Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

The Impact of Esketamine on Depression: Targeting Oxidative Stress and Neuronal Apoptosis Through BDNF/TrkB/PI3K/AKT Pathway Activation

Yueyi Zhang[1,*], Qianqian Cai[2,*], Lingshan Wang[3], Bei Zhang[4]

[1]Tianjin Medical College Pharmacy and Biotechnology Department, Tianjin, People's Republic of China; [2]The Rehabilitation Center of Tianjin Hospital in Tianjin, Tianjin, People's Republic of China; [3]Department of Human Resources, Tianjin Medical University General Hospital Airport Hospital, Tianjin, People's Republic of China; [4]Department of Pharmacy, Tianjin First Central Hospital, Tianjin, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yueyi Zhang, Pharmacy and Biotechnology Department, Tianjin Medical College, No. 1, Chenglindao Dongjuzi, Dongli District, Tianjin, People's Republic of China, Email zhangyueran93@163.com

Background & Aims: Esketamine, a promising treatment for treatment-resistant depression, has shown potential advantages over traditional antidepressants. However, its mechanisms remain unclear. This study explores how esketamine alleviates depressive behaviors through activation of the BDNF/TrkB/PI3K/AKT signaling pathway.

Methods: Using a Chronic Unpredictable Mild Stress (CUMS) rat model, behavioral assays (Sucrose Preference Test, Morris Water Maze Test) and histological analyses (HE and Nissl's staining) were performed. Esketamine (5 mg/kg) treatment was administered to evaluate its antidepressant effects, and the PI3K inhibitor, PI3K-IN-6, was used to investigate the role of the PI3K/AKT pathway in the underlying mechanism.

Results: Esketamine treatment improved depressive behaviors, enhanced neuronal structure, and reduced apoptosis and oxidative stress. These effects were linked to the activation of the BDNF/TrkB/PI3K/AKT pathway. PI3K-IN-6 reversed the effects, confirming the pathway's involvement.

Conclusion: Esketamine alleviates depressive behaviors by activating the BDNF/TrkB/PI3K/AKT signaling pathway, reducing oxidative stress and inhibiting neuronal apoptosis. These findings highlight the therapeutic potential of esketamine in treating depression, particularly in cases where traditional treatments fail.

Keywords: esketamine, BDNF/TrkB/PI3K/AKT, CUMS rats, depression, oxidative stress

Introduction

Depression, also known as major depressive disorder (MDD), is a mood disorder characterized by persistent sadness and a loss of interest in activities previously enjoyed.[1] This condition significantly affects emotions, thoughts, and behaviors, leading to a range of physical and emotional complications.[2] Common symptoms include feelings of sadness, hopelessness, irritability, and frequent outbursts of anger or frustration.[3] More than just a temporary mood disturbance, depression is a major mental health disorder that affects millions worldwide across various demographic groups.[4] The complex nature of its underlying mechanisms highlights the need for continued research to identify novel therapeutic strategies capable of addressing its multifaceted causes and alleviating its symptoms.

Recent studies suggest that, in addition to traditional antidepressant mechanisms, the activation of the brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB)/phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway holds significant promise in the treatment of depression.[5] This pathway plays a key role in regulating neuronal survival,[6] synaptic plasticity,[7] and neuroprotection,[8] and modulates cellular responses to various

Received: 21 October 2024
Accepted: 30 July 2025
Published: 25 August 2025

neurotrophic factors, including BDNF.[9] BDNF, a crucial neurotrophin for brain growth and function, primarily exerts its effects through the TrkB receptor.[10] TrkB activation triggers downstream signaling cascades, including the PI3K/AKT pathway,[11] which is vital for promoting cell survival,[12] differentiation,[13] and the prevention of apoptosis.[14]

The pathogenesis of depression is closely linked to oxidative stress and neuronal death, and targeting these factors may offer novel therapeutic avenues.[15] Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses,[16] can lead to neuronal damage and contribute to the development and progression of depressive disorders.[17] Excessive neuronal apoptosis, or programmed cell death, disrupts neural circuits[18] and exacerbates depressive symptoms.[19]

Esketamine, a specific enantiomer of ketamine,[20] has recently emerged as a promising treatment for treatment-resistant depression (TRD)[21] and MDD.[22] Esketamine, a novel antidepressant, exerts its effects by modulating glutamatergic signaling, particularly through the NMDA receptor.[23] Compared to other new antidepressants, esketamine stands out for its ability to enhance neuroplasticity and reduce neuronal death, offering a unique therapeutic approach.[24] A randomized, placebo-controlled study and meta-analysis by Vázquez et al demonstrated that esketamine is an effective adjunct to antidepressants for managing acute major depressive episodes.[25] Similarly, a meta-analysis by Bahji et al confirmed esketamine's efficacy, safety, and tolerability as a therapy for depression.[26] Furthermore, esketamine has been shown to increase levels of heterogeneous nuclear ribonucleoprotein K (hnRNPK), which regulates dendritic spine density and synaptic plasticity in MDD patients via the ERK-BDNF pathway.[27] Supporting these findings, Zhuang et al demonstrated that esketamine significantly improved molecular, behavioral, and electrophysiological characteristics of MDD in a mouse model.[28]

Moreover, the BDNF/TrkB/PI3K/AKT pathway does not act in isolation. This signaling cascade is interconnected with other important pathways involved in inflammation and mitochondrial function, which are also implicated in depression.[29] Chronic inflammation has been shown to exacerbate depression symptoms, with inflammatory cytokines influencing neuroplasticity and promoting neuronal death.[30] Additionally, mitochondrial dysfunction, often characterized by altered oxidative phosphorylation and increased ROS production, is increasingly recognized as a key player in the pathophysiology of depression.[31]

This study aims to examine the effects of esketamine on mood and neuroprotection, focusing on its role in reducing oxidative stress and preventing neuronal apoptosis through activation of the BDNF/TrkB/PI3K/AKT signaling pathway. By investigating these molecular mechanisms, the research seeks to deepen our understanding of depression and contribute to the development of more effective treatment strategies.

Materials and Methods

Animals and Chronic Unpredictable Mild Stress (CUMS) Model

The CUMS model was used to induce behavioral and biological changes in rats that closely mimic those observed in human depression. This model serves as an effective tool for studying the mechanisms underlying depression and for screening potential antidepressant agents. In this study, male Sprague-Dawley (SD) rats, aged 6 weeks and weighing between 180–220 g, were purchased from SPF (Beijing) Biotechnology Co., Ltd. (China) and allowed to acclimatize for one week prior to experimentation. Over a 42-day period, the rats were exposed to a series of unpredictable stressors, including water deprivation, food deprivation, 12 hours of nocturnal illumination, wet bedding, restraint, tail pinching, electrical shocks to the soles, swimming in ice water (4°C for 5 minutes), cage closure, heat stress, diurnal reversal, and braking.[32] To prevent anticipatory responses, no single stressor was applied consecutively, with each stressor being applied on average three times.

A total of 30 rats were randomly divided into five groups of six animals each: Normal, CUMS, Esketamine (E610187, Aladdin, China), PI3K-IN-6 (HY-101115, MedChemExpress, USA), and Esketamine+PI3K-IN-6. All groups except the Normal group were subjected to CUMS treatment. The experimental groups received intraperitoneal injections of either esketamine, PI3K-IN-6, or a combination of both, for 21 consecutive days at a dosage of 5 mg/kg. The Normal and CUMS groups were given placebo (normal saline) injections at the same time points.

Sample collection (Immediately after behavioral tests): Immediately after the behavioral tests, blood samples were collected through laparotomy by extracting 4 mL from the abdominal aorta of each rat. The blood was centrifuged at 3,000 rpm for 15 minutes at low temperature. The serum was stored at −80°C. For prefrontal cortex tissue collection, the brain was carefully extracted, and the prefrontal cortex was removed on ice, rinsed with pre-cooled saline, and divided into two segments. One part was fixed in 4% paraformaldehyde, and the other was stored at −80°C. This study was approved by the ethics committee of Tianjin Medical College Pharmacy and Biotechnology Department (Approval number: TJYX2309007). Animal experiments were performed in strict accordance with the Chinese national guidelines, including the Regulations for the Administration of Affairs Concerning Experimental Animals and the standard GB/T 14925–2001 (Laboratory Animal-Requirements of Environment and Housing Facilities). The guidelines for the care and use of experimental animals refer to previous literature.[33]

Behavioral Tests

Behavioral testing commenced on the 21st day after model establishment. Sucrose preference test: Three days prior to the test, the rats were acclimatized to drinking a 1% glucose solution. Two drinking bottles were placed in each cage—one containing the glucose solution and the other containing purified water, with equal volumes in each bottle. The positions of the bottles were alternated every 6 hours over a 24-hour period. The sucrose preference for each group was calculated using the following formula: Sucrose preference = 1% sucrose intake/(sucrose intake + pure water intake).

Morris water maze test: The Morris water maze test was specifically designed to assess spatial memory in rodents. This test was a widely recognized behavioral paradigm aimed at evaluating the animal's ability to remember the platform location using spatial cues, where the animal had to find a hidden platform in a water maze. The Morris water maze test assessed the rats' learning and memory. Starting on day 21, rats were placed in the water (facing the wall) and allowed to search for a submerged platform. The time taken to find the platform (within a maximum of 2 minutes) was recorded. If the rat failed to find the platform, a time of 2 minutes was recorded, and the rat was guided to the platform, where it remained for 30 seconds. The training phase lasted one week, with four trials per day, each separated by 30-minute intervals. In the formal testing phase, the platform was removed, and the rats were placed in the pool at predetermined entry points. The following parameters were recorded: platform latency (in seconds), the number of entries into the platform area, and the percentage of time spent in the platform quadrant.[34]

Western Blotting (WB)

The cells were lysed with radioimmunoprecipitation assay buffer (Beyotime, Shanghai, China), and total protein was measured using a bicinchoninic acid protein assay kit (7780S, Cell Signaling, Danvers, MA, USA). The proteins were separated by 10% SDS-PAGE gel (P0012A, Beyotime, Shanghai, China) and transferred to a polyvinylidene fluoride (PVDF) membrane (ab133411, Abcam, UK). Membranes were blocked and incubated with primary antibodies against β-actin (1:10000, AC004, ABclonal), Caspase-3 (1:1000, A2156, ABclonal), Caspase-9 (1:1000, A2636, ABclonal), BDNF (1:500, A11028, ABclonal), phosphorylated-TrkB (p-TrkB; 1:500, C50F3, Cell Signaling), TrkB (1:1000, 80878-6-RR, Proteintech), PI3K (1:1000, 4292S, Cell Signaling), AKT (1:500, A11016, ABclonal), phosphorylated-AKT (p-AKT; 1:2000, AP0637, ABclonal), GSK-3β (1:1000, A2081, ABclonal), and phosphorylated-GSK-3β (p-GSK-3β; 1:1000, 5558S, Cell Signaling) overnight at 4°C. The membranes were then treated the next day with an HRP-conjugated secondary antibody (1:5000, SA00001-1, SA00001-2, Proteintech, Wuhan, China). Ultimately, protein bands were visualized using a chemiluminescent substrate and quantified with ImageJ software (National Institutes of Health, USA). Using β-actin as an internal control, the relative expression levels of the proteins were measured.

TUNEL

Following the sacrifice of the animals, the tissues were fixed in 4% formalin for 24 hours before being embedded and sliced (5 μm). Following that, the sections and apoptotic cells were identified using a TUNEL assay kit (NBP2-31164, Novus Biologicals, USA) as directed by the manufacturer. An optical microscope was applied to acquire pictures of TUNEL staining in various fields. TUNEL-positive cells were identified using Image J software (V1.8.0.112, NIH,

Madison, WI, USA). To calculate the apoptosis rate, the number of apoptotic cells and all cells were counted (apoptosis rate = number of TUNEL positive cells/total number of cells × 100%).

Biochemical Analysis

Levels of GSH (S0057S, Beyotime), SOD (S0101S, Beyotime), and MDA (S0131S, Beyotime) were quantified using corresponding Assay Kit, as recommended by the manufacturer. Measure absorbance at 540 nm using a microplate reader (VLBLATGD2, ThermoFisher Scientific, Waltham, USA) to determine concentrations.

Nissl's Staining

Tissue sections were processed, dehydrated, embedded, and sectioned. Nissl staining was performed following the manufacturer's instructions. The number of neurons was quantified using a light microscope (DM2500; Leica Microsystems, Germany).

HE Staining

Tissues were fixed in 4% paraformaldehyde, dehydrated in a graded alcohol series, cleared in xylene, and embedded in paraffin. After deparaffinization and rehydration, tissues were stained with Hematoxylin and Eosin according to the manufacturer's instructions (C0105S, Beyotime, China). Pathological changes in tissues were observed and photographed under a light microscope. Five random fields were captured per slide, and image backgrounds were optimized based on threshold intensity across all groups.

Statistical Analysis

Statistical analyses were performed using Prism 9 software (GraphPad, USA). Data are presented as mean ± SD (Standard Deviation). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for comparisons among three or more groups. A p-value of < 0.05 was considered statistically significant. The sample size (n=6 per group) was determined based on a priori power analysis using GraphPad Prism, with the following parameters: the anticipated effect size estimated from preliminary data, significance level (α) = 0.05, and statistical power ($1-\beta$) = 0.8. The analysis indicated that a sample size of six animals per group was sufficient to detect statistically meaningful differences while adhering to ethical principles of minimizing animal use.

Results

Esketamine Modifies Behavioral Effects in CUMS Rats

Behavioral testing revealed that CUMS rats exhibited a significant reduction in sucrose preference compared to the control group, which was partially reversed by esketamine treatment. However, the therapeutic effects of esketamine were negated by PI3K-IN-6 (Figure 1A). Similarly, in the Morris water maze test, CUMS rats demonstrated significantly longer latencies, fewer platform crossings, and less time spent in the platform quadrant, but these impairments were alleviated by esketamine treatment, and reversed upon PI3K-IN-6 administration (Figure 1B–E). These findings suggest that esketamine has potential antidepressant effects in the CUMS rat model.

Effect of Esketamine on BDNF/TrkB/PI3K/AKT Pathway and GSK-3β Phosphorylation in the Hippocampus

In CUMS rats, BDNF expression was significantly decreased, but esketamine treatment restored BDNF levels without affecting the PI3K-IN-6 intervention (Figure 2A–F). The expression of total TrkB remained relatively stable across all experimental conditions; however, TrkB phosphorylation was diminished in the CUMS group. This reduction was reversed after esketamine administration, with PI3K-IN-6 not influencing the therapeutic effect of esketamine (Figure 2A–F). Furthermore, in the CUMS group, the levels of PI3K and phosphorylated AKT (p-AKT) were significantly decreased. Esketamine treatment restored the levels of both PI3K and p-AKT; however, this recovery was significantly antagonized by subsequent PI3K-IN-6 administration (Figure 2A–F). Phosphorylated-GSK-3β (p-GSK-3β)

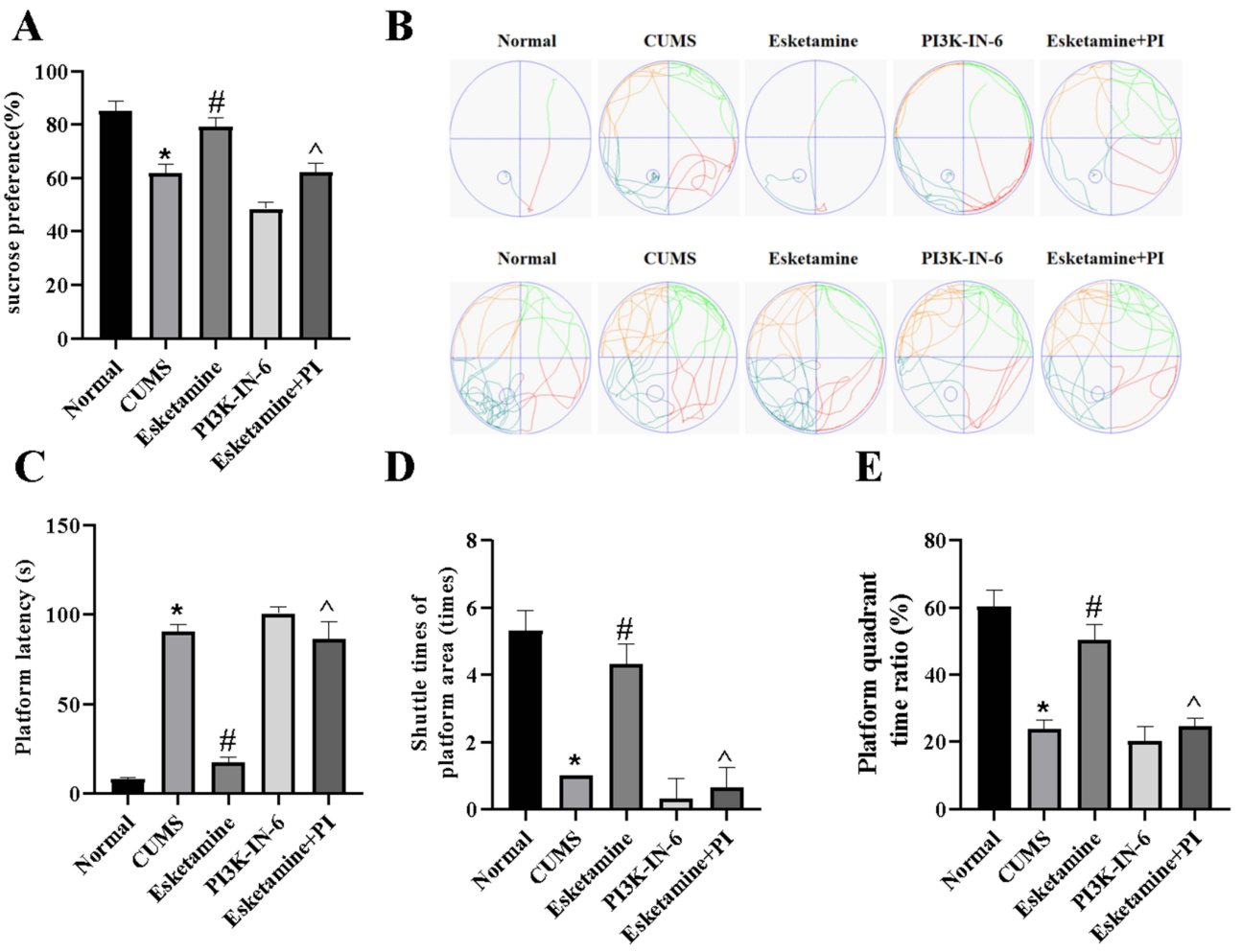

Figure 1 Esketamine modifies behavioural effects in CUMS rats. (**A**) Sugar water preference test: Used to assess anhedonia by measuring the preference for sugar water over regular water; (**B-E**) Morris water maze test (**B**) for the platform latency (**C**), shuttle times of platform area (**D**) and platform quadrant time ratio (**E**). *$p < 0.05$ vs the Normal group, #$p < 0.05$ vs the CUMS group, and ^$p < 0.05$ vs the Esketamine group. Results are shown as mean ± SD. N=6.

is the inactive form of GSK-3β, and in the CUMS group, levels of p-GSK-3β were decreased. Esketamine treatment restored p-GSK-3β levels, but this recovery was significantly reversed following PI3K-IN-6 treatment (Figure 2A–F). In summary, our findings demonstrate that esketamine exerts its therapeutic effects on CUMS by activating the BDNF/TrkB/PI3K/AKT signaling pathway, elucidating the upstream and downstream relationships among TrkB, PI3K, and AKT.

Esketamine Inhibits Oxidative Stress and Improves Histopathological Morphology in the Prefrontal Cortex

Oxidative stress plays a key role in the pathophysiology of depression, and we observed significant reductions in the levels of antioxidant markers in CUMS rats. Specifically, GSH levels (Figure 3A) and SOD activity (Figure 3B) were significantly lower in the CUMS group compared to controls, reflecting heightened oxidative stress. In contrast, the concentration of MDA, a marker of lipid peroxidation, was markedly elevated (Figure 3C). Esketamine treatment successfully restored GSH levels and increased SOD activity, while reducing MDA levels, thus mitigating oxidative stress. However, the therapeutic effect of esketamine on oxidative stress markers was abolished following the administration of PI3K-IN-6, indicating that the activation of the BDNF/TrkB/PI3K/AKT signaling pathway is essential for its antioxidative action.

https://doi.org/10.2147/NDT.S502090

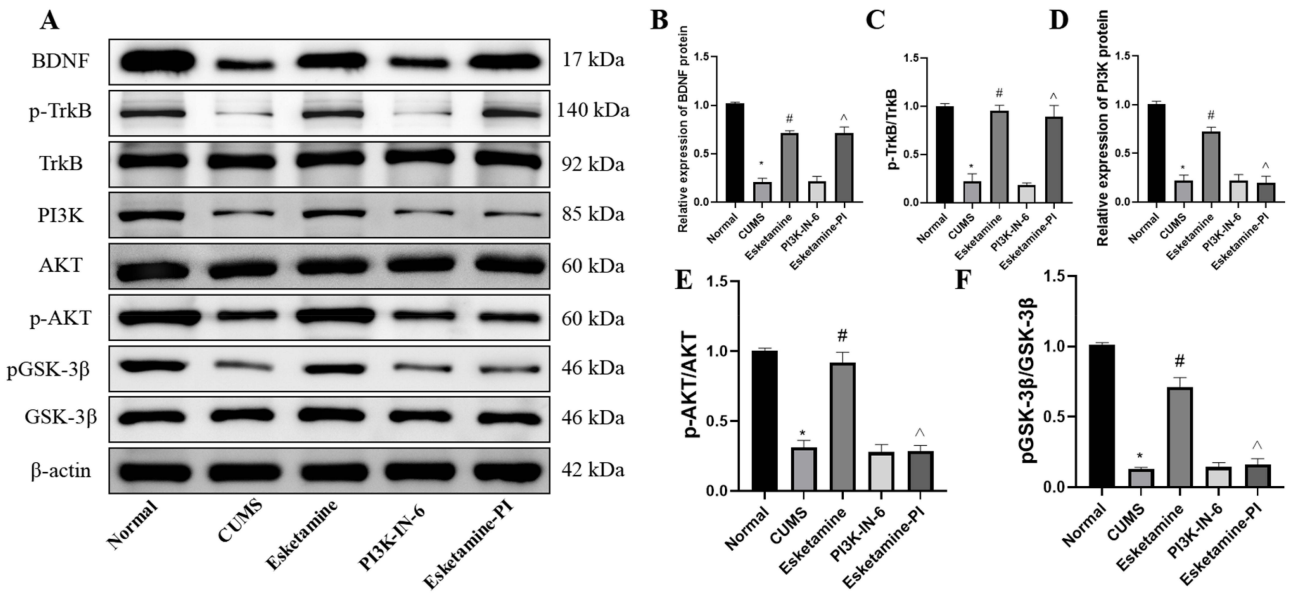

Figure 2 Effect of Esketamine on BDNF/TrkB/PI3K/AKT Pathway and GSK-3β Phosphorylation in the Hippocampus. (**A**) WB bands: Shows the Western blot band images for BDNF, p-TrkB/TrkB, PI3K, p-AKT/AKT, and p-GSK-3β/GSK-3β. (**B**) Relative expression of BDNF: Presents the statistical graph of the relative expression of BDNF protein. (**C**) Relative expression of p-TrkB/TrkB: Presents the statistical graph of the relative expression of p-TrkB/TrkB protein. (**D**) Relative expression of PI3K: Presents the statistical graph of the relative expression of PI3K protein. (**E**) Relative expression of p-AKT/AKT: Presents the statistical graph of the relative expression of p-AKT/AKT protein. (**F**) Relative expression of p-GSK-3β/GSK-3β: Presents the statistical graph of the relative expression of p-GSK-3β/GSK-3β protein. *$p < 0.05$ vs the Normal group and #$p < 0.05$ vs the CUMS group, and ^$p < 0.05$ vs the Esketamine group. Results are shown as mean ± SD. N=6. The experiments were performed in triplicate.

Histopathological examination of the prefrontal cortex using HE staining (Figure 3D) and Nissl staining (Figure 3E) revealed significant neuronal damage in the CUMS group. Neurons exhibited necrosis, reduced density, and disorganized arrangements, with diminished Nissl bodies. Following esketamine administration, neuronal morphology was improved, with an increase in neuronal density and a more symmetrical arrangement of cells, accompanied by visible Nissl bodies in the cytoplasm. These histopathological improvements were reversed by PI3K-IN-6, further supporting the role of the BDNF/TrkB/PI3K/AKT signaling pathway in mediating the therapeutic effects of esketamine.

Esketamine Reduces Neuronal Apoptosis and Modulates Apoptotic Pathway Proteins by Activating the BDNF/TrkB/PI3K/AKT Pathway in CUMS Rats

Increased neuronal apoptosis is a hallmark of depression, and our data indicate that CUMS rats showed a significant increase in TUNEL-positive cells, indicative of DNA fragmentation (Figure 4A and B). Additionally, the expression levels of the key apoptotic proteins, caspase-9 and caspase-3, were significantly elevated in the CUMS group (Figure 4C–E). Esketamine treatment reversed these alterations, significantly reducing the number of TUNEL-positive cells and the expression of apoptotic proteins, suggesting its potential to inhibit neuronal apoptosis. The protective effects of esketamine on apoptosis were reversed by PI3K-IN-6, suggesting that the beneficial effects of esketamine on apoptosis are mediated through the activation of the BDNF/TrkB/PI3K/AKT signaling pathway. These findings demonstrate that esketamine exerts its antidepressant effects, at least in part, by reducing apoptosis in neuronal cells, through modulation of apoptotic proteins such as caspase-9 and caspase-3.

Discussion

In this study, we investigated the potential antidepressant effects of esketamine in a CUMS rat model of depression. Our results indicate that esketamine exerts its antidepressant effects by modulating the BDNF/TrkB/PI3K/AKT signaling pathway, which plays a critical role in enhancing neuroplasticity, providing neuroprotection, regulating oxidative stress, and inhibiting apoptotic pathways. By binding to TrkB receptors, BDNF regulates neuroplasticity and stimulates various downstream signaling pathways, including the PI3K/AKT pathway.[5] This pathway is vital for regulating critical

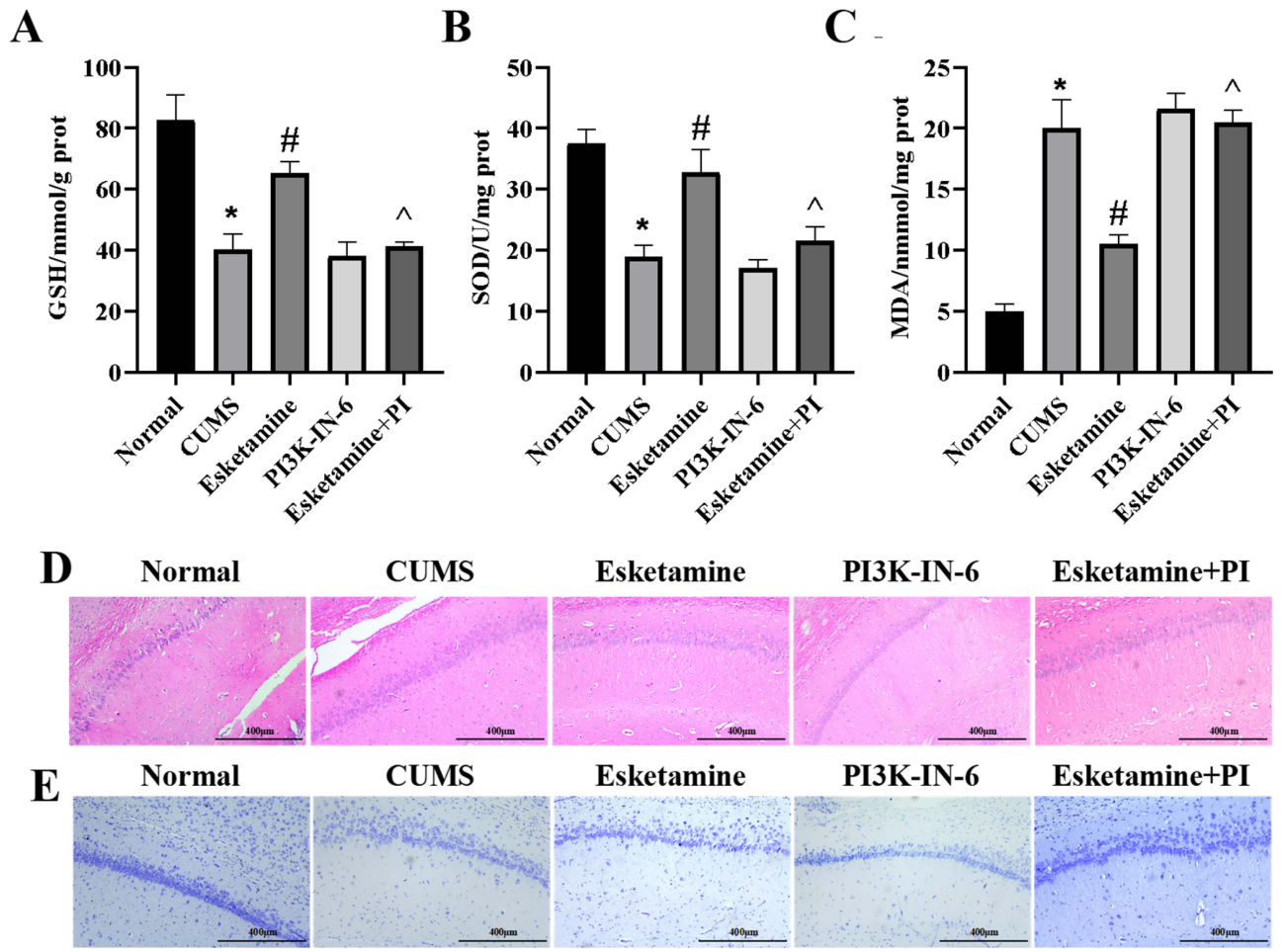

Figure 3 Esketamine Inhibits Oxidative Stress and Improves Histopathological Morphology in the Prefrontal Cortex. (**A**) shows the GSH levels in each group, (**B**) represents the SOD activity in each group, and (**C**) illustrates the MDA levels. (**D**) presents HE staining of tissue sections with a magnification of 100×, and (**E**) shows Nissl staining of tissue sections with a magnification of 100×. *$p < 0.05$ vs the Normal group, #$p < 0.05$ vs the CUMS group, and ^$p < 0.05$ vs the Esketamine group. Results are shown as mean ± SD. N=6.

biological activities such as cell survival, proliferation, differentiation, and metabolism.[35,36] An increasing body of research has conclusively demonstrated that BDNF-TrkB signaling is involved in the mechanisms underlying antidepressant efficacy in animal models.[37] Clinical investigations have also linked depression to lower levels of BDNF and its receptor TrkB in the brain.[38] Furthermore, the BDNF-PI3K/AKT signaling pathway has been shown to regulate neuronal growth, survival, and proliferation in the hippocampus, thereby influencing the development of depressive disorders.[39–42] Our findings imply that esketamine stimulates the BDNF/TrkB/PI3K/AKT signaling pathway and exerts a corrective impact on the activity of GSK-3β, a downstream target of the PI3K/AKT pathway that can induce neuronal damage when activated.[43] Notably, GSK-3β activity can be inhibited by the PI3K/AKT pathway through phosphorylation.[44]

Additionally, our study revealed the effects of esketamine on pleasure perception, learning, and memory through behavioral assessments. The observed improvements in sucrose preference and performance in the Morris water maze test following esketamine treatment suggest its potential in restoring normal learning and exploratory behaviors disrupted by depressive conditions. These findings are consistent with the study conducted by Hu et al.[45] However, the therapeutic effects of esketamine on the behavior of CUMS rats were reversed by the addition of a PI3K inhibitor.

Histopathological analyses demonstrated the potential of esketamine to ameliorate prefrontal cortex histomorphology, indicating structural improvements accompanying its antidepressant effects. Interestingly, the presence of a PI3K inhibitor reversed the beneficial effects of esketamine on abnormal tissue morphology in CUMS rats.

https://doi.org/10.2147/NDT.S502090

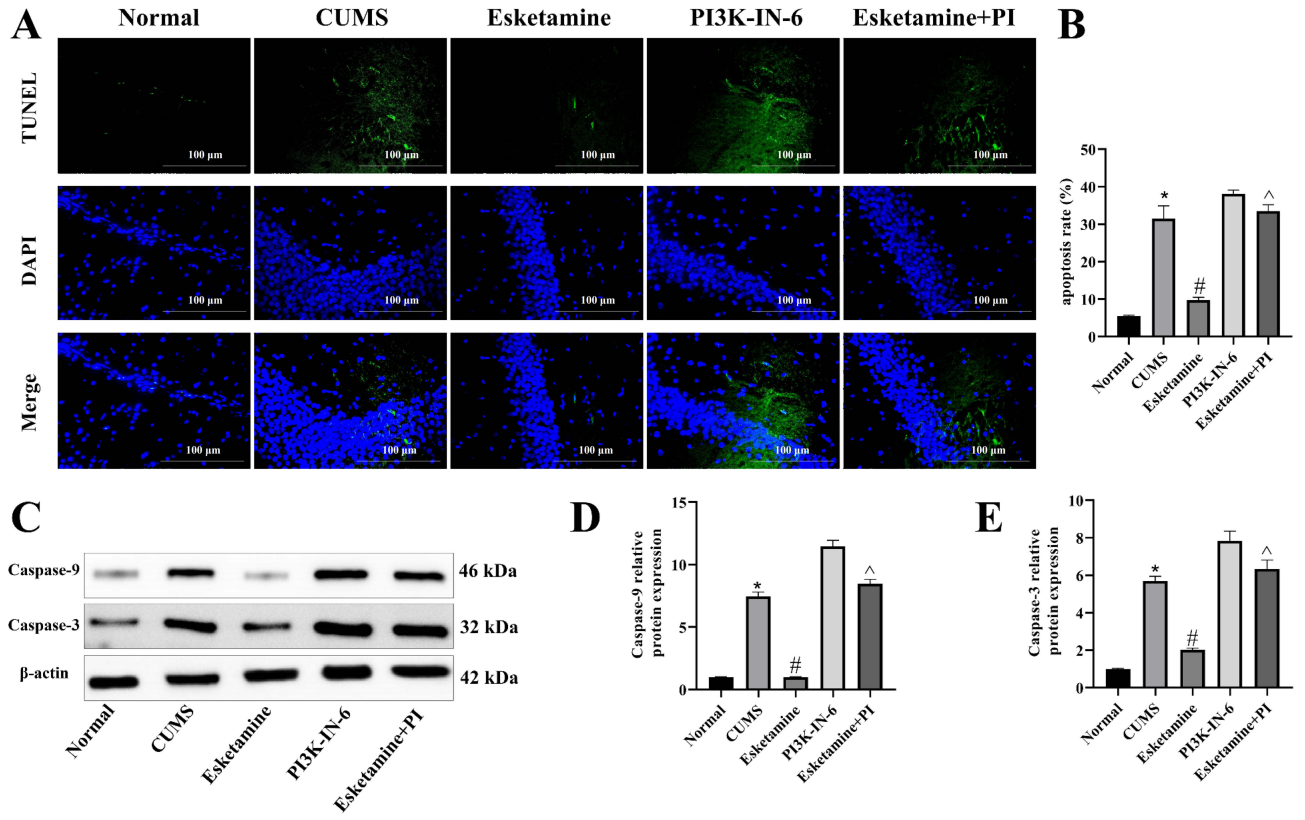

Figure 4 Esketamine Reduces Neuronal Apoptosis and Modulates Apoptotic Pathway Proteins by Activating the BDNF/TrkB/PI3K/AKT Pathway in CUMS Rats. (**A**) Shows the TUNEL staining image for detecting apoptotic cells, magnified at 400×; (**B**) displays the corresponding statistical chart of TUNEL staining, reflecting the relative level of apoptosis in different groups; (**C**) presents the Western blot bands for Caspase-9 and Caspase-3 proteins, illustrating their expression levels; (**D**) shows the statistical chart of the relative expression of Caspase-9 protein, and (**E**) displays the statistical chart of the relative expression of Caspase-3 protein in different experimental groups. *$p < 0.05$ vs the Normal group, #$p < 0.05$ vs the CUMS group, and ^$p < 0.05$ vs the Esketamine group. Results are shown as mean ± SD. N=6. The experiments were performed in triplicate.

Moreover, oxidative stress plays a critical role in the development of depression, and the efficacy of antidepressant medications may be mediated through improvements in oxidative stress and antioxidant function.[46] Esketamine treatment was shown to reduce oxidative stress by enhancing antioxidant molecule levels and decreasing oxidative damage-related parameters, corroborating findings from Tang et al.[47] Our results indicate that the alleviating effects of esketamine on oxidative stress in CUMS rats were reversed by the addition of a PI3K inhibitor.

Furthermore, GSK-3β acts as a pro-apoptotic factor in neuronal cells.[48] Our findings suggest that esketamine has the capacity to prevent apoptosis in neuronal cells, as indicated by the reduction of apoptotic markers following esketamine intervention. This suggests a protective role in cell death prevention, which is crucial for understanding the antidepressant effects of esketamine. The addition of a PI3K inhibitor reversed the inhibitory effect of esketamine on neuronal apoptosis in CUMS rats.

Collectively, the findings from various aspects of this study—including animal behavior, oxidative stress, and neuronal apoptosis—suggest that esketamine exerts its antidepressant effects by modulating the BDNF/TrkB/PI3K/AKT pathway. While our study provides valuable insights into the antidepressant effects of esketamine through the modulation of the BDNF/TrkB/PI3K/AKT signaling pathway, several limitations need to be considered. Firstly, the sample size per group (n=6) may limit the statistical power and reliability of the findings, as smaller sample sizes can increase the risk of Type I and Type II errors. A larger sample size would enhance the robustness of our conclusions. Additionally, although the study focuses on depressive behaviors, we did not include tests to evaluate anxiety, which often co-occurs with depression and could provide a more comprehensive understanding of esketamine's therapeutic potential. The sucrose preference test was used as a measure of depressive-like behaviors; however, additional validated depression assessments, such as the forced swimming test, would offer a broader perspective on the antidepressant effects

of esketamine. Moreover, the study did not assess other types of memory, such as working or emotional memory, which are also important in understanding the full scope of esketamine's impact on cognitive functions. Future studies addressing these limitations could provide a more thorough and balanced interpretation of esketamine's therapeutic potential in depression and related disorders.

Conclusion

In summary, our research provides insight into the potential antidepressant mechanisms of esketamine in a CUMS-induced depression model. Esketamine appears to exert its effects by modulating the BDNF/TrkB/PI3K/AKT signaling pathway, resulting in improved neuroplasticity, effective management of oxidative stress, and inhibition of apoptosis. Further studies are warranted to elucidate the underlying molecular pathways and evaluate the therapeutic potential of targeting this pathway in the treatment of depression.

Data Sharing Statement

The original data underlying all statistical graphs presented in this study have been compiled and are available in the supplementary materials. Specifically, the original data can be found in Table S1.

Ethics Statement

This study was approved by the ethics committee of Tianjin Medical College Pharmacy and Biotechnology Department. (Approval number: TJYX2309007).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no fund support from any Institution or Individual for this Research.

Disclosure

The authors declare that they have no conflicts of interest.

References

1. Cahill B, Poelker-Wells S, Prather JF, et al. A glimpse into the sexual dimorphisms in major depressive disorder through epigenetic studies. *Front Neural Circuits*. 2021;15:768571. doi:10.3389/fncir.2021.768571
2. Meshkat S, Haikazian S, Di Vincenzo JD, et al. Oral ketamine for depression: an updated systematic review. *World J Biol Psychiatry*. 2023;24 (7):545–557. doi:10.1080/15622975.2023.2169349
3. Janting ME, Whye Lian C, Hazmi H. Depression level and its associated factors among postpartum working women in Kuching, Sarawak—a cross-sectional study. *Malays J Med Sci*. 2023;30(4):147–156. doi:10.21315/mjms2023.30.4.13
4. Lin J, Liu W, Guan J, et al. Latest updates on the serotonergic system in depression and anxiety. *Front Synaptic Neurosci*. 2023;15:1124112. doi:10.3389/fnsyn.2023.1124112
5. Luo L, Liu X-L, Li J, et al. Macranthol promotes hippocampal neuronal proliferation in mice via BDNF-TrkB-PI3K/Akt signaling pathway. *Eur J Pharmacol*. 2015;762:357–363. doi:10.1016/j.ejphar.2015.05.036
6. Wang X, Ma W, Wang T, et al. BDNF-TrkB and proBDNF-p75NTR/sortilin signaling pathways are involved in mitochondria-mediated neuronal apoptosis in dorsal root ganglia after sciatic nerve transection. *CNS Neurol Disord Drug Targets*. 2020;19(1):66–82. doi:10.2174/1871527319666200117110056
7. Kowianski P, Lietzau G, Czuba E, et al. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. *Cell Mol Neurobiol*. 2018;38(3):579–593. doi:10.1007/s10571-017-0510-4
8. Palasz E, Wysocka A, Gasiorowska A, et al. BDNF as a promising therapeutic agent in Parkinson's disease. *Int J Mol Sci*. 2020;21(3):1170. doi:10.3390/ijms21031170
9. Golka B, Święch-Sabuda E, Gołka D, et al. The changes in neurotrophic properties of the peripheral nerves extracts following blocking of BDNF activity. *Neurol Res*. 2007;29(5):500–505. doi:10.1179/016164107X164111

10. Maryam K, Ali H. Aerobic and resistance exercises affect the BDNF/TrkB signaling pathway, and hippocampal neuron density of high-fat diet-induced obese elderly rats. *Physiol Behav*. 2023;264:114140. doi:10.1016/j.physbeh.2023.114140
11. Huang P, Liu A, Song Y, et al. Optical activation of TrkB signaling. *J Mol Biol*. 2020;432(13):3761–3770. doi:10.1016/j.jmb.2020.05.002
12. Gunduz D, Troidl C, Tanislav C, et al. Role of PI3K/Akt and MEK/ERK signalling in cAMP/Epac-mediated endothelial barrier stabilisation. *Front Physiol*. 2019;10:1387. doi:10.3389/fphys.2019.01387
13. Liu CS, Xia T, Luo Z-Y, et al. Network pharmacology and pharmacokinetics integrated strategy to investigate the pharmacological mechanism of Xianglian pill on ulcerative colitis. *Phytomedicine*. 2021;82:153458. doi:10.1016/j.phymed.2020.153458
14. Tan X, Gong L, Li X, et al. Promethazine inhibits proliferation and promotes apoptosis in colorectal cancer cells by suppressing the PI3K/AKT pathway. *Biomed Pharmacother*. 2021;143:112174. doi:10.1016/j.biopha.2021.112174
15. Casaril AM, Dantzer R, Bas-Orth C. Neuronal mitochondrial dysfunction and bioenergetic failure in inflammation-associated depression. *Front Neurosci*. 2021;15:725547. doi:10.3389/fnins.2021.725547
16. Zhang X, Zhou H, Liu H, et al. Role of oxidative stress in the occurrence and development of cognitive dysfunction in patients with obstructive sleep apnea syndrome. *Mol Neurobiol*. 2024;61(8):5083–5101. doi:10.1007/s12035-023-03899-3
17. Bai G, Jing S, Cao H, et al. Kai-Xin-San protects depression mice against CORT-induced neuronal injury by inhibiting microglia activation and oxidative stress. *Evid Based Complement Alternat Med*. 2022;2022:5845800. doi:10.1155/2022/5845800
18. Guo X, Mao R, Cui L, et al. PAID study design on the role of PKC activation in immune/inflammation-related depression: a randomised placebo-controlled trial protocol. *Gen Psychiatr*. 2021;34(2):e100440. doi:10.1136/gpsych-2020-100440
19. Du Y, Yan T, Wu B, et al. Research on the mechanism of antidepressive effect of Suanzaoren decoction through TLR4/MyD88/NF-kappaB pathway and Wnt/beta-catenin pathway. *J Ethnopharmacol*. 2024;319(Pt 1):117190. doi:10.1016/j.jep.2023.117190
20. d'Andrea G, Pettorruso M, Lorenzo GD, et al. Rethinking ketamine and esketamine action: are they antidepressants with mood-stabilizing properties? *Eur Neuropsychopharmacol*. 2023;70:49–55. doi:10.1016/j.euroneuro.2023.02.010
21. McIntyre RS, Rosenblat JD, Nemeroff CB, et al. Synthesizing the evidence for ketamine and esketamine in treatment-resistant depression: an international expert opinion on the available evidence and implementation. *Am J Psychiatry*. 2021;178(5):383–399. doi:10.1176/appi.ajp.2020.20081251
22. Dean RL. Ketamine and other glutamate receptor modulators for depression in adults with unipolar major depressive disorder. *Cochrane Database Syst Rev*. 2021;9(9):CD011612.
23. Yavi M, Lee H, Henter ID, et al. Ketamine treatment for depression: a review. *Discov Ment Health*. 2022;2(1):9. doi:10.1007/s44192-022-00012-3
24. Xu M, Wang J, Shi J, et al. Esketamine mitigates endotoxin-induced hippocampal injury by regulating calcium transient and synaptic plasticity via the NF-alpha1/CREB pathway. *Neuropharmacology*. 2025;269:110362. doi:10.1016/j.neuropharm.2025.110362
25. Vazquez GH, Bahji A, Undurraga J, et al. Efficacy and tolerability of combination treatments for major depression: antidepressants plus second-generation antipsychotics vs. Esketamine vs. *Lithium J Psychopharmacol*. 2021;35(8):890–900. doi:10.1177/02698811211013579
26. Bahji A, Zarate CA, Vazquez GH. Efficacy and safety of racemic ketamine and esketamine for depression: a systematic review and meta-analysis. *Expert Opin Drug Saf*. 2022;21(6):853–866. doi:10.1080/14740338.2022.2047928
27. Zhuang LP, Gao W-J, Fang -L-L, et al. HnRNPK is involved in stress-induced depression-like behavior via ERK-BDNF pathway in mice. *Neurochem Int*. 2023;169:105589. doi:10.1016/j.neuint.2023.105589
28. Zhuang L, Gao W, Chen Y, et al. LHPP in glutamatergic neurons of the ventral hippocampus mediates depression-like behavior by dephosphorylating CaMKIIalpha and ERK. *Biol Psychiatry*. 2024;95(5):389–402. doi:10.1016/j.biopsych.2023.08.026
29. Li LX, Chu J-H, Chen X-W, et al. Selenium ameliorates mercuric chloride-induced brain damage through activating BDNF/TrKB/PI3K/AKT and inhibiting NF-kappaB signaling pathways. *J Inorg Biochem*. 2022;229:111716. doi:10.1016/j.jinorgbio.2022.111716
30. Hassamal S. Chronic stress, neuroinflammation, and depression: an overview of pathophysiological mechanisms and emerging anti-inflammatories. *Front Psychiatry*. 2023;14:1130989. doi:10.3389/fpsyt.2023.1130989
31. Song Y, Cao H, Zuo C, et al. Mitochondrial dysfunction: a fatal blow in depression. *Biomed Pharmacother*. 2023;167:115652. doi:10.1016/j.biopha.2023.115652
32. Chen Y, Zhang Y, Wang J, et al. Anti-neuroinflammation effects of transcutaneous auricular vagus nerve stimulation against depression-like behaviors via hypothalamic alpha7nAchR/JAK2/STAT3/NF-kappaB pathway in rats exposed to chronic unpredictable mild stress. *CNS Neurosci Ther*. 2023;29(9):2634–2644. doi:10.1111/cns.14207
33. MacArthur Clark JA, Sun D. Guidelines for the ethical review of laboratory animal welfare people's republic of china national standard GB/T 35892-2018 [Issued 6 February 2018 effective from 1 September 2018]. *Anim Model Exper Med*. 2020;3(1):103–113. doi:10.1002/ame2.12111
34. Nunez J. Morris water maze experiment. *J Vis Exp*. 2008;(19). doi:10.3791/897.
35. Liu B, Zhang Y, Yang Z, et al. omega-3 DPA protected neurons from neuroinflammation by balancing microglia M1/M2 polarizations through inhibiting NF-kappaB/MAPK p38 signaling and activating neuron-BDNF-PI3K/AKT pathways. *Mar Drugs*. 2021;19(11):587. doi:10.3390/md19110587
36. Yi Z, Yi Z, Huang K, et al. Propofol attenuates mast cell degranulation via inhibiting the miR-221/PI3K/Akt/Ca(2+) pathway. *Exp Ther Med*. 2018;16(2):1426–1432. doi:10.3892/etm.2018.6317
37. Jiang C, Sakakibara E, Lin W-J, et al. Grape-derived polyphenols produce antidepressant effects via VGF- and BDNF-dependent mechanisms. *Ann NY Acad Sci*. 2019;1455(1):196–205. doi:10.1111/nyas.14098
38. Pan F, Mou T, Shao J, et al. Effects of neuronavigation-guided rTMS on serum BDNF, TrkB and VGF levels in depressive patients with suicidal ideation. *J Affect Disord*. 2023;323:617–623. doi:10.1016/j.jad.2022.11.059
39. Qian Y, Li X, Fan R, et al. MicroRNA-31 inhibits traumatic brain injury-triggered neuronal cell apoptosis by regulating hypoxia-inducible factor-1A/vascular endothelial growth factor A axis. *Neuroreport*. 2022;33(1):1–12. doi:10.1097/WNR.0000000000001741
40. Sharma A, Singh AK. Molecular mechanism of caloric restriction mimetics-mediated neuroprotection of age-related neurodegenerative diseases: an emerging therapeutic approach. *Biogerontology*. 2023;24(5):679–708. doi:10.1007/s10522-023-10045-y
41. Qiao W, Zang Z, Li D, et al. Liensinine ameliorates ischemia-reperfusion-induced brain injury by inhibiting autophagy via PI3K/AKT signaling. *Funct Integr Genomics*. 2023;23(2):140. doi:10.1007/s10142-023-01063-7
42. Li S, Lu C, Kang L, et al. Study on correlations of BDNF, PI3K, AKT and CREB levels with depressive emotion and impulsive behaviors in drug-naive patients with first-episode schizophrenia. *BMC Psychiatry*. 2023;23(1):225. doi:10.1186/s12888-023-04718-8

43. Zong W, Zeng X, Chen S, et al. Ginsenoside compound K attenuates cognitive deficits in vascular dementia rats by reducing the Abeta deposition. *J Pharmacol Sci*. 2019;139(3):223–230. doi:10.1016/j.jphs.2019.01.013
44. Zhang Q, Li R-L, Wang L-Y, et al. Hydroxy-alpha-sanshool isolated from Zanthoxylum bungeanum Maxim. has antidiabetic effects on high-fat-fed and streptozotocin-treated mice via increasing glycogen synthesis by regulation of PI3K/Akt/GSK-3beta/GS signaling. *Front Pharmacol*. 2022;13:1089558. doi:10.3389/fphar.2022.1089558
45. Hu N, Chen X, Chen C, et al. Exploring the role of esketamine in alleviating depressive symptoms in mice via the PGC-1alpha/irisin/ERK1/2 signaling pathway. *Sci Rep*. 2023;13(1):16611. doi:10.1038/s41598-023-43684-9
46. Jimenez-Fernandez S, Gurpegui M, Díaz-Atienza F, et al. Oxidative stress and antioxidant parameters in patients with major depressive disorder compared to healthy controls before and after antidepressant treatment: results from a meta-analysis. *J Clin Psychiatry*. 2015;76(12):1658–1667. doi:10.4088/JCP.14r09179
47. Tang Y, Liu Y, Zhou H, et al. Esketamine is neuroprotective against traumatic brain injury through its modulation of autophagy and oxidative stress via AMPK/mTOR-dependent TFEB nuclear translocation. *Exp Neurol*. 2023;366:114436. doi:10.1016/j.expneurol.2023.114436
48. Li L, Klebe D, Doycheva D, et al. G-CSF ameliorates neuronal apoptosis through GSK-3beta inhibition in neonatal hypoxia-ischemia in rats. *Exp Neurol*. 2015;263:141–149. doi:10.1016/j.expneurol.2014.10.004