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Neuroprotective Effects of *Nigella sativa* Oil against Dibutyl Phthalate-induced Neurotoxicity in Prefrontal Cortex of Wistar Rats

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ABSTRACT

The prefrontal cortex regulates executive functions, including decision-making, emotion regulation, and behavior. Environmental toxicants like di-butyl phthalate (DBP), a commonly used plasticizer, have been implicated in oxidative stress and neuronal dysfunction. This research explored the protective role of *Nigella sativa* oil (NSO) against DBP-induced neurotoxicity in the prefrontal cortex of male Wistar rats. Forty rats were randomly assigned into five groups: control, NSO only (2 mL/kg b.w.), DBP only (500 mg/kg b.w.), DBP + NSO, and DBP withdrawal. Treatments were administered orally for 35 days, except the withdrawal group, which received DBP for 21 days followed by no treatment. Behavioral performance was evaluated using the open field test. Prefrontal cortex weights were recorded, and oxidative stress biomarkers, including malondialdehyde (MDA) and catalase, were assessed in tissue homogenates. Histological analysis was performed using cresyl violet staining. DBP administration caused significant reductions in body and brain weights, impaired exploratory behavior, increased MDA, reduced antioxidant enzyme activity, and induced neuronal degeneration. Co-administration of NSO preserved body and brain weights, improved behavioral outcomes, restored oxidative balance, and maintained normal neuronal architecture, while DBP withdrawal showed partial but incomplete recovery. These results demonstrated that NSO protected the neurons in the prefrontal cortex from the toxic effects of DBP. This was primarily due to its antioxidant action. Therefore, NSO can be considered a natural therapeutic agent against environmental neurotoxins.

Keywords: neurotoxicity, *Nigella sativa* oil, prefrontal cortex, oxidative stress

INTRODUCTION

Phthalates are synthetic esters of phthalic acid widely used as plasticizers in consumer goods, including food packaging, cosmetics, medical devices, and household products [1]. Their pervasive use has led to extensive human exposure through ingestion, inhalation, and dermal absorption, raising concerns about their long-term effects on health. More and more evidence shows that phthalates have endocrine-disrupting effects and thus interfere with the normal hormonal regulation, which in turn can cause developmental, reproductive, and neurological disorders. One of the most concerning compounds is di-butyl phthalate (DBP), a low-molecular-weight phthalate that is frequently present in the environment and can disrupt neuronal homeostasis after crossing the blood-brain barrier[6]. Animal studies have demonstrated that DBP exposure can impair neural development and function through mechanisms involving oxidative stress, mitochondrial dysfunction, and disruption of neurotransmitter systems [3,4]. For example, DBP-induced neurotoxicity has been associated with anxiety-like behaviors and impaired cognitive performance in rodents [5,6]. Histological evaluations have further revealed DBP-induced alterations in brain morphology, including neuronal loss and disrupted neurogenesis, suggesting that phthalate exposure may compromise structural and functional integrity of critical brain regions such as the hippocampus and prefrontal cortex [7]. Despite these findings, the neurotoxic potential of DBP in higher-order cortical areas remains insufficiently studied.

In parallel, natural antioxidants are increasingly being investigated for their capacity to mitigate toxicant-

induced neural injury. *Nigella sativa* oil (NSO), derived from black seed, has gained attention due to its bioactive compounds, particularly thymoquinone, which exhibit strong antioxidant, anti-inflammatory, and neuroprotective properties [8, 9]. These findings highlight its potential as a therapeutic intervention against chemically induced brain damage. Despite growing evidence that phthalates compromise neural health, there is still limited understanding of how DBP specifically impacts higher-order brain regions such as the prefrontal cortex, which governs executive function and emotional regulation. At the same time, natural antioxidants like *Nigella sativa* oil have gained recognition as promising neuroprotective agents, yet their potential against DBP-induced cortical neurotoxicity remains relatively underexplored. This study was therefore designed to investigate the impact of chronic DBP exposure on growth, behavior, oxidative balance, and neuronal integrity in the prefrontal cortex of rats, while also evaluating whether *Nigella sativa* oil can mitigate these toxic effects. By addressing this gap, the study aims to provide more insights into the mechanisms of phthalate-induced brain injury and the therapeutic promise of plant-derived interventions.

MATERIALS AND METHODS

Ethical consideration

All experimental procedures were conducted in compliance with the Ethical Review Committee guidelines of Al-Hikmah University, Ilorin, Nigeria, with approval number HUI-FHS-ERC-25-0037. The principles of the 3Rs (Replacement, Reduction, and Refinement) were strictly followed in the handling, housing, and care of animals to ensure animal welfare and scientific validity.

Chemicals and reagents

Dibutyl phthalate (DBP) was obtained from Central Drug House (P) Ltd., New Delhi, India (Batch No: 171019), while *Nigella sativa* oil (NSO) was procured from Kahira Pharmaceutical and Chemical Industries Company, Egypt. All other reagents, including sucrose, formalin, ethanol, and cresyl fast violet, were of analytical grade.

Experimental animals

Forty adult male Wistar rats weighing 180-220g were purchased from the University of Ilorin animal facility. The animals were acclimated for two weeks before the commencement of the study and were maintained in plastic cages under a 12-hour light/dark cycle at 22-25°C and 50-60% humidity. Standard rat chow and water were provided ad libitum.

Experimental design and grouping

The animals were randomly divided into five groups of eight rats each. Group A served as the control and received normal saline, Group B received *Nigella sativa* oil (2 ml/kg b.w., orally), Group C received DBP (500 mg/kg b.w., orally), and Group D received a combination of DBP (500 mg/kg b.w.) and NSO (2 ml/kg b.w., orally). In contrast, Group E received DBP (500 mg/kg b.w., orally) for 21 days followed by withdrawal without further treatment. Groups A to D received their respective treatments daily for a period of 35 days. The dose of *Nigella sativa* oil (2 ml/kg body weight) was selected based on previous experimental studies in which this concentration was used as an effective dose and reported to exert neuroprotective and antioxidant effects without observable toxicity in rodent models [10, 11].

Neurobehavioral assessment

Neurobehavioral performance was evaluated using the open field test (OFT) conducted 24 hours after the last administration. Each rat was placed individually in the center of a 100 × 100 cm arena divided into 16 equal squares, with the central square outlined in red. Exploratory behavior, including locomotion, center square entries, and time spent in the center square, was recorded for 5 minutes using video tracking. The apparatus was wiped with ethanol after each trial to eliminate residual odor cues.

Termination of experiment and tissue collection

At the end of the study, animals were euthanized by cervical dislocation. Brains were excised immediately, and the prefrontal cortex (PFC) was carefully dissected and divided for histological and biochemical investigations.

Biochemical analysis

For biochemical assays, PFC tissues (n = 5 per group) were homogenized in 0.25 M sucrose solution (1:4 w/v), centrifuged at 5000 rpm for 10 minutes at 4 °C, and the supernatants were stored at –20 °C. Oxidative stress biomarkers were assessed: malondialdehyde (MDA) levels were determined using the thiobarbituric acid reactive substances (TBARS) assay at 532 nm, catalase activity was measured by the rate of hydrogen peroxide decomposition at 520 nm, and total glutathione (GSH) levels were quantified using a colorimetric assay kit according to the manufacturer's instructions.

Histological analysis

For histological assessment, PFC samples (n = 3 per group) were fixed in 10% formalin, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. Tissue sections of 4 µm thickness were obtained with a rotary microtome and stained with Cresyl Fast Violet (CFV) to demonstrate Nissl substance integrity.

Statistical analysis

Data were expressed as mean ± SEM and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p<$

0.05, and all analyses were performed using ezAnova software.

RESULTS

Effect of di-butyl phthalate and Nigella sativa oil administration on body weight

Morphological assessment revealed significant changes in body weight across the experimental groups (Figure 1)**.** Control and *Nigella sativa* oil–treated rats exhibited normal weight gain over the study period, whereas dibutyl phthalate exposure resulted in impaired growth. Co-administration of *Nigella sativa* oil attenuated DBP-induced weight loss, while withdrawal from DBP exposure led to partial recovery of body weight.

Effect of di-butyl phthalate and Nigella sativa oil administration on brain weight

Organ weight assessment demonstrated significant differences in brain weights across the experimental groups (Figure 2). Rats in the control and *Nigella sativa*–treated groups maintained normal brain weights, with the NS-treated group showing a modest but significant increase relative to control. In contrast, exposure to dibutyl phthalate resulted in a marked reduction in brain weight. Co-administration of DBP with *Nigella sativa* significantly attenuated this reduction, although brain weight remained lower than in the control and NS-only groups. Animals in the withdrawal group exhibited partial recovery of brain weight; however, values remained significantly reduced compared to the control group.

Effect of di-butyl phthalate and NSO administration on open field test performance

Exploratory behavior in the open field test showed significant ($P< 0.05$) differences among groups.

Time spent in center square and outer edge

The number of center square entries varied significantly among the experimental groups. Control rats exhibited higher exploratory activity, whereas DBP-treated rats showed a marked reduction in center square entries, suggestive of anxiety-like behavior. Rats administered *Nigella sativa* oil alone demonstrated the highest exploratory activity. Co-administration of DBP with NSO significantly improved center square entries compared with DBP alone, although values remained lower than those of the control group. The DBP withdrawal group showed partial recovery but did not fully normalize exploratory behavior (Figure 3).

Centre square entry

Time spent in the center square differed across groups, with NSO-treated rats exhibiting the greatest central exploration, followed by the control group. In contrast, DBP-treated rats showed reduced center occupancy accompanied by increased preference for the outer edges, indicative of anxiety-like behavior. Co-administration with NSO ameliorated this effect relative to DBP alone, while the withdrawal group demonstrated modest improvement without full normalization (Figure 4).

Effect of DBP and NSO administration on oxidative stress

Levels of malondialdehyde (MDA)

The mean MDA concentration differed significantly among the experimental groups (Figure 5). DBP exposure increased lipid peroxidation, with the DBP withdrawal and DBP-only groups showing the highest levels. Rats treated with *Nigella sativa* oil alone exhibited a marked reduction in MDA, while co-administration with DBP further attenuated lipid peroxidation. The control group maintained the lowest MDA levels.

Activities of catalase

Catalase activity differed significantly among the experimental groups. DBP exposure markedly reduced catalase activity, with the DBP-only group showing the lowest levels. The DBP withdrawal group demonstrated partial recovery, while co-administration of DBP with *Nigella sativa* oil significantly improved catalase activity relative to DBP alone. Rats treated with NSO alone exhibited the highest catalase activity, and the control group maintained elevated activity, higher than DBP but lower than NSO alone. (Figure 6).

Histological findings

Histological evaluation of the prefrontal cortex revealed distinct neuronal changes across the experimental groups. Control and NSO-treated rats exhibited normal cortical architecture with well-preserved neurons and prominent Nissl granules (Figures 7 [×100] and 8 [×400]). In contrast, DBP exposure induced marked neuropathological alterations, including neuronal degeneration, shrunken cell bodies, and reduced Nissl substance. Co-administration of DBP with NSO attenuated these changes, showing improved neuronal preservation, while the DBP withdrawal group demonstrated partial recovery with moderate reappearance of Nissl bodies compared to DBP alone.

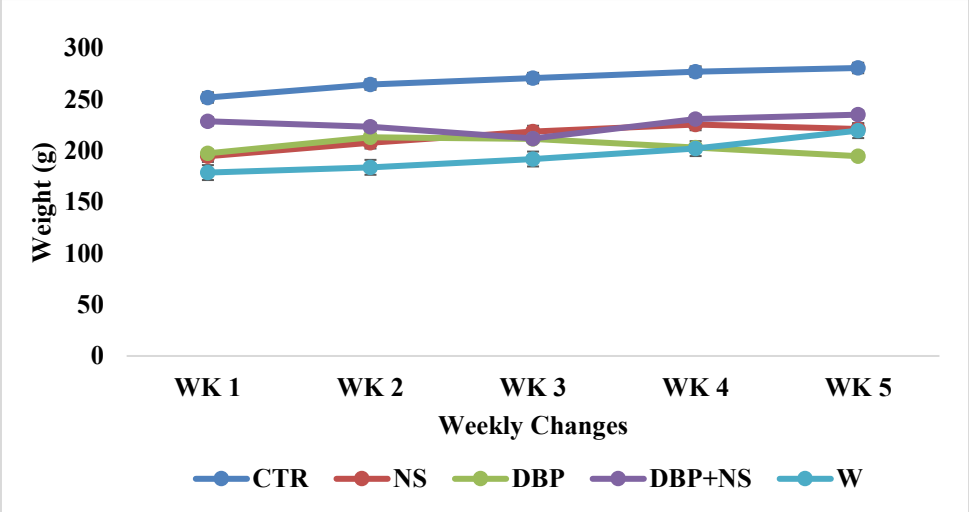

Figure 1: Graph showing the effect of DBP and NSO administration on the body weight of experimental animals. (*) indicates statistical significance compared to the control group, (±) indicates statistical significance compared to the NS group, while CTR = control (normal saline), NS = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NS = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and W = di-butyl phthalate withdrawal group (500 mg/kg b.w., 21 days)

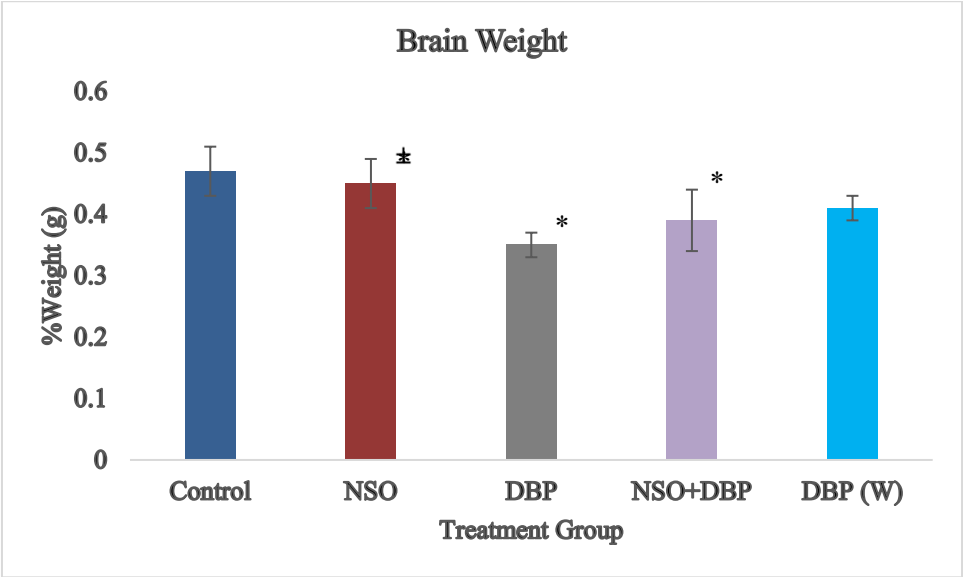

Figure 2: Graph showing the effect of DBP and NSO administration on brain weight of experimental animals. (*) indicates statistical significance compared to the control group, while (±) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NSO = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and DBP W = di-butyl phthalate withdrawal group (500 mg/kg b.w. 21 days)

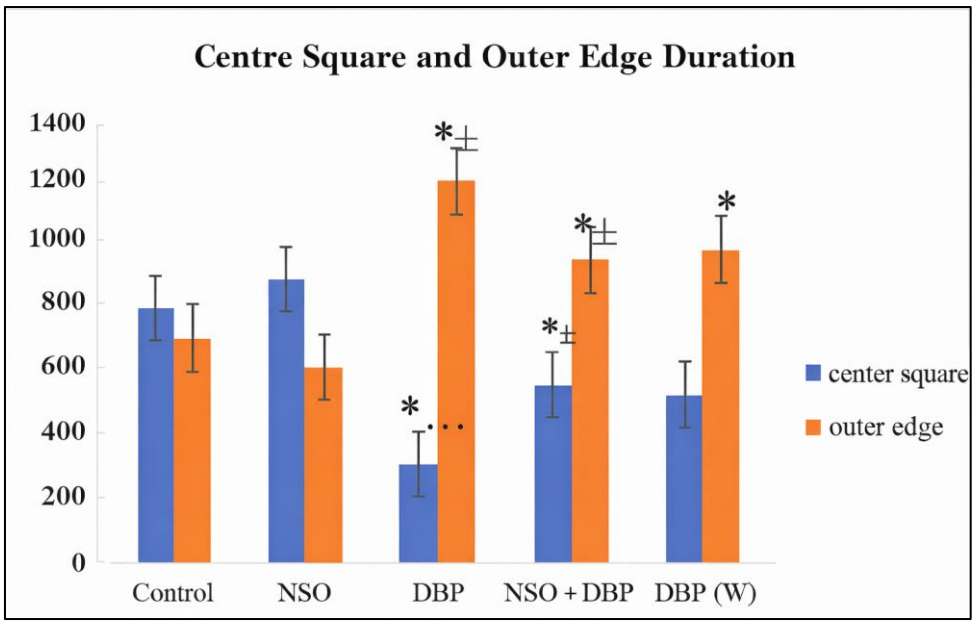

Figure 3: Graph showing the Centre Square vs Outer Edge duration in the open field test. (*) indicates statistical significance compared to the control group, while (±) indicates statistical significance compared to the NS group; CTR = control (normal saline), NS = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NS = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and W = di-butyl phthalate withdrawal group (500 mg/kg b.w., 21 days)

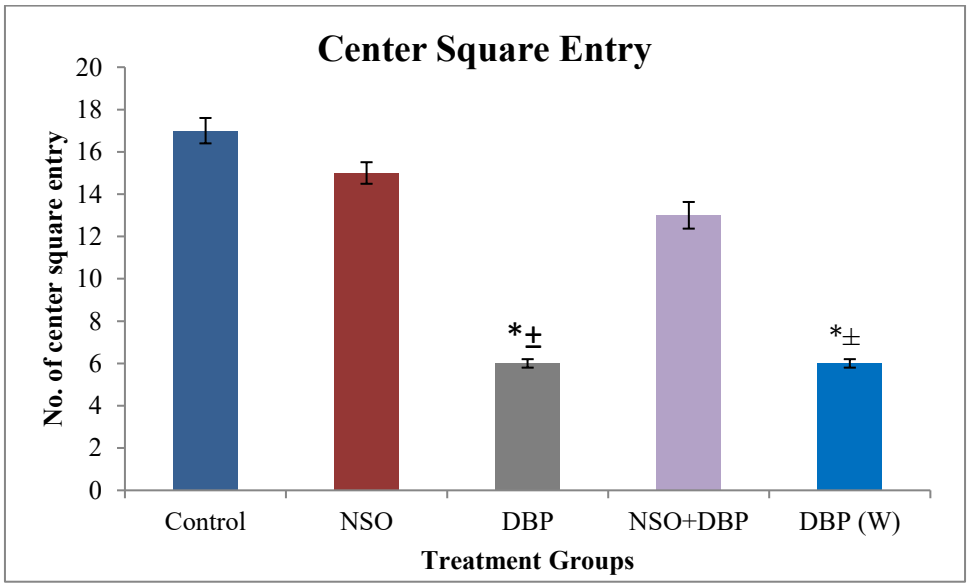

Figure 4: Graph showing the Centre square entry in the open field test. (*) indicates statistical significance compared to the control group, while (±) indicates statistical significance compared to the NS group; CTR = control (normal saline), NS = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NS = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and W = di-butyl phthalate withdrawal group (500 mg/kg b.w., 21 days

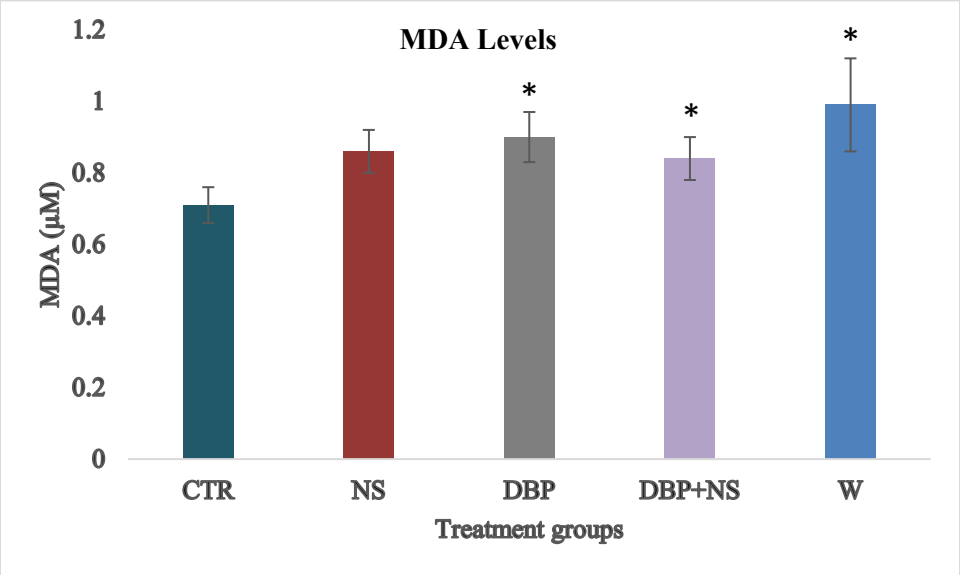

Figure 5: Graph showing the effect of DBP and NSO on MDA levels across experimental groups. (*) indicates statistical significance compared to the control group. CTR = control (normal saline), NS = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NS = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and W = di-butyl phthalate withdrawal group (500 mg/kg b.w., 21 days).

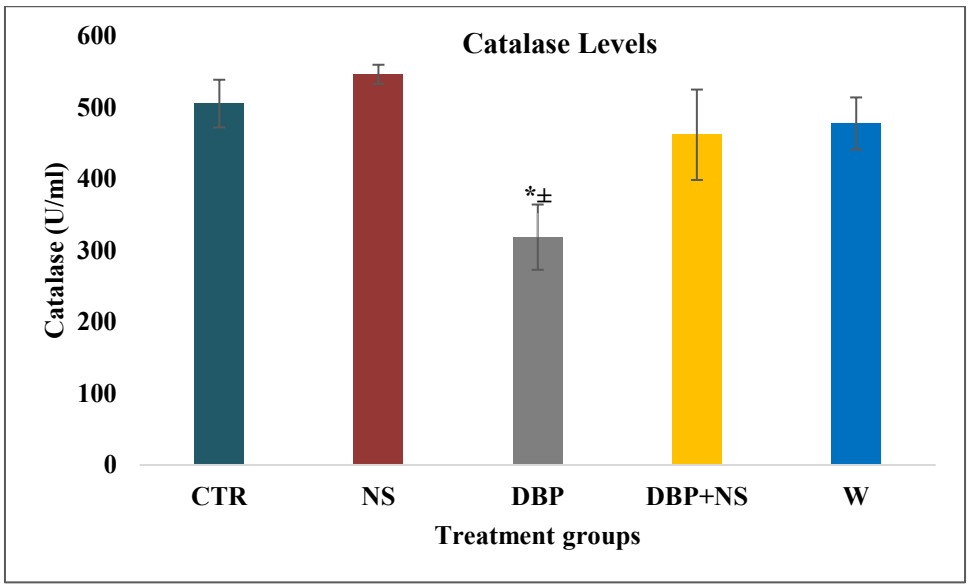

Figure 6: Graph showing the effect of DBP and NSO on catalase activities across experimental groups. (*) indicates statistical significance compared to the control group, while (±) indicates statistical significance compared to the NS group; CTR = control (normal saline), NS = Nigella sativa oil (2 ml/kg b.w.), DBP = di-butyl phthalate (500 mg/kg b.w.), DBP+NS = di-butyl phthalate (500 mg/kg b.w.) + Nigella sativa oil (2 ml/kg b.w.), and W = di-butyl phthalate withdrawal group (500 mg/kg b.w., 21 days).

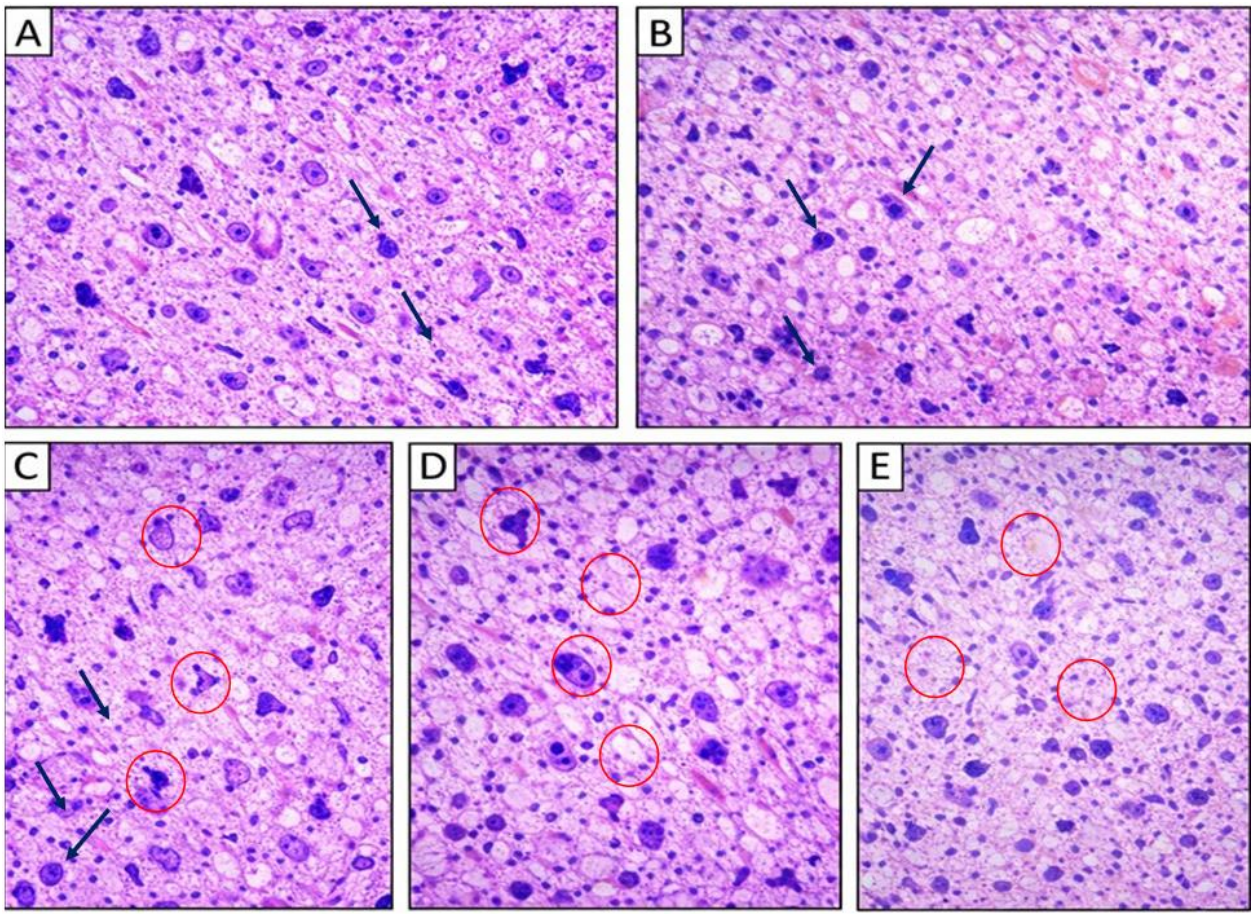

Figure 7: Photomicrographs of Cresyl Violet (CFV)-stained sections of the prefrontal cortex at ×100 magnification. Control group (A) and NSO group (B) showing normal Nissl profiles with deeply stained pyramidal cells (blue arrows). DBP group (C) showing reduced staining intensity with loss of its substance with significant degenerative changes (red circles). DBP+NSO group (D) demonstrating preserved neurons (blue arrows) with minimal degenerative changes (red circles). (E) DBP withdrawal group showing partial recovery, with some intact neurons (blue arrows) but residual vacuolation, and a loss of Nissl substance (red circles).

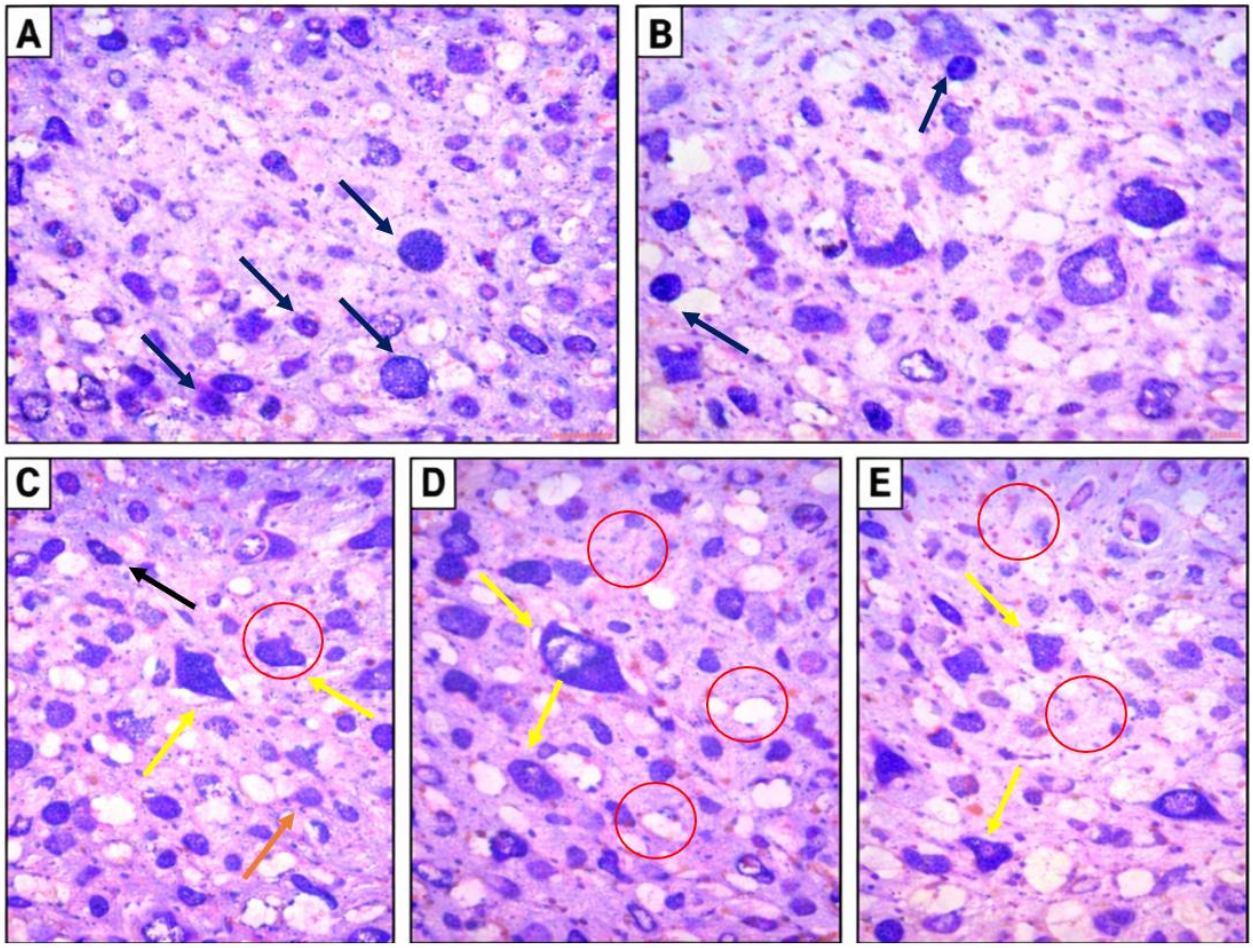

Figure 8: Photomicrographs of Cresyl Violet (CFV)-stained sections of the prefrontal cortex at ×400 magnification. (A) Control group showing normal pyramidal neurons with well- preserved Nissl granules (blue arrows). (B) The NSO group displayed intact neuronal architecture similar to that of the control (blue arrows). (C) DBP group showing neuronal shrinkage (black arrows), chromatolysis, and vacuolation (red circles). (D) DBP+NSO group demonstrating a few preserved neurons with minimal degenerative changes (yellow arrows) and vacuolation (red circles). (E) DBP withdrawal group showing partial recovery, with some intact neurons (red arrows) but residual vacuolation (red circles).

DISCUSSION

Chronic exposure to dibutyl phthalate (DBP) in the present study resulted in significant impairments in somatic growth, brain weight, and behavioral performance, indicating systemic and neurotoxic effects. The observed reduction in body and brain weights following DBP administration is consistent with earlier reports demonstrating that phthalates interfere with growth regulation and neurodevelopment through endocrine disruption and metabolic dysregulation [12, 13]. Phthalates have been shown to impair thyroid hormone signaling and growth hormone pathways, both of which are critical for brain maturation and overall somatic development [14]. Although withdrawal from DBP exposure led to partial recovery in body and brain weights, the incomplete restoration suggests persistent neurobiological alterations, in agreement with studies reporting long-lasting effects of phthalate exposure even after cessation [15].

Behavioral assessment using the open-field paradigm further demonstrated DBP-induced neurobehavioral deficits. The significant reduction in time spent in the center and center square entries (Figure 4) among DBP-treated rats reflects heightened anxiety-like behavior and reduced exploratory drive. Similar behavioral alterations have been reported in rodents exposed to DBP and other phthalates, where increased thigmotaxis and avoidance of open spaces were attributed to altered neurotransmitter signaling and oxidative stress within the prefrontal cortex and limbic structures [16, 17]. The withdrawal group exhibited modest improvement in exploratory behavior, suggesting some degree of functional recovery;

however, performance remained inferior to controls, supporting evidence that DBP-induced behavioral disturbances may persist beyond exposure periods [18].

In contrast, rats treated with Nigella sativa oil (NSO) alone exhibited enhanced exploratory activity, while co-administration of NSO with DBP significantly improved behavioral outcomes relative to DBP alone. These findings are consistent with reports describing the anxiolytic and neurobehavioral benefits of NSO, which have been linked to modulation of GABAergic transmission, attenuation of oxidative stress, and stabilization of neuronal membranes [19]. The partial behavioral recovery observed in the DBP + NSO group suggests that NSO mitigates, but does not entirely reverse, DBP-induced neurobehavioral alterations, particularly when exposure has already occurred.

At the biochemical level, DBP exposure induced a pronounced oxidative imbalance in the prefrontal cortex, evidenced by elevated malondialdehyde (MDA) concentrations and reduced catalase activity. Increased MDA reflects enhanced lipid peroxidation resulting from excessive reactive oxygen species (ROS) generation, a well-documented mechanism underlying phthalate-induced neurotoxicity [20, 21]. Further reduced catalase activity indicates impaired antioxidant defense, rendering neuronal tissue more vulnerable to oxidative injury. Similar oxidative profiles have been reported in DBP-exposed rodents, in which oxidative stress has been implicated in mitochondrial dysfunction, neuronal apoptosis, and synaptic impairment [22, 23].

Interestingly, the DBP withdrawal group exhibited even higher MDA levels than the DBP-only group, suggesting a rebound or delayed oxidative response following cessation of exposure. This phenomenon has been described in toxicological studies where withdrawal unmasks latent oxidative damage due to incomplete detoxification or sustained mitochondrial dysfunction [24]. In contrast, NSO administration significantly reduced MDA levels and restored catalase activity, both in NSO-only and DBP + NSO groups (Figure 6). These findings align with previous studies demonstrating that NSO and its principal bioactive constituent, thymoquinone, exert potent antioxidant effects by scavenging free radicals, enhancing endogenous antioxidant enzymes, and preserving membrane integrity [25–27].

Histological evaluation of the prefrontal cortex further substantiated the biochemical and behavioral findings. DBP-treated rats exhibited marked neuropathological alterations, including neuronal shrinkage, chromatolysis, vacuolation, and loss of Nissl substance. These features are characteristic of oxidative stress–mediated neuronal injury and have been widely reported in cortical and hippocampal regions following phthalate exposure [28, 29]. Such structural damage likely underlies the observed behavioral deficits, given the critical role of the prefrontal cortex in anxiety regulation, decision-making, and exploratory behavior.

In contrast, cortical sections from the control and NSO-only groups displayed preserved cytoarchitecture with intact pyramidal neurons and prominent Nissl granules, indicating normal protein synthesis and neuronal viability. Co-treatment with NSO markedly attenuated DBP-induced histological damage, as evidenced by reduced neuronal degeneration and improved preservation of Nissl substance. This histological protection corroborates earlier reports showing that NSO preserves neuronal integrity by limiting oxidative injury, stabilizing intracellular calcium homeostasis, and suppressing neuroinflammatory signaling [30–32]. The withdrawal group demonstrated partial histological recovery, with moderate reappearance of Nissl granules but persistent vacuolation, suggesting incomplete structural repair in the absence of antioxidant intervention [33].

Taken together, the present findings demonstrate that DBP induces neurotoxicity through interconnected mechanisms involving oxidative stress, behavioral dysregulation, and structural neuronal damage within the prefrontal cortex. The ameliorative effects of NSO across behavioral, biochemical, and histological parameters strongly support its role as a neuroprotective agent. The observed benefits are likely mediated through antioxidant and cytoprotective pathways rather than complete reversal of established damage, highlighting the importance of early intervention during toxicant exposure.

CONCLUSION

Chronic exposure to dibutyl phthalate induced anxiety-like behavior, oxidative imbalance, and neuronal degeneration in the prefrontal cortex. At the same time, co-administration of *Nigella sativa* oil mitigated these alterations, indicating a neuroprotective effect. These findings underscore the neurotoxic potential of DBP in cortical brain regions and support further investigation into its mechanisms and relevance to chronic environmental exposure.

Conflict of interests: The authors have no conflicts of interest to declare

Author's contribution: ATA: concept, result interpretation, and critical review of manuscript; **AOA:** data collection, and draft of manuscript; **BJD:** conceptual guidance and result interpretation; **IAL:** result interpretation and manuscript review; **AAS** and **MB:** data collection and analysis; **SOI** and **SME:** methodology refinement and result interpretation;

FOH: methodology refinement and manuscript draft review; **AA:** data analysis and result interpretation.

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