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Antidepressant Role of Stachydrine in LPS Induced Depression by Targeting Neuroinflammatory Signaling

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Abstract

Depression is a multifactorial neuropsychiatric disorder frequently associated with neuroinflammatory processes. Docking analyses revealed a favorable binding affinity of stachydrine, a natural pyrrolidine alkaloid, to the NLRP3 receptor, suggesting its potential modulatory effects on inflammasome activity. To investigate its antidepressant-like effects, adult male Sprague–Dawley rats were randomly assigned to four groups. Saline control, LPS-induced depression, stachydrine treated (50 mg/kg), and fluoxetine treated (20 mg/kg). Behavioral assessments were performed using the forced swim test, while molecular analyses focused on NF-κB and NLRP3 expression via real time polymerase chain reaction, key mediators of neuroinflammation. LPS administration significantly increased immobility time and reduced struggling time, indicating depressive-like behavior. Stachydrine treatment effectively reversed these behavioral deficits, demonstrating antidepressant like effects comparable to fluoxetine. At the molecular level, stachydrine markedly attenuated LPS-induced upregulation of NF-κB and NLRP3, indicating suppression of inflammasome-mediated neuroinflammatory pathways. These results suggest that stachydrine exerts neuroprotective and antidepressant like effects through modulation of inflammatory

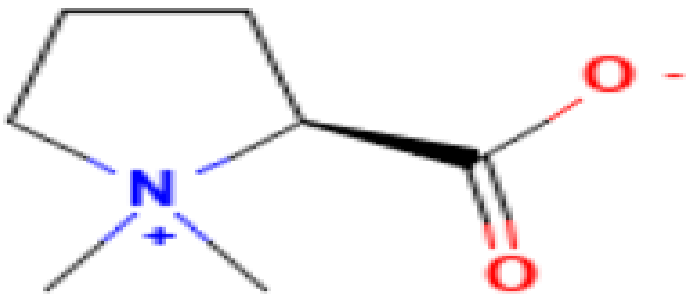
signaling. Overall, stachydrine emerges as a promising natural compound for further development as a neuroimmune targeted therapeutic agent in depression. Future investigations incorporating protein level validation, cytokine profiling and additional behavioral analysis are required to fully elucidate its pharmacological potential.

Introduction

Depression is a prevalent and debilitating mental disorder characterized by persistent low mood, loss of interest in daily activities, and impaired cognitive and emotional functioning (Zhu et al., 2016). Depression is a widespread mental health disorder that affects millions of individuals across the globe and is associated with a significant risk of suicide. According to the World Health Organization, nearly 300 million people worldwide experience different levels of depression. This condition impacts a large portion of the population close to one-fifth resulting in a considerable burden on families, healthcare systems, and society as a whole (Yin et al., 2023). The lipopolysaccharide (LPS)-induced depression like model in rats is widely used to investigate the underlying mechanisms of inflammation related depression. This experimental model also helps evaluate the potential antidepressant effects of therapeutic compounds. It provides a reliable approach for understanding how inflammatory processes contribute to depressive behaviors (Connor et al., 2009). Several pharmacological therapies are currently available for the management of depression. Antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs), are widely prescribed because they are effective in treating both depressive and anxiety-related symptoms. Benzodiazepines may also be used as adjunct therapy to relieve associated anxiety or insomnia in some patients. However, due to limitations in long-term efficacy and potential dependence, modern treatment strategies primarily rely on antidepressant medications for sustained management of depressive disorders Buller & Legrand et al., 2001).

Stachydrine (Figure 1) is a natural pyrrolidine alkaloid found in several medicinal plants. The major plant sources include Leonurus japonicus. Stachydrine is a naturally occurring alkaloid that has attracted increasing attention due to its diverse pharmacological properties. Previous studies have reported its significant anti-inflammatory, antioxidant, and anti-ulcer activities in various experimental models. These findings suggest that stachydrine possesses important therapeutic potential in inflammatory and gastrointestinal disorders. Despite these well-documented biological effects, its possible role in the management of depression has not yet been clearly explored. Considering the growing evidence linking inflammation with depressive disorders, investigating the antidepressant potential of stachydrine may provide valuable insights. Therefore, the present study aims to evaluate the potential antidepressant effects and underlying mechanisms of stachydrine in an experimental model.

This present study meets with the United Nations sustainable development Goal 3.



Material and Methods

Chemicals

Stachydrine and lipopolysaccharide were purchased from Shanghai Macklin Biochemical Co., Ltd., China. Dimethyl sulfoxide, fluoxetine, phosphate-buffered saline, and chloroform were obtained from a local pharmaceutical supplier. Kits for NLRP3 and NF-κB were procured from Elabscience. All chemicals were analytically graded.

Animals

Adult male Sprague–Dawley rats weighing 180–200 g were obtained from the National Institute of Health Islamabad and housed in groups of five per cage. The animals were maintained under standard laboratory conditions with a temperature of 25 ± 1 °C, relative humidity of 50 ± 10%, and a 12-hour light–dark cycle. Food and water were provided ad libitum throughout the experimental period.

Insilico Evaluation

The three-dimensional structure of the test compound was prepared using BIOVIA Discovery Studio Visualizer. The standard antidepressant Fluoxetine was used as a reference drug. The 3D structure of the target protein associated with depression was retrieved from the Research Collaboratory for Structural Bioinformatics. The selected protein target was NLRP3. During protein preparation, ligands and water molecules were removed, polar hydrogen atoms were added, and the structure was saved in PDB format. Molecular docking analysis was performed using PyRx. The docking results were evaluated based on atomic contact energy (ACE) values expressed in kcal/mol, and the pose with the lowest energy was selected as the best interaction model. Finally, both 2D and 3D interaction visualizations were analyzed to interpret the binding pattern (Noman et al., 2022).

Experimental design

The rats were randomly divided into four groups, with n = 5 animals in each group
Saline group received 10 mL/kg of saline.
Disease group received LPS (500 μg/kg) to induce depression-like behavior.
Treatment group treated with stachydrine (50 mg/kg).
Standard group treated with fluoxetine (20 mg/kg).
LPS and fluoxetine were dissolved in normal saline containing 5% DMSO, while the doses were selected based on previous studies. The treatment protocol lasted 14 days. The saline group received normal saline on alternate days, whereas LPS was administered on alternate days to induce the depression model. Fluoxetine and stachydrine were given once daily via intraperitoneal injection, 1 hour after LPS administration, for 14 consecutive days. Following the treatment period, behavioral assessment was conducted using the Forced Swim Test (FST). After the experiments, tissue samples were collected, some were fixed in 4% paraformaldehyde, while others were immediately frozen and stored at −80 °C for subsequent biochemical analyses (Mir et al., 2024).

Behavioral testing

The Forced Swim Test (FST) was performed to assess depression-like behavior in rats. Each animal was placed individually in a cylinder filled with water at 25 ± 1 °C for a total of 6 minutes. During the test, struggling time and immobility time were recorded, with immobility defined as minimal movement to keep the head above water. Increased immobility indicates depression-like behavior, whereas longer struggling time reflects antidepressant-like effects (Lapiz et al., 2008).

Real-Time Polymerase Chain Reaction

Total RNA was isolated from brain tissues using a commercial extraction kit according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from the extracted RNA. Real-time RT-PCR was then performed using specific primers for target genes and a SYBR Green master mix on a real-time PCR system. Gene expression levels were normalized to a housekeeping gene (β-actin), and relative changes were calculated using the 2–ΔΔCt method (Noman et al., 2026).

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Graphical representations were generated using GraphPad Prism (version 9.5.0 for macOS; GraphPad Software, San Diego, CA, USA), and statistical analyses were conducted with SPSS (version 25 for macOS; IBM Corp., Armonk, NY, USA). Group differences were evaluated using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Computational Analysis

In this study, stachydrine demonstrated binding to the NLRP3 receptor with measurable affinitiy. The atomic contact energy (ACE) values for the best docking poses of stachydrine and the reference drug, along with the amino acid residues involved in hydrogen bonding, π–π interactions, and other hydrophobic contacts, were analyzed. Figures 2 and 3 present 2D and 3D visualizations of the interactions between stachydrine and fluoxetine with NLRP3. Stachydrine exhibited an E-value of −5.4 kcal/mol, whereas fluoxetine showed a slightly stronger binding with an E-value of −6.6 kcal/mol against the NLRP3 receptor (Table 1).

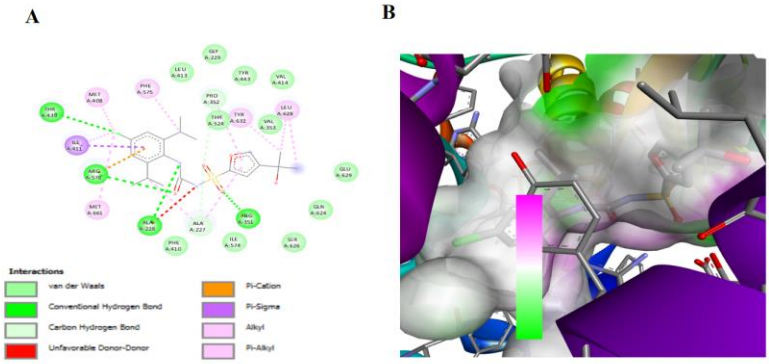


Figure 2: 2D and 3D interactions of stachydrine with NLRP3

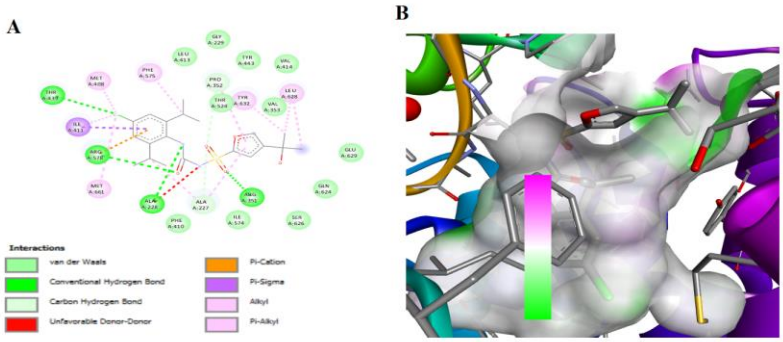


Figure 3: 2D and 3D interactions of Fluoxetine with NLRP3

Table 1: Binding energy values of stachydrine and fluoxetine with NLRP3.

Compounds	Receptors	PDB ID	Energy Values (Kcal/mol)
Stachydrine	NLRP3	7ALV	-5.4
Fluoxetine	NLRP3	7ALV	-6.6

Force Swim Test

The effects of stachydrine and fluoxetine on LPS-induced behavioral changes in rats. LPS administration (500 µg/kg) significantly decreased struggling time and increased immobility time compared to the saline group ($^{###}p < 0.001$), indicating depressive-like behavior. Treatment with stachydrine (50 mg/kg) significantly restored struggling time (**$p < 0.01$) and reduced immobility time (**$p < 0.01$) relative to the LPS group. Fluoxetine (20 mg/kg) also improved these behavioral parameters (***$p < 0.001$), confirming its antidepressant effect. Both stachydrine and fluoxetine mitigated LPS-induced depressive-like behavior. Overall, stachydrine exhibits comparable antidepressant-like effects to fluoxetine as shown in (Figure 4).

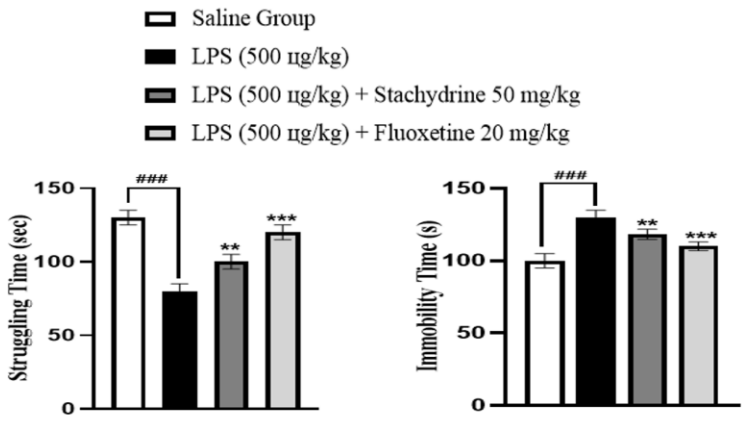
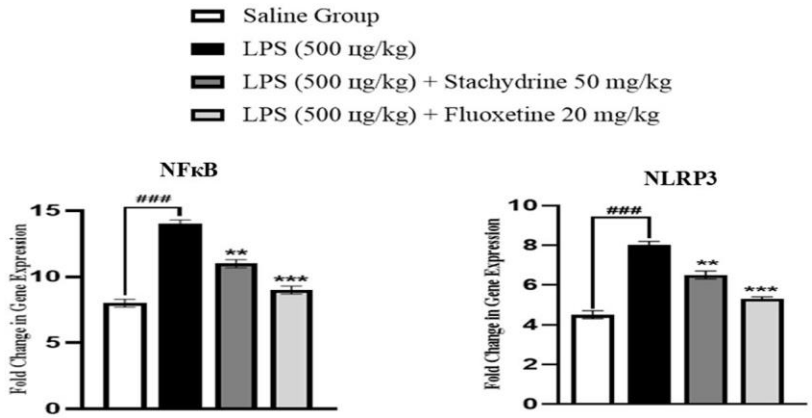


Figure 4: Effect of stachydrine and fluoxetine against struggling time and immobility time in rat's Force swim test. Values expressed as mean ± SEM (n = 5).

Gene Expression via RT-PCR

LPS administration (500 µg/kg) significantly upregulated NFκB and NLRP3 gene expression compared to the saline group (###, $p<0.001$). Treatment with Stachydrine (50 mg/kg) markedly reduced the LPS-induced increase in NFκB and NLRP3 expression (**$p<0.01$ for NFκB, **$p<0.01$ for NLRP3). Similarly, Fluoxetine (20 mg/kg) significantly attenuated the elevated expression of both genes (***$p<0.001$). The results indicate that Stachydrine exhibits comparable anti-inflammatory effects to Fluoxetine in this model. These findings suggest that Stachydrine effectively suppresses LPS-induced activation of key inflammatory mediators. Overall, the data support the potential of Stachydrine as a modulator of neuroinflammation (Figure 5).

Figure 5: Effects of stachydrine and fluoxetine against NFkB and NLRP3 using RT-PCR. Values expressed as mean ± SEM (n = 5).



Discussion

In the present study, the antidepressant-like potential of stachydrine was evaluated using a LPS-induced model of depression, a validated inflammatory model that mimics several behavioral and biochemical features of clinical depression (Ding et al., 2025; Xu et al., 2014). Docking analysis showed stable binding energy value of stachydrine against NLRP3 receptor. Our behavioral data showed that LPS significantly increased immobility time and decreased struggling time in the forced Swim Test, indicating the successful induction of depressive-like behavior. Treatment with stachydrine markedly reversed these effects, suggesting an antidepressant-like response that mirrors the effects seen with fluoxetine, a well-established antidepressant in both preclinical and clinical settings. A key focus of this study was the involvement of the NLRP3 inflammasome and NF-κB, both molecular mediators implicated in inflammation-associated depression. Growing evidence supports that inflammatory processes contribute centrally to depressive symptoms, particularly through microglial activation and inflammasome signaling (Bian et al., 2022; Ding et al., 2025). LPS initiates an innate immune response via Toll-like receptor 4 (TLR4), which activates downstream NF-κB and facilitates transcriptional upregulation of pro-inflammatory cytokines and inflammasome components such as NLRP3 (Ding et al., 2025; Zhang et al., 2014). Indeed, in our study, LPS elevated NF-κB and NLRP3 expression, consistent with evidence that chronic inflammation can amplify depressive-like behavior by engaging inflammasome pathways (Zhang et al., 2014). Fluoxetine ability to modulate the NLRP3 inflammasome has been documented in other rodent models of stress-induced depression. It was shown to reduce NLRP3 expression and associated inflammatory mediators, in parallel with improvements in depressive behaviors (Alcocer-Gomez et al., 2015). Our results extend this mechanism by showing that stachydrine also significantly reduced NF-κB and NLRP3 gene expression relative to the LPS group. These molecular changes likely underpin the behavioral improvements observed, underscoring inflammation modulation as a shared path through which both conventional antidepressants and natural compounds can exert therapeutic effects. Importantly, stachydrine is recognized for its anti-inflammatory properties in non-CNS models, including reductions in pro-inflammatory cytokines and inhibition of NF-κB signaling in LPS-stimulated peripheral tissues (Meng et al., 2019; Zhou et al., 2021). In sepsis and cardiac injury models, stachydrine was shown to attenuate systemic inflammation by decreasing IL-1β, IL-6, and TNF-α expression, and by activating anti-oxidative pathways (Zhang et al., 2024), further supporting its utility in inflammatory conditions. While these studies did not specifically address behavioral endpoints, they provide a mechanistic framework for our depression-related findings. The suppression of NF-κB and NLRP3 expression in the stachydrine group suggests that its antidepressant-like effects may involve a reduction in neuroinflammatory signaling cascades that are activated in response to LPS challenge. Consistent with this, recent inhibitors targeting NLRP3 directly have demonstrated antidepressant-like effects in LPS models, with reduced inflammasome activation and ameliorated behavioral symptoms (Wan et al., 2022). Taken together, this evidence strengthens the proposition that modulation of inflammasome signaling whether through classical antidepressants or natural products like stachydrine represents a viable strategy for mitigating inflammation-associated depression. Beyond inflammasome regulation, fluoxetine and other antidepressants are also known to influence downstream neurotrophic factors and neuroplasticity, which were not directly measured in the present study but remain relevant pathways intertwined with immune signaling (Ding et al., 2025). Chronic inflammation can impair brain-derived neurotrophic factor (BDNF) expression and synaptic plasticity, both of which are implicated in the pathophysiology of depression (Karger Publishers, 2020). Therefore, stachydrine anti-inflammatory effects could indirectly preserve neuroplasticity and support neuronal resilience, further contributing to antidepressant-like outcomes. Several limitations should be acknowledged. First, this study assessed gene expression of

NF-κB and NLRP3 but did not quantify corresponding protein levels or cytokine concentrations, which would provide more direct evidence of inflammasome activation. Additionally, while the forced swim test is a widely used for depressive-like behavior, additional behavioral paradigms such as sucrose preference or novelty-suppressed feeding would provide a more comprehensive evaluation of anhedonic and anxiety-related aspects. In conclusion, the present findings reveal that stachydrine exerts significant antidepressant-like effects in an LPS-induced model of depression, likely mediated by down-regulation of pro-inflammatory signaling pathways involving NF-κB and the NLRP3 inflammasome. These results support the expanding view that targeting neuroinflammation represents a promising approach for the treatment of depression and highlight stachydrine as a candidate for further development as a neuroimmune modulator in depressive disorders.

Conclusion

Stachydrine exhibits significant antidepressant-like effects in an LPS-induced depression model, likely through suppression of NF-κB and NLRP3-mediated neuroinflammation. Behavioral improvements and downregulation of inflammatory mediators suggest its effects are comparable to fluoxetine. These findings highlight stachydrine as a promising natural compound for further investigation as a neuroimmune targeted antidepressant.

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