a natural product, offer advantages such as fewer side effects, a faster onset of action, and convenient administration, making them a promising candidate for an ideal antidepressant therapy. Future research should further elucidate the molecular mechanisms of MSEOs' antidepressant effects, such as identifying the specific targets and signaling pathways of their active components. Furthermore, clinical trials are warranted to evaluate the safety and efficacy of MSEOs in humans.

In conclusion, this study demonstrates that MSEOs exert significant antidepressant effects. The underlying mechanisms may involve the modulation of the serotonergic signaling pathway, the promotion of neuronal proliferation, and the regulation of the HPA axis. As a natural product, MSEOs hold considerable potential for treating depression and could become an effective and safe alternative therapeutic agent.

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Author Contributions

Conceptualization, Writing review and editing, and Investigation, Tianchan Yun; Methods, Visualization and Resources, Yue Xiao; Validation, Supervising and Data management, Yanxian Lai and Shiya Huang; Formal analysis, Management and Writing and manuscript preparation, Yanmei Gong and Cong Deng. All authors have read and agreed to the published version of the manuscript.

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Clinical trial number

Not applicable

Institutional Review Board Statement

All animals in this study were from Southern Medical University (License number: SCXK (Guangdong) 2016-0041). All animal experiments were performed by the Guide for the Care and Use of Laboratory Animals of Guangdong University of Technology (Guangzhou, China) and approved by the Animal Ethics Committee of Guangdong University of Technology (Guangzhou, China) (approval documents: 20221015011).

Informed Consent Statement

Not applicable.

Data Availability Statement

All data used for the analyses in this report are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Consent to Publish declaration:

Not applicable.

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