Pakistan J. Zool., pp 1-8, 2026. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20251201074741

Free Radical Scavenging Activity of Clove Oil and *Nigella sativa* Oil Diminishes Anxiety and Depression in Restrained Rats: A Comparative Study

Sana Sadaf[1,2]*, Mahinn Ali[3], Shaista Emad[2,4], Yousra Sarfaraz[2,5], Sarwat Yousuf[2], Sara Qadeer[2,6], Sheeza Sheikh[2], Zehra Batool[7] and Tahira Perveen[2]

[1]*Department of Biochemistry, Faculty of Health and Medical Sciences, Hamdard University, Karachi*
[2]*Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi*
[3]*Department of Surgery Unit IV, Dr. Ruth K.M Pfau Civil Hospital, Karachi.*
[4]*Department of Biochemistry, Jinnah Medical and Dental College, Sohail University, Karachi*
[5]*Department of Pathology and Laboratory Medicine, The Aga Khan University, Karachi*
[6]*Department of Allied Health & Medical Sciences, Al-Kawthar University, Karachi*
[7]*The Panjwani Centre for Molecular Medicine and Drug Research, The International Centre for Chemical and Biological Sciences, University of Karachi, Karachi*

Article Information
Received 01 December 2025
Revised 20 January 2026
Accepted 10 February 2026
Available online 17 August 2026
(early access)

Authors' Contribution
SS involved in conceptualization and writing original draft. AM and ES involved in data curation. YS and YS performed statistical analysis of data. QS, SS and BZ were involved in review and editing of the article. TP supervised and critically evaluate the research. All authors have read and agreed to the published version of the manuscript.

Key words
Clove oil, *Nigella sativa* L. oil, Oxidative stress, Antioxidant enzymes, Anxiety, Depression

ABSTRACT

Stress has now been known as an imperative aspect in the development of major depressive, bipolar as well as anxiety disorders. Antioxidant potential of clove and *Nigella sativa* L. oil (NSO) have become more popular in folk medicine as a preventive measure to treat many diseases around the globe. Study was intended to explore antioxidant potential of clove oil and NSO on restraint stress and also stress-associated psychological and biochemical amendment in rats. Therapeutic effects of clove oil and NSO were observed in unrestrained and 2 h restrained rats. Light dark box activity (LDA) and forced swim test (FST) models were facilitated to monitor anxiety and depression. Stress-induced biochemical and hormonal changes were estimated by measuring lipid peroxidation (LPO), antioxidant enzyme levels as well as plasma corticosterone levels. The results revealed that 2 h restraint stress significantly boosted anxiety and depression in rats ($p<0.01$). Pretreatment with clove and NSO significantly reduced anxiety and depression in restrained and unrestrained rats ($p<0.01$). It was also observed that stress-induced increase in LPO and corticosterone levels significantly decreased in clove and NSO treated restrained rats. Similarly, the activities of endogenous antioxidant enzymes are enhanced in restrained rats pretreated with clove and NSO. To conclude that administration of clove and NSO may have beneficial effects to improve overall stress related psychological ailments. Since these oils have lesser side effects, they may be a better choice for therapeutic aspects.

INTRODUCTION

Depression is the utmost mental disorders deteriorate physical function, increased disability, caused devastating health problem around globe and clues to substantial surge in suicide chances (Li *et al*., 2020). According to the WHO, depression leads nearly 4.3% of the worldwide ailment encumbrance and is estimated to be overgrown in 2030 (Dochert *et al*., 2017). The pharmacological effectiveness of numerous plant species

* Corresponding author: sana.sadaf@hamdard.edu.pk
0030-9923/2026/0001-0001 $ 9.00/0

has served as reliable sources of therapy and have been employed as complementary and alternative therapies for managing the psychiatric conditions (Tang and Tang, 2019). Several plants based essential oils are known to work efficiently in emotion homeostasis and to recover physical and mental well-being (Fung *et al*., 2021).

Numerous evidences revealed that the stress has substantial role in progression of neuro-psychiatric complications comprising depression and anxiety (Giglberger *et al*., 2025). The consequences of stressful actions change the physiological, psychological, and neurobehavioral responses that increase vulnerability to dissimilar psychiatric diseases (Giovanniello *et al*., 2023). Acute stress provokes the activation of hypothalamic-pituitary-adrenal (HPA) axis, raises corticosterone level and leads to significant production of free radicals (Spiers *et al*., 2015). The reactive oxygen species (ROS) cause oxidative stress. The free radicals have damaging effects on brain causing psychotic ailments. Management with antioxidant compounds have shown to diminish damaging pathological conditions (Tumilaar *et al*., 2024). The damaging effects of oxidative stress can be normalized by using plant-based antioxidants components and their phenolic and flavonoids compounds. Increasing the antioxidant levels will potentially provide protection from neuronal damage and mitigation from depression and anxiety symptoms (Jomova *et al*., 2023).

Within the spectrum of medicinal flora, cloves *(Syzium aromaticum, Eugenia caryophyllata* and *Eugenia aromaticum*) belonging to Mytraceae family, are known to have curative effects. Chief active components of the clove are eugenol, acetate derivative and beta-caryophyllene (Batiha *et al*., 2020). Clove oil may protect neurons, stimulate CNS, and enhance memory (Akbar *et al*., 2021). Other therapeutic properties of clove oil include anti-inflammatory, anti-hyperglycemic, antitoothache, anesthetic and hypotensive activities (Kaur and Kaushal, 2019).

Nigella sativa Linn., taxonomically belong to the Ranunculaceae family. A diverse sorts of flavonoids and bioactive constituents notably thymoquinone (TQ), thymohydroquinone, dithymoquinone, thymol and carvacrol have been isolated form essential oils of *Nigella* seeds (Ashfaq *et al*., 2021). Therapeutic activities of *Nigella sativa* include increased cognitive functions, analgesic, antidiabetic, antihypertensive, anticancer, antiinflammatory, LDL cholesterol decreasing, hepatoprotective, and antimicrobial activities (Alberts, 2024; Yimer *et al*., 2019). The study aimed to evaluate the antioxidant effects of clove oil and *Nigella sativa* oil (NSO) against restraint stress in rats. It is focused on assessing their ability to reduce oxidative stress, improve stress-induced behavioural and biochemical alterations. The study also compared the effectiveness of both oils and explored their potential as natural therapeutic agents for stress management. Therefore, current study in rats was intended to explore antioxidant activity of clove and NSO proceeding stress related psychological and biochemical alterations.

MATERIALS AND METHODS

Animals

Albino Wistar male rats (150-200g) were purchased from Husein Ebrahim Jamal Research Institute of Chemistry, University of Karachi, Pakistan. Social contact of animals was avoided by kept them separately with ad libitum admittance to good quality rodent food and normal drinking water exposed to 12:12 h light/dark regime at maintained ambient temperature (22±2 °C).

Oils and reagent

Nigella sativa oil was acquired from (Amir's oil), Karachi. Clove oil (Roghan-E-Long) was supplied from (Hamdard Laboratories, Waqf) Pakistan. Further chemicals were purchased from Sigma-Aldrich Company, USA.

Experimental protocol

Rats (36) were divided into three groups like control, clove oil and NSO groups. Once a day till three weeks the oral doses of 0.2 ml/kg/day of clove oil and NSO (Ozturk and Ozbek, 2005; Mehmet *et al*., 2005) were administered to the oil treated groups. At the end of the treatment, each group was divided into unrestrained (n=6) and restrained rats (n=6). Restrained group were restraint for two days (2 h/day) in specially designed closed aired plastic tubes that limited the sidewise movement of animals (Sadaf *et al*., 2017). Unrestrained group was housed in their cages during course of the experiment. Behavioral analysis was performed 24 h following the stress in both restrained and unrestrained rats. After the behavioral analysis rats were guillotined, and plasma samples were taken. After guillotining, immediately whole brains were removed from the cranium, kept at -70 °C for biochemical testing.

Behavioral assessment

Light dark box activity test (LDA)

LDA model described anxiety behavior in rats as described by Yousuf *et al*. (2018). The LDA comprised two sections: One enclosed by black plastic and the other by transparent material, both boxes having a size of 26 x 26 x 26 cm, and are attached through a middle gate 12 x 12 cm. The time spent by animals in a black box were supposed to depict anxiety like behavior. Therefore, animals first

located in a light box and time consumed for 5 minutes were recognized as an anxiolytic effect.

Forced swim test (FST)

Depressive behaviour of rats was assessed by the method defined by Lopez-Rodriguez *et al*. (2004). The FST setup used a 53 × 19 × 28 cm glass tank containing 18 cm of water. The water level was sufficient to prevent the rats from standing or maintaining balance and faces inescapable stress condition comparable to depressive state of a living person. Therefore, rats were individually housed in the tank for 5 min and their struggling time was noted as an extent of depressive behavior.

Biochemical estimations

Corticosterone (CORT) estimation

The corticosterone concentration (µg/dL) was measured in plasma by flurometry method (Misiak *et al*., 2020; Sadaf et al., 2026). In a glass stopper test tube 200 µL plasma and 1500 µL dichloromethane were poured and then centrifuged. After centrifugation aspirated layers were moved to other tubes and poured 1 mL of ethyl sulfate reagent was added in it. After an hour of incubation the upper organic layers were separated from bottom layer of ethyl sulfate reagent. The fluorescence of lower layer was determined at 460 nm while that of upper layer at 570 nm.

Estimation of LPO, SOD, CAT and GPx activities

For estimation of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), the procedure described by Sadaf *et al*. (2017) were followed.

Statistical analysis

Values are mean±SD. Two-way analysis of variance (ANOVA) was done by using SPSS version 23. Post hoc analyses through Tukey's test showed $p>0.05$ was measured as non-significant.

RESULTS AND DISCUSSION

An acute stress response can be a consequence of several underlying mechanisms, including increased exposure to glucocorticoid levels. Corticosterone cascade involves in the dysregulation of HPA-axis and may cause several psychological disorders (Perveen *et al*., 2014). Results of the current study demonstrated that 2 h of restraint stress significantly increased ($F=87.505$; $df=1,20$; $p<0.01$) corticosterone levels in rat plasma. In restrained group clove oil and NSO pretreatment attenuated the stressed-induced increase of corticosterone level ($F=113.912$; $df=1,20$; $p<0.01$). Whereas, in unrestrained group these oils have also significant modulation ($F=324.198$; $df=1,20$; $p<0.01$) on corticosterone levels with respect to their control. Current result showed that NSO has more activity in reducing corticosterone levels in restrained group as compared to clove oil (Fig. 1). Results of current study also aligned with the previous research which indicated that an active components eugenol of clove oil and thymoquinone of NSO reduced the stress increased plasma corticosterone levels via HPA axis modulation (Jain *et al*, 2017; Siyall *et al*, 2020). Thus, clove oil and NSO reduce corticosterone levels and decrease the effects of stress in restrained groups.

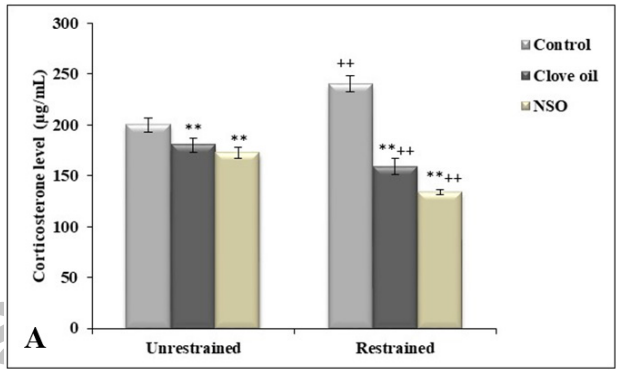

Fig. 1. Effect of clove oil and NSO on corticosterone level in 2h restrained rats.
Values are mean+SD (n=6). Significant differences by Tukey's test ++p <0.01 from their respective unrestrained control.

In current study, 2 h restraint stress increased anxiety and depression-like symptoms as it is evident by significant reduction of time spent in light compartment of LDA ($F=11.976$; $df=1,20$; $p<0.01$) and decreased struggling time in FST ($F=140.0$; $df=1,20$; $p<0.01$) in restrained rats. Pretreatment with clove oil and NSO provided significant protections against restraint stress by enhanced time spent in the light compartment of LDA ($F=30.814$; $df=1,20$; $p<0.01$). Whereas, in unrestrained group both oils showed significant reduction of time spent ($F=15.196$; $df=1,20$; $p<0.01$).) in light compartment as compared to their controls. NSO showed more time spent in restrained and unrestrained groups as compared to clove oil (Fig. 2A). The pretreated clove oil and NSO rats significantly increased struggling time in restrained ($F=37.998$; $df=1,20$; $p<0.01$) and unrestrained groups ($F=1391.345$; $df=1,20$; $p<0.01$). However, the elevated struggling time in FST were recorded by clove oil and NSO had similar effects in unrestrained and restrained group (Fig. 2B). Therefore, the results of current study showed anxiolytic

and antidepressant activity of these oils with respect to the results of restrained group. The daily use of NSO has been shown to help in coping with stressful situations (Perveen et al., 2014). Stress affects the regulation of 5-hydroxytryptamine, 5-HT (serotonin). Stress-induced behavioural deficits in animals are widely used as the animal model of depression. 5-HT plays an important role in the modulation of various behaviours. The conventional hypothesis of serotonergic functions alteration in the brain associated with depression and anxiety. However, increased 5-HT levels produce antidepressant effects. (Perveen et al., 2014). It has previously been shown that repeated treatment of NSO boosted tryptophan and 5-HT levels while decreasing 5-Hydroxyindoleacetic acid levels (Mesole et al., 2020). Stress-induced changes in brain regional 5-HT levels could also be reversed by clove oil component eugenol (Cecerska et al., 2022).

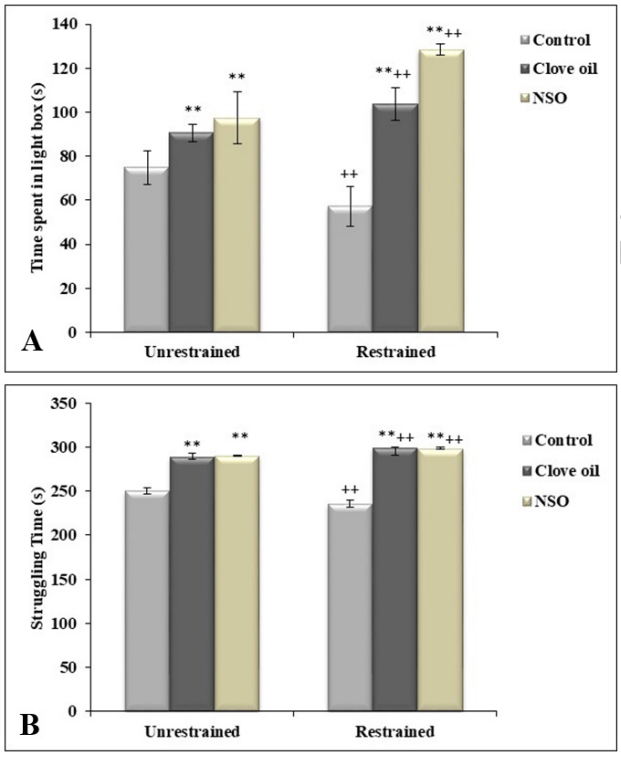

Fig. 2. Effect of clove oil and NSO on light and dark box activity (A) and forced swim test (B) in 2h restrained rats. See Figure 1 for statistical details.

The pathogenesis of numerous neuropsychiatric ailments including anxiety and depression has been linked to oxidative stress (Abdelkhalek *et al.*, 2020). This study further illustrated that 2 h of restraint stress induces oxidative damage by significantly augmented (F=80.74; df=1,20; p<0.01) LPO in rat's brain. MDA is a biomarker of brain lipid peroxidation. Restrained rats had higher MDA levels, which is one of the indicator of brain lipid peroxidation, indicating that 2 h of restraint stress compromised the integrity of brain tissues. However, clove oil and NSO pretreated rats significantly reduced (F=181.968; df=1,20; p<0.01) LPO levels and reversed the effects of 2 h of restraint stress. The MDA lowering effect of these oils (F=105.860; df=1,20; p<0.01) were also comparable with respect to their control in unrestrained group. However, reduction in MDA level was observed more in NSO treated rats in both groups (Fig 3A). Clove oil reduced oxidative damage by lowering MDA level (Rahim *et al.*, 2022). NSO contains bioactive components and necessary fatty acids that inhibit cell membrane oxidation and reduced LPO levels (Zhang *et al.*, 2021). Figure 3B, C and D showed modulatory impact of clove oil and NSO on antioxidant enzyme activities. The result for SOD, showed significant effect of stress (F=30.24; df=1,20; p<0.01), significant effect of clove oil and NSO in unrestrained group (F=42.579; df=1,20; p<0.01) and significant association among stress, clove oil and NSO (F=157.844; df=1,20; p<0.01) in restrained group (Fig 3B). Data analysis for CAT revealed significant effect of stress (F=65.237; df=1,20; p<0.01), significant effect of clove oil and NSO (F=80.548; df=1,20; p<0.01) in unrestrained group, and significant effect among stress, clove oil and NSO (F=88.470; df=1,20; p<0.01) in restrained group (Fig 3C). Data analysis for GPx showed significant effects of stress (F=72.476; df=1,20; p<0.01) significant effect of clove oil and NSO (F=82.90; df=1,20; p<0.01) in unrestrained group and significant relation among stress, clove oil and NSO (F=92.81; df=1,20; p<0.01) (Fig 3D).

The current study showed that 2 h restraint stress decreased SOD and increased CAT and GPx activities. Clove oil suppressed SOD generation and more significantly scavenged superoxide radicals and significantly improved the levels of SOD, CAT and GPx in restrained group and unrestrained group as compared to NSO (Nisar et al., 2021). Result of current study demonstrated that NSO has also significant activity to enhanced SOD activity in restrained group. It was observed that clove oil significantly increased CAT and GPx activities in restrained group whereas, NSO significantly enhanced the activities of CAT and GPx in unrestrained groups which shown the potential antioxidant activity of both oils. Antioxidant properties of eugenol stops propagation of oxidative stress and can cross blood brain barrier because of its hydrophobic nature that raises endogenous antioxidant enzymes levels in the brain (Pottoo et al., 2022). Repeated administration of NSO decreased SOD level and increased CAT and GPx levels in rats brain (Sadaf et al., 2017). The quinone structure of TQ has strong redox properties. It can cross the blood

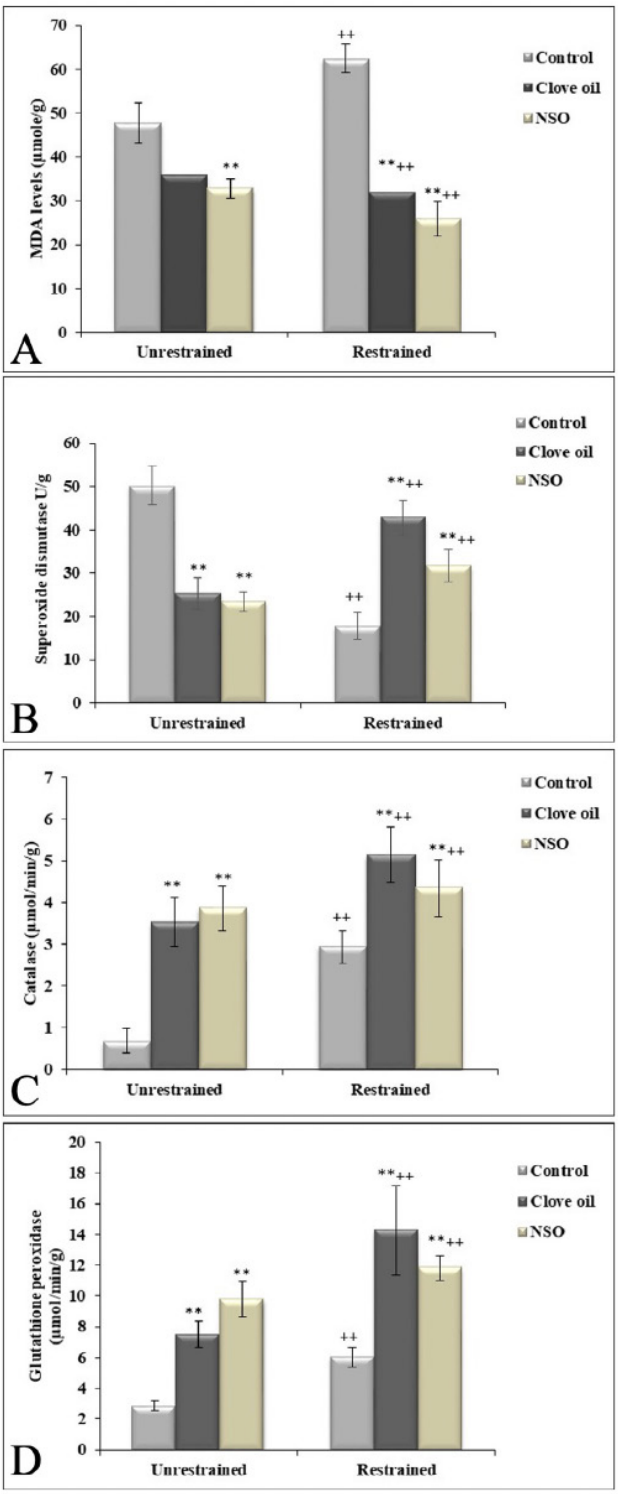

Fig. 3. Effect of clove oil and NSO on brain MDA levels (A), SOD (B) and CAT (C) and GPx (D) activities in 2 h restrained rats.
See Figure 1 for statistical details.

brain barrier and protects CNS from damaging effects of stress. Additionally, it has the competency to cross morphophysiological barriers, leading to its effortless access to subcellular compartments and enabling the radical scavenging effects (Isaev et al., 2023).

Despite the fact that clove oil and NSO have been studied for their diverse behavioral effects, only a few studies outlined the effect of these oils on anxiety and depression caused by oxidative stress (Sulakhiya *et al*., 2016; Sung *et al*., 2024). Consequently, the result of the present study demonstrated that 2 h restraint stress induced oxidative stress and produced anxiety and depression which were attenuated by antioxidant potential of clove oil and NSO. Studies reported that eugenol and β-caryophyllene of clove oil have antidepressant effect due to its antioxidant activity by decreasing LPO Sung *et al*., 2024). TQ also exhibits antidepressant activity, which is attributed to its antioxidant properties, notably through the reduction of thiobarbituric acid reactive substances (TBARS) levels (Bahi *et al*., 2014). The current outcomes of the research proposed that NSO possesses stronger anxiolytic effects. Both NSO and clove oil exhibited significant antidepressant activity. Notably, clove oil exhibited superior antioxidant potential in the restrained group as compared to NSO. In addition, clove oil and NSO showed significant anxiolytic, antidepressive and antioxidant activities in rats may be due to possible modulation of corticosterone, MDA, and antioxidant enzymes level.

CONCLUSION

Stress induced oxidative stress linked with anxiogenic and depressive behaviors in rats ameliorated by antioxidant potency of clove and NSO. These oils were found to be more operative to scavenge free radicals in rats brain and also exhibited imperative role to declined free radical generated anxiety and depression. Further investigations are needed to explore on active components of clove oil and NSO for their antioxidant activities.

DECLARATIONS

Acknowledgments

Research support from the University of Karachi, Pakistan, is gratefully acknowledged.

Funding

Financial support provided by University of Karachi, Pakistan.

Ethical statement and IRB approval

Before starting the experiment, an official consent

was taken through Institutional Review Board (IRB) Of University of Karachi, No.03363/SC-2015 and adhering to the animal care principles established in the revised 1985 edition of the National Institute of Health NIH Guide (Publication No. 85-23).

Data availability

The current study is available from the corresponding author upon reasonable request. The authors declare full data transparency.

Generative AI and AI-assisted technology statement

The authors confirm that no generative AI or AI-assisted technologies were used in the writing, data analysis, or preparation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdelkhalek, N.K., Risha, E., Mohamed, A., Salama, M.F. and Dawood, M.A., 2020. Antibacterial and antioxidant activity of clove oil against *Streptococcus iniae* infection in Nile tilapia (*Oreochromis niloticus*) and its effect on hepatic hepcidin expression. *Fish Shellfish Immunol.,* **104:** 478–488. https://doi.org/10.1016/j.fsi.2020.05.064

Akbar, L., Juliandi, B., Boediono, A., Batubara, I. and Subangkit, M., 2021. Effects of eugenol on memory performance, neurogenesis, and dendritic complexity of neurons in mice analyzed by behavioral tests and Golgi staining of brain tissue. *J. Stem Cells Regen. Med*., **17**: 3-5. https://doi.org/10.46582/jsrm.1701005

Alberts, A., Moldoveanu, E.T., Niculescu, A.G. and Grumezescu, A.M., 2024. *Nigella sativa:* A comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications. *Int. J. mol. Sci.,* **25:** 13410. https://doi.org/10.3390/ijms252413410

Ashfaq, S., Khan, N.T. and Ali, G.M., 2021. *Nigella sativa* (Kalonji), its essential oils and their therapeutic potential. *Biomed. J. Sci. Tech. Res.,* **33:** 25448–25454. https://doi.org/10.26717/BJSTR.2021.33.005335

Bahi, A., Al-Mansouri, S., Al-Memari, E., Al-Ameri, M., Nurulain, S.M. and Ojha, S., 2014. β-Caryophyllene, a CB2 receptor agonist, produces multiple behavioral changes relevant to anxiety and depression in mice. *Physiol. Behav.,* **135:** 119–124. https://doi.org/10.1016/j.physbeh.2014.06.003

Batiha, G.E., Alkazmi, L.M., Wasef, L.G., Beshbishy, A.Z., Nadwa, E.H. and Rashwan, E.K., 2020. *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules.,* **10**: 352. https://doi.org/10.3390/biom10020202

Cecerska-Heryć, E., Polikowska, A., Serwin, N., Roszak, M., Grygorcewicz, B., Heryć, R., Michalczyk, A. and Dołęgowska, B., 2022. Importance of oxidative stress in the pathogenesis, diagnosis, and monitoring of patients with neuropsychiatric disorders: A review. *Neurochem. Int.,* **153:** 105269. https://doi.org/10.1016/j.neuint.2021.105269

Docherty, A.R., Edwards, A.C., Yang, F., Peterson, R.E., Sawyers, C., Adkins, D.E., Moore, A.A., Webb, B.T., Bacanu, S.A., Flint, J. and Kendler, K.S., 2017. Age of onset and family history as indicators of polygenic risk for major depression. *Depress. Anxiety.,* **34:** 446–452. https://doi.org/10.1002/da.22607

Fung, T.K., Lau, B.W., Ngai, S.P. and Tsang, H.W., 2021. Therapeutic effect and mechanisms of essential oils in mood disorders: Interaction between the nervous and respiratory systems. *Int. J. mol. Sci.,* **22:** 4844. https://doi.org/10.3390/ijms22094844

Giglberger, M., Peter, H.L., Henze, G.I., Bärtl, C., Konzok, J., Kirsch, P., Kudielka, B.M., Kreuzpointner, L. and Wüst, S., 2025. Associations between the neural stress response and symptoms of anxiety and depression. *J. Neurosci. Res.,* **103**: e70019. https://doi.org/10.1002/jnr.70019

Giovanniello, J., Bravo-Rivera, C., Rosenkranz, A. and Lattal, K.M., 2023. Stress, associative learning, and decision-making. *Neurobiol. Learn. Mem.,* **204:** 107812. https://doi.org/10.1016/j.nlm.2023.107812

Isaev, N.K., Genrikhs, E.E. and Stelmashook, E.V., 2023. Antioxidant thymoquinone and its potential in the treatment of neurological diseases. *Antioxidants,* **12:** 433. https://doi.org/10.3390/antiox12020433

Jomova, K., Raptova, R., Alomar, S.Y., Alwasel, S.H., Nepovimova, E., Kuca, K. and Valko, M., 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Arch. Toxicol.,* **97:** 2499–2574. https://doi.org/10.1007/s00204-023-03562-9

Kaur, K. and Kaushal, S., 2019. Phytochemistry and pharmacological aspects of *Syzygium aromaticum*: A review. *J. Pharmacogn. Phytochem*., **8:** 398–406.

Li, M., Fu, X., Xie, W., Guo, W., Li, B., Cui, R. and Yang,

W., 2020. Effect of early life stress on the epigenetic profiles in depression. *Front. Cell Dev. Biol.,* **8:** 867. https://doi.org/10.3389/fcell.2020.00867

Lopez-Rodriguez, F., Kim, J. and Poland, R.E., 2004. Total sleep deprivation decreases immobility in the forced-swim test. *Neuropsychopharmacology,* **29:** 1105. https://doi.org/10.1038/sj.npp.1300406

Mehmet, K., Omer, C.O. and Budancamanak, M., 2005. Hepatoprotective effects of *Nigella sativa* oil L. and Urtica dioca L. on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride treated rats. *World J. Gastroenterol*., **11:** 6684–6688. https://doi.org/10.3748/wjg.v11.i42.6684

Mesole, S.B., Alfred, O.O., Yusuf, U.A., Lukubi, L. and Ndhlovu, D., 2020. Apoptotic inducement of neuronal cells by aluminium chloride and the neuroprotective effect of eugenol in Wistar rats. *Oxid. Med. Cell. Longev.,* **2020:** 8425643. https://doi.org/10.1155/2020/8425643

Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L. and Samochowiec, J., 2020. The HPA axis dysregulation in severe mental illness: Can we shift the blame to gut microbiota? *Prog. Neuropsychopharmacol. Biol. Psych.,* **102:** 109951. https://doi.org/10.1016/j.pnpbp.2020.109951

Nisar, M.F., Khadim, M., Rafiq, M., Chen, J., Yang, Y. and Wan, C.C., 2021. Pharmacological properties and health benefits of eugenol: A comprehensive review. *Oxid. Med. Cell. Longev*., **2021**: 2497354. https://doi.org/10.1155/2021/2497354

Ozturk, A. and Ozbek, H., 2005. The anti-inflammatory activity of *Eugenia caryophyllata* essential oil: An animal model of anti-inflammatory activity. *Eur. J. Gen. Med.,* **2:** 159–163. https://doi.org/10.29333/ejgm/82334

Perveen, T., Haider, S., Zuberi, N.A., Saleem, S., Sadaf, S. and Batool, Z., 2014. Increased 5-HT levels following repeated administration of *Nigella sativa* L. (black seed) oil produce antidepressant effects in rats. *Sci. Pharm*., **82:** 161–170. https://doi.org/10.3797/scipharm.1304-19

Pottoo, F.H., Ibrahim, A.M., Alammar, A., Alsinan, R., Aleid, M., Alshehhi, A., Alshehri, M., Mishra, S. and Alhajri, N., 2022. Thymoquinone: Review of its potential in the treatment of neurological diseases. *Pharmaceutical,* **15:** 408. https://doi.org/10.3390/ph15040408

Rahim, M.A., Shoukat, A., Khalid, W., Ejaz, A., Itrat, N., Majeed, I., Koraqi, H., Imran, M., Nisa, M.U., Nazir, A. and Alansari, W.S., 2022. A narrative review on various oil extraction methods, encapsulation processes, fatty acid profiles, oxidative stability, and medicinal properties of black seed (*Nigella sativa*). *Foods,* **11:** 2826. https://doi.org/10.3390/foods11182826

Sadaf, S., Emad, S., Siddiqui, N.A., Ghafoor, S., Yousuf, S., Jabeen, B., Haider, S. and Perveen, T., 2017. Enhancement of memory function by antioxidant potential of *Nigella sativa* L. oil in restrained rats. *Pakistan J. Pharm. Sci.,* **30:** 2039–2046.

Sadaf, S., Shazia., D., Shaista., E., Sarwat., Y., Qadeer, S., Yousra., S., Sheeza S. and Perveen T., 2026. Impact of clove oil on behavioral and biochemical parameters in restrained rats by using docking and experimental approaches. *Pak. J. Pharm. Sci.,* **39**: 381-388.

Spiers, J.G., Chen, H.J.C., Sernia, C. and Lavidis, N.A., 2015. Activation of the hypothalamic–pituitary–adrenal stress axis induces cellular oxidative stress. *Front. Neurosci*., **8:** 456. https://doi.org/10.3389/fnins.2014.00456

Sulakhiya, K., Patel, V.K., Saxena, R., Dashore, J., Srivastava, A.K. and Rathore, M., 2016. Effect of Beta vulgaris Linn. leaves extract on anxiety- and depressive-like behavior and oxidative stress in mice after acute restraint stress. *Pharmacogn., Res*., **8:** 1–7. https://doi.org/10.4103/0974-8490.171100

Sung, W.W., Yeh, T.M. and Shih, W.L., 2024. Additive effect of clove essential oil combined with hydrogen inhalation improves psychological harm caused by lipopolysaccharide in mice. *BMC Complement. Med. Ther*., **24:** 399. https://doi.org/10.1186/s12906-024-04682-0

Tang, S.W. and Tang, W.H., 2019. Opportunities in novel psychotropic drug design from natural compounds. *Int. J. Neuropsychopharmacol*., **22:** 601–607. https://doi.org/10.1093/ijnp/pyz042

Tumilaar, S., Hardianto, A., Dohi, H. and Kurnia, D., 2024. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *J. Chem.*, **2024:** 5594386. https://doi.org/10.1155/2024/5594386

Yimer, E.M., Tuem, K.B., Karim, A., Ur-Rehman, N. and Anwar, F., 2019. *Nigella sativa* L. (Black Cumin): A promising natural remedy for wide range of illnesses. *Evid. Based Complement. Altern. Med.,* **2019:** 1–17. https://doi.org/10.1155/2019/1528635

Yousuf, S., Emad, S., Ahmad, S., Qadeer, S., Sadaf, S., Sheikh, S., Sarfaraz, Y., Mehdi, B.J. and Perveen,

T., 2018. Alteration in redox profile and behavioral effects following repeated administration of citral in rats. *Pak. J. Pharm. Sci*., **31:** 2639–2644.

Zhang, L., Gu, B. and Wang, Y., 2021. Clove essential oil confers antioxidant activity and lifespan extension in *C. elegans* via the DAF-16/FOXO transcription factor. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*., **242:** 108938. https://doi.org/10.1016/j.cbpc.2020.108938