RJNMSR
Review Journal of Neurological & Medical Sciences Review

Volume 4, Issue 3, 2026

ALOPERINE SUPPRESSES NEUROINFLAMMATION AND DEPRESSIVE-LIKE BEHAVIOR VIA TLR4/NF-ΚB INHIBITION

Sufyan Siraj[1], Rubia Anwer[2], Muhammad Adnan[*3], Muhammad Noman[4], Muhammad Faheem[5], Abida Shamim[6], Samran[7], Rukhshanda Habib[8], Abu Bakr Ahmad[9], Fahim Hilal[10,11]

*[1,2,*3,6,7,9]Faculty of Pharmacy, IBADAT International University Islamabad, Pakistan.*
[4]Faculty of Pharmacy, MY University Islamabad, Pakistan.
[5]Department of Pharmacy, University of Swabi, Swabi 23561, Pakistan
[8]Department of Pharmacology, Gomal Center of Pharmaceutical Sciences, Gomal University, Dera Ismael Khan, Khyber Pakhtunkhwa, Pakistan
[10]Department of Pharmacy, Sarhad University of Science and Information Technology, Islamabad Campus
[11]Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad Pakistan

[*3]muhammad.adnan@pharm.iiui.edu.pk

Corresponding Author: *
Muhammad Adnan
DOI: http://doi.org/10.5281/zenodo.19348979

Received	Accepted	Published
31 January 2026	14 March 2026	31 March 2026

ABSTRACT

The present study provides the first evidence that aloperine exerts significant antidepressant-like effects in a lipopolysaccharide-induced model of depression, primarily through modulation of insilico and molecular neuroinflammatory pathways. LPS administration reliably induced depressive-like behavior, characterized by increased immobility and reduced struggling time in the Forced Swim Test, consistent with activation of innate immune responses via TLR4 signaling. This activation promotes NF-κB translocation and subsequent release of pro-inflammatory cytokines, contributing to neuroinflammation and behavioral deficits. The observed upregulation of TLR4 and NF-κB expression in the LPS group aligns with established mechanisms linking inflammation to depression. Treatment with aloperine significantly attenuated both behavioral impairments and molecular alterations induced by LPS, suggesting its potential role in suppressing neuroinflammatory signaling. These findings are supported by previous evidence demonstrating that aloperine inhibits TLR4/NF-κB-mediated inflammatory responses. Comparable effects have been reported with other anti-inflammatory agents, further emphasizing the importance of targeting inflammatory pathways in depression. Additionally, fluoxetine demonstrated similar protective effects, reinforcing the concept that antidepressants may exert therapeutic benefits partly through anti-inflammatory mechanisms. Despite these promising findings, limitations include the lack of assessment of downstream cytokines and neuroplasticity-related markers, which warrant further investigation. Overall, the study highlights aloperine as a potential therapeutic candidate for inflammation-associated depression by modulating key neuroinflammatory mediators.

Keywords: *Aloperine; Depression; Neuroinflammation; TLR4; NF-κB*

INTRODUCTION

Depression is a complex and debilitating neuropsychiatric disorder characterized by persistent low mood, loss of interest or pleasure, and cognitive and functional impairments. It significantly affects an individual's emotional, social, and physical well-being, making it one of

the leading contributors to global disability (Marasine et al., 2021) . The disorder is multifactorial and involves intricate interactions between biological, psychological, and environmental factors. The causative factors of depression are diverse and include genetic predisposition, neurotransmitter imbalance (particularly serotonin, norepinephrine, and dopamine), neuroinflammation, hormonal dysregulation, and psychosocial stressors such as trauma, chronic stress, and socioeconomic difficulties. Emerging evidence also highlights the role of inflammatory pathways and immune system activation in the pathophysiology of depression, linking peripheral inflammation with central nervous system dysfunction (Yuan et al., 2020) . Globally, depression represents a major public health burden with high prevalence and recurrence rates. According to global health estimates, it affects hundreds of millions of people worldwide and is a leading cause of disability-adjusted life years (DALYs), contributing substantially to morbidity and mortality (Marasine et al., 2021) . The increasing prevalence of depression has intensified the need for effective therapeutic strategies and improved understanding of its underlying mechanisms. Currently, several pharmacological and non-pharmacological treatment options are available for managing depression. Pharmacotherapy primarily includes antidepressants such as selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and atypical antidepressants. Among these, SSRIs are considered first-line agents due to their favorable safety and tolerability profiles (Marasine et al., 2021; Chu & Wadhwa, 2021) . In addition to pharmacological interventions, psychotherapy, lifestyle modifications, and emerging therapies targeting neuroinflammation and neuroplasticity are increasingly being explored to improve treatment outcomes.

Aloperine (Figure 1) is a naturally occurring quinolizidine alkaloid obtained from medicinal plants, mainly belonging to the genus Sophora. The major source of aloperine is Sophora alopecuroides commonly known as “Ku Dou Zi” in traditional Chinese medicine Family Fabaceae. Various pharmacological activities of aloperine include antiapoptotic (Ren et al., 2017), anti-inflammatory (Yang et al., 2015), antiviral and antifungal (Hu et al., 2024).Till now no antidepressant activity of aloperine has been reported. However, its potential antidepressant effects have not yet been systematically investigated. Therefore, the present study aims to explore the antidepressant-like role of aloperine in a lipopolysaccharide-induced model of depression, with a focus on neuroinflammatory mechanisms.

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

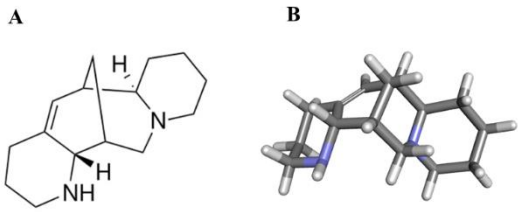

Figure 1: A and B represent 2D and 3D structures of aloperine.

Material and Methods

Chemicals

Aloperine and LPS were obtained from Shanghai Macklin Biochemical Co., Ltd., China, ensuring high purity suitable for experimental use. Additional reagents, including dimethyl sulfoxide (DMSO), fluoxetine, phosphate-buffered saline (PBS), and

chloroform, were procured from a certified local pharmaceutical supplier and were used as received without further purification. Commercially available assay kits for the quantification of TLR4 and NFkB were purchased from Elabscience, following the manufacturer's protocols for all experimental procedures. All chemicals and reagents utilized in the present study were of analytical grade, ensuring reliability and reproducibility of the experimental outcomes.

Experimental Animals

Healthy adult male Sprague–Dawley rats, weighing between 150 and 200 g, were procured from the National Institute of Health Islamabad. The animals were acclimatized and housed in standard polypropylene cages, with five rats allocated to each cage to ensure proper social housing conditions. The animals were kept under controlled environmental conditions, including a regulated temperature of 25 ± 1 °C, relative humidity maintained at 50 ± 10%, and a consistent 12-hour light and dark cycle. Standard laboratory diet and clean drinking water were made freely available throughout the duration of the study.

Blood Brain Permeability

Aloperine ability to penetrate the blood brain barrier was evaluated using the SwissADME online prediction tool. The compound's physicochemical properties, including lipophilicity, molecular size, and polarity, were analyzed to estimate its CNS permeability. The BOILED-Egg model within SwissADME was specifically applied to predict gastrointestinal absorption and brain access (Daina & Zoete et al., 2016).

Docking Study

The three-dimensional structure of the selected drug candidate was retrieved and visualized using BIOVIA Discovery Studio Visualizer (DSV). Protein targets relevant to depression were identified, and their crystallographic structures were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The selected targets included serotonin (PDB ID: 7E2Y) and dopamine receptor D1 (PDB ID: 7LJD). Prior to docking, all co-crystallized ligands and water molecules were removed, and polar hydrogen atoms were added using DSV. The prepared protein structures were then saved in PDB format. Molecular docking analysis was performed using AutoDock version 1.5.6 and PyRx version 0.8. Binding interactions were evaluated based on atomic contact energy (ACE) values (kcal/mol), and the most stable binding conformation was selected based on the lowest ACE score for further analysis (Noman et al., 2022).

Experimental Model

Animals were randomly assigned into four groups (n = 5 per group). The control group received saline (10 mL/kg). The disease group was administered lipopolysaccharide (LPS; 500 µg/kg) to induce depression-like behavior. The treatment group received aloperine (30 mg/kg), while the standard group was treated with fluoxetine (20 mg/kg). LPS and fluoxetine were prepared in normal saline supplemented with 5% DMSO, and dosing regimens were selected based on previously reported studies. The experimental protocol was conducted over 14 days. The control group was given saline on alternate days, whereas LPS was administered on alternate days to establish the depressive model. Both aloperine and fluoxetine were administered intraperitoneally once daily, 1 hour following LPS injection, throughout the treatment period. At the end of the experimental duration, behavioral evaluation was performed using the Forced Swim Test (FST). Subsequently, animals were sacrificed, and brain tissues were collected. A portion of the samples was fixed in 4% paraformaldehyde for histological analysis, while the remaining tissues were rapidly frozen and stored at −80 °C for further biochemical investigations (Adnan et al., 2025).

Behavioral Evaluation

Depression-like behavior in rats was evaluated using the Forced Swim Test (FST). Each animal was individually placed in a cylindrical container filled with water maintained at 25 ± 1 °C and observed for a total duration of 6 minutes. During the test, both active (struggling) and passive (immobility) behaviors were recorded. Immobility was defined as the absence of active escape-directed movements, with only minimal

activity required to keep the head above the water surface. An increase in immobility time was considered indicative of depression-like behavior, whereas a reduction in immobility accompanied by increased struggling time was interpreted as an antidepressant-like response (Yankelevitch et al., 2015).

Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from brain tissue samples using a commercially available extraction kit, following the manufacturer's protocol. The isolated RNA was then reverse transcribed to synthesize complementary DNA (cDNA). Quantitative real-time PCR (RT-PCR) was carried out using gene-specific primers along with a SYBR Green master mix on a real-time PCR system. The expression levels of target genes were normalized against the housekeeping gene β-actin. Relative gene expression was determined using the 2^−ΔΔCt method (Noman et al., 2026).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS (version 25), while graphs were generated with GraphPad Prism (version 9.5.0). Group comparisons were conducted using one-way ANOVA followed by the LSD post hoc test. A p-value < 0.05 was considered statistically significant.

Results

Blood Brain Barrier Penetration

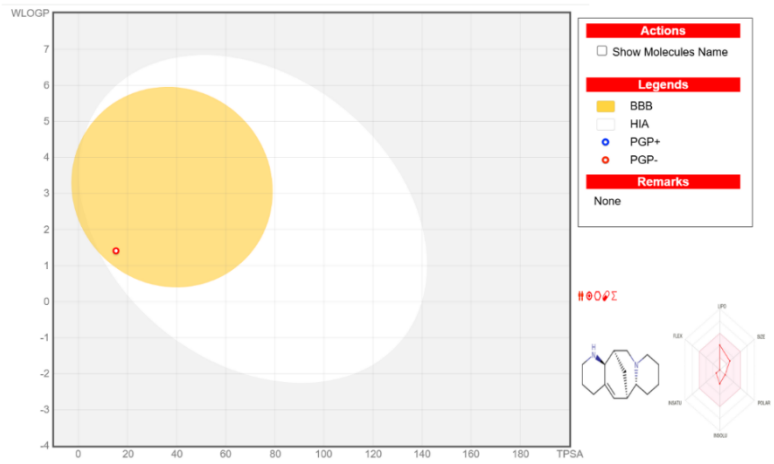

Figure 2: Blood brain barrier permeability of aloperine.

The BOILED-Egg model demonstrated that aloperine is positioned within the yolk region, indicating a high probability of blood–brain barrier penetration. The compound also falls within the white region, suggesting favorable gastrointestinal absorption. The WLOGP and TPSA values of aloperine lie within the optimal range required for CNS-active compounds. Additionally, the compound is predicted to be P-glycoprotein negative (PGP−), indicating a lower likelihood of efflux from the brain. These properties collectively support efficient brain accessibility and systemic absorption. Overall, the results suggest that aloperine possesses suitable pharmacokinetic characteristics for central nervous system activity (Figure 2)

Computational Study

In this study, aloperine was found to interact with both serotonin and dopamine 1 receptors, showing significant binding affinity. The atomic contact energy (ACE) values for the most favorable docking poses were evaluated, along with the key amino acid residues involved in hydrogen bonding, π–π interactions, and other hydrophobic contacts. (Figures 2 and 3)

illustrate the 2D and 3D representations of these interactions with serotonin and dopamine 1 receptors. Aloperine exhibited a binding energy of −7.5 kcal/mol for the serotonin receptor and −7.0 kcal/mol for the dopamine 1 receptor, as summarized in (Table 1).

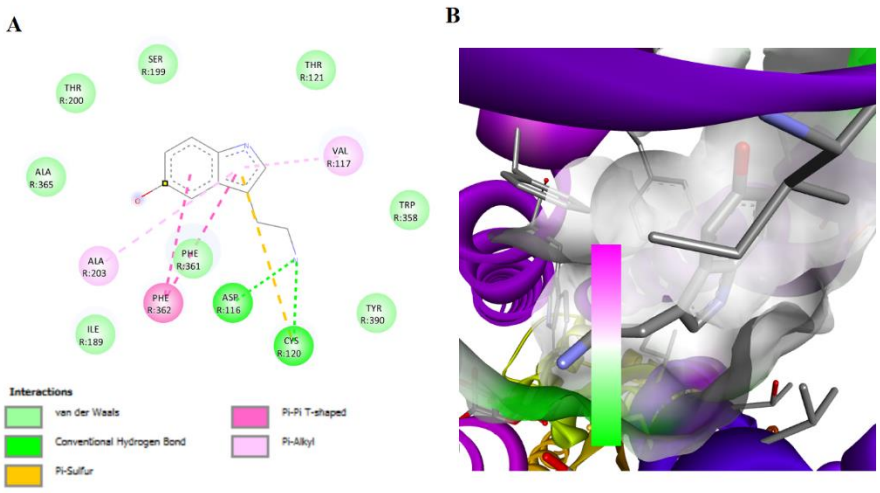

Figure 3: A and B reperesent 2D and 3D interactions of aloperine with serotonin.

Table 1: Binding energy values of aloperine with serotonin and dopamine 1.

Compound	Receptors	PDB ID	Energy Values Kcal/mol
Aloperine	Serotonin	7E2Y	-7.5
Aloperine	Dopamine 1	7LJD	-7.0

Forced Swim Test

The impact of aloperine and fluoxetine on LPS-induced behavioral alterations in rats was evaluated using the forced swim test. LPS administration (500 µg/kg) led to a significant reduction in struggling time and an increase in immobility time compared to the saline-treated group (###p < 0.001), reflecting depressive-like behavior. Treatment with aloperine (30 mg/kg) significantly increased struggling time (**p < 0.01) and decreased immobility time (**p < 0.01) relative to the LPS group. Similarly, fluoxetine (20 mg/kg) effectively improved these behavioral measures (***p < 0.001), validating its established antidepressant effect. Both aloperine and fluoxetine attenuated LPS-induced depressive-like behaviors, with aloperine demonstrating antidepressant-like efficacy comparable to fluoxetine, as illustrated in (Figure 5).

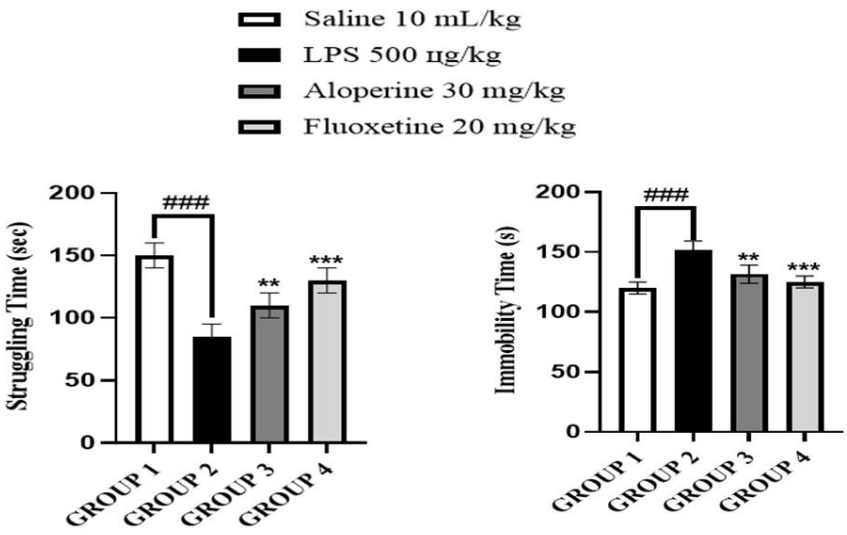

Figure 5: Impact of aloperine and fluoxetine on struggling and immobility durations in the rat Forced Swim Test. Data are presented as mean ± SEM (n = 5).

Gene Expression Analysis

LPS administration (500 µg/kg) significantly upregulated the expression of TLR4 and NFkB genes compared to the saline-treated group (###, $p < 0.001$). Treatment with aloperine (30 mg/kg) effectively suppressed this LPS-induced elevation in TLR4 and NFkB expression. Likewise, fluoxetine (20 mg/kg) significantly reduced the overexpression of both genes (***$p < 0.001$). These results indicate that aloperine exhibits anti-inflammatory effects comparable to fluoxetine in this model. Overall, the findings suggest that aloperine can effectively inhibit LPS-induced activation of key inflammatory mediators, highlighting its potential as a modulator of neuroinflammation (Figure 6).

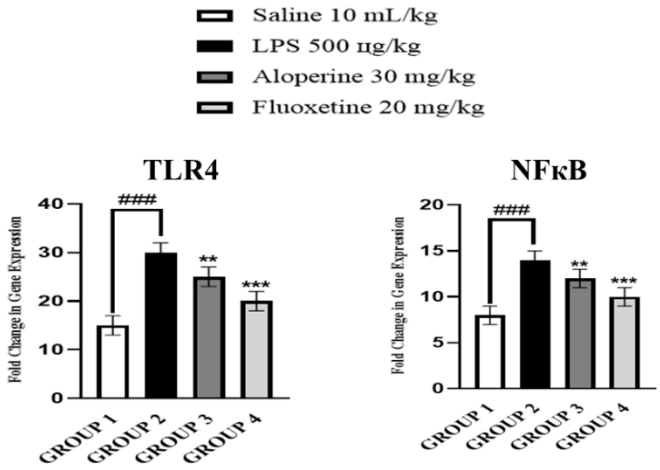

Figure 6: Effects of aloperine and fluoxetine on TLR4 and NFkB expression analyzed by RT-PCR. Data are presented as mean ± SEM (n = 5).

Discussion

The current study demonstrates for the first time that aloperine exerts significant antidepressant-like effects in a LPS-induced model of depression, and that this effect is associated with the modulation of key neuroinflammatory mediators. These findings provide compelling preclinical evidence that the anti-inflammatory properties of aloperine may underline its behavioral and molecular effects in this paradigm. In this study, systemic administration of LPS reliably induced depressive-like behaviors, as evidenced by increased immobility and decreased struggling time in the FST. Such behavioral changes are a robust indicator of despair-like states in rodents and are widely used in inflammation-associated depression research (Yankelevitch et al., 2015). LPS is a gram-negative bacterial endotoxin that activates the innate immune system primarily via TLR4 signaling, catalyzing a cascade of intracellular responses that lead to NF-κB activation and elevated pro-inflammatory cytokines such as TNF-α and IL-1β (da Silva et al., 2024; Yuan et al., 2020). The link between LPS administration and depression-like behavior is well supported in the literature: peripheral LPS triggers central neuroinflammatory responses, microglial activation, and cytokine release, which correlate with anhedonia and behavioral despair in animal models (He et al., 2020; Couch et al., 2016). The observed increase in TLR4 and NF-κB mRNA expression following LPS administration in our study is consistent with the known mechanisms of LPS-induced neuroinflammation. TLR4 is a pattern-recognition receptor that detects pathogen-associated molecular patterns (PAMPs) like LPS and activates MyD88-dependent signaling pathways culminating in NF-κB translocation to the nucleus and induction of pro-inflammatory gene transcription (da Silva et al., 2024). This signaling pathway has been implicated in both preclinical models and clinical observations of major depressive disorder, where elevated inflammatory markers are associated with symptom severity (Miller et al., 2009; He et al., 2020). Importantly, aloperine treatment significantly attenuated both the behavioral effects of LPS and the LPS-induced upregulation of TLR4 and NF-κB. These results suggest that the antidepressant-like effects of aloperine are likely mediated, at least in part, through suppression of neuroinflammatory signaling pathways. Previous in vitro studies have shown that aloperine can suppress macrophage activation via inhibition of the TLR4/NF-κB pathway (Ye et al., 2020), supporting the present in vivo findings and indicating a potential molecular mechanism (Ye et al., 2020). The anti-inflammatory actions of aloperine may thus parallel those seen with other natural compounds and pharmacological agents in similar models. For example, paeoniflorin attenuates LPS-induced depression-like behaviors through suppression of the NLRP3 inflammasome and activation of antioxidative signaling (He et al., 2025), and inhibition of RIPK1 has been shown to mitigate neuroinflammation and depressive behavior in LPS models (Gong et al., 2024). These studies collectively emphasize that targeting inflammatory signaling pathways, particularly TLR4/NF-κB and downstream cytokines, is an effective strategy for alleviating inflammation-driven depressive phenotypes. Moreover, the capacity of fluoxetine a well-established SSRI to reverse LPS-induced behavioral and molecular changes in this study aligns with previous reports suggesting that certain antidepressants possess anti-inflammatory properties aside from their monoaminergic effects (Batey, 2024). This supports the notion that effective antidepressant treatments can exert therapeutic effects by reducing neuroinflammation in addition to modulating monoamine neurotransmission. Nevertheless, this study has limitations that should be considered. While the molecular analyses focused on TLR4 and NF-κB expression, further investigations into downstream cytokines (TNF-α, IL-6) and microglial activation markers would provide a more comprehensive understanding of the neuroimmune changes mediated by aloperine. Additionally, evaluation of other depression-related molecular markers, such as BDNF or serotonergic/dopaminergic receptor expression, could illuminate potential neuroplasticity mechanisms linked to behavioral outcomes. Overall the present findings indicate

that aloperine exhibits antidepressant-like effects in a LPS-induced model of depression, likely mediated by its suppression of pro-inflammatory pathways involving TLR4 and NF-κB signaling. These results offer promising preclinical evidence supporting further exploration of aloperine as a potential therapeutic agent for inflammation-associated depression.

Conclusion

In conclusion, aloperine demonstrates significant antidepressant-like effects in a LPS-induced model of depression by attenuating behavioral despair and reducing neuroinflammatory signaling. Its modulatory effects on the TLR4/NF-κB pathway suggest a key role in suppressing inflammation-driven depressive phenotypes. These findings highlight aloperine potential as a novel therapeutic agent targeting neuroinflammation in depression. Further studies are warranted to explore its effects on downstream cytokines, neuroplasticity markers, and long-term behavioral outcomes. Overall, aloperine represents a promising candidate for the development of anti-inflammatory strategies in managing depression.

REFERENCES

Marasine, N. R., Sankhi, S., Lamichhane, R., Marasini, N. R., & Dangi, N. B. (2021). Use of antidepressants among patients diagnosed with depression: A scoping review. *BioMed Research International, 2021*, 6699028. https://doi.org/10.1155/2021/6699028

Yuan, Z., Chen, Z., Xue, M., Zhang, J., & Leng, L. (2020). Application of antidepressants in depression: A systematic review and meta-analysis. *Journal of Clinical Neuroscience, 80*, 169–181. https://doi.org/10.1016/j.jocn.2020.08.013

Chu, A., & Wadhwa, R. (2021). Selective serotonin reuptake inhibitors and adverse effects: A narrative review. *Mental Illness, 13*(3), 38.

Ren, D., Ma, W., Guo, B., & Wang, S. (2017). Aloperine attenuates hydrogen peroxide-induced injury via anti-apoptotic activity and suppression of the nuclear factor-κB signaling pathway. *Experimental and Therapeutic Medicine*, *13*(1), 315-320.

Yang, Y., Jin, S. J., Wang, H. L., Li, Y. X., Du, J., Zhou, R., ... & Yu, J. Q. (2015). Effects of aloperine on acute and inflammatory pain models in mice. *Scandinavian journal of pain*, *8*(1), 28-34.

Hu, Z. X., Zhang, J., Zhang, T., Tian, C. Y., An, Q., Yi, P., ... & Hao, X. J. (2024). Aloperine-type alkaloids with antiviral and antifungal activities from the seeds of Sophora alopecuroides L. *Journal of Agricultural and Food Chemistry*, *72*(14), 8225-8236.

Noman, M., Qazi, N. G., Rehman, N. U., & Khan, A. U. (2022). Pharmacological investigation of brucine anti-ulcer potential. *Frontiers in pharmacology*, *13*, 886433.

Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, *11*(11), 1117-1121.

Adnan, M., Noman, M., Anwer, R., Siraj, S., Shamim, A., Faheem, M., ... & Azher, S. (2025). Antidepressant Role of Stachydrine in LPS Induced Depression by Targeting Neuroinflammatory Signaling. *Pakistan Journal of Medical & Cardiological Review*, *4*(4), 2664-2672.

Yankelevitch-Yahav, R., Franko, M., Huly, A., & Doron, R. (2015). The forced swim test as a model of depressive-like behavior. Journal of visualized experiments: JoVE, (97), 52587.

Batey, L. (2024). *Lipopolysaccharide effects on neurotransmission: Understanding implications for depression*. ACS Chemical Neuroscience.

Couch, Y., Trofimov, A., Markova, N., Nikolenko, V., Steinbusch, H. W., Chekhonin, V., & Strekalova, T. (2016). *Low-dose lipopolysaccharide augments depressive behaviours in mice. Journal of Neuroinflammation*, 13, 108.

da Silva, A. A. F., Fiadeiro, M. B., Bernardino, L. I., Fonseca, C. S. P., Baltazar, G. M. F., & Cristóvão, A. C. B. (2024). *Lipopolysaccharide-induced animal models for neuroinflammation – An overview. Journal of Neuroimmunology*, 387, Article 578273.

Gong, Q., Ali, T., Hu, Y., Gao, R., Mou, S., Luo, Y., ... Li, X. (2024). *RIPK1 inhibition mitigates neuroinflammation and rescues depressive-like behaviors in a mouse model of LPS-induced depression. Cell Communication and Signaling*, 22, Article 427.

He, Y., Li, W., Wang, Y., Tian, Y., Chen, X., Wu, Z., ... Lan, T. (2025). *Paeoniflorin alleviates LPS-induced neuroinflammation and depression through Keap1/Nrf2/HO-1 signaling pathway. Antioxidants*, 14(5), 585.

Miller, A. H., Maletic, V., & Raison, C. L. (2009). *Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. Biological Psychiatry*, 65, 732–741.

Ye, Y., Wang, Y., Yang, Y., & Tao, L. (2020). *Aloperine suppresses LPS-induced macrophage activation through inhibiting the TLR4/NF-κB pathway. Inflammation*, 69(4), 375–383.

Yuan, F., et al. (2020). *Emerging evidence on inflammation and depression. Journal of Affective Disorders.*