

Research Paper

Neuroprotective Effects of *Nigella sativa* Oil Against Prenatal Di-Butyl Phthalate-Induced Cerebellar Neurotoxicity in Wistar Rat Offspring

Aminat Temitayo Atoyebi, Abdulmuiz Oluwatobi Adebiyi*, Aisha Abdulrauf, Abdullah Musliamat, Kafilat Alasirin, Ismail Adetayo Lawal, Fatima Omowumi Hamzat, Solihu Olashile Ibrahim, Sulaiman Muhammad Eze and Babatunde Joseph Dare

Department of Human Anatomy,
Faculty of Basic Medical Sciences, Al-Hikmah University, Ilorin, Nigeria.

*Corresponding Author Email: aadebiyi@alhikmah.edu.ng

ABSTRACT

Background:

Di-butyl phthalate (DBP) is a widely used plasticizer and environmental endocrine disruptor known to induce oxidative stress and neurodevelopmental toxicity. The developing brain, particularly regions involved in motor coordination and behavior, is highly vulnerable to prenatal toxicant exposure. Natural antioxidants such as *Nigella sativa* oil (NSO) have shown promise in mitigating chemically induced neural damage.

Aim:

This study investigated the neurobehavioral, biochemical, and histological effects of prenatal DBP exposure and evaluated the protective potential of *Nigella sativa* oil in Wistar rat offspring.

Materials and Methods:

Twenty pregnant Wistar rats were randomly assigned to four groups: control, NSO (1 mL/kg b.w.), DBP (500 mg/kg b.w.), and DBP + NSO. Treatments were administered orally from gestational day 4 to 21. Offspring were assessed for neurobehavioral performance using the open field test on postnatal day 35. Cerebellar tissues were analyzed for oxidative stress markers including malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GPx), and processed for histological evaluation using hematoxylin and eosin and cresyl fast violet staining.

Results:

Prenatal DBP exposure resulted in reduced locomotor activity, elevated MDA levels, and decreased CAT and GPx activities, indicating oxidative stress and impaired antioxidant defense. Histological analysis revealed neuronal degeneration, vacuolation, disrupted cortical layering, and loss of Nissl substance in the cerebellum. Co-administration of NSO significantly improved behavioral performance, reduced lipid peroxidation, restored antioxidant enzyme activity, and preserved cerebellar cytoarchitecture.

Conclusion:

Prenatal DBP exposure induces significant neurobehavioral deficits, oxidative stress, and cerebellar neurodegeneration in rat offspring. *Nigella sativa* oil confers substantial neuroprotection, likely through its

antioxidant and cytoprotective properties, highlighting its potential as a natural intervention against environmental neurotoxins.

KEYWORDS: Di-butyl phthalate; *Nigella sativa* oil; Neurotoxicity; Oxidative stress; Cerebellum; Neurobehavior.

INTRODUCTION

Phthalates are synthetic esters of phthalic acid widely used as plasticizers in numerous consumer and industrial products, including plastics, food packaging, toys, cosmetics, medical devices, and pharmaceuticals¹. Their widespread application has resulted in continuous human exposure through ingestion, inhalation, and dermal absorption, raising serious concerns regarding their long-term health effects^{1,2}. Accumulating evidence indicates that phthalates function as endocrine-disrupting chemicals, capable of interfering with hormonal regulation and contributing to reproductive, developmental, and neurological disorders^{2,3}. Among these compounds, Di-butyl phthalate (DBP), a low-molecular-weight phthalate commonly encountered in daily environments, has drawn particular attention due to its environmental prevalence and documented toxicity affecting multiple organs, including the nervous system^{3,4}.

Experimental and animal studies have demonstrated that DBP exposure disrupts neural development and brain function through mechanisms involving oxidative stress, endocrine imbalance, altered gene expression, and mitochondrial dysfunction^{5,6}. DBP-induced neurotoxicity has been associated with neuronal apoptosis, synaptic dysfunction, impaired neurogenesis, and behavioral abnormalities⁶. Prenatal exposure is of particular concern, as DBP and its metabolites can cross the placental barrier and interfere with hormone-dependent neurodevelopmental processes^{3,5}. Histopathological studies have revealed DBP-induced neuronal loss and structural alterations in developing brain regions, with the cerebellum being especially vulnerable due to its critical role in motor coordination, balance, and cognitive processing^{3,5}.

In parallel, increasing attention has been directed toward natural antioxidants as potential protective agents against toxicant-induced neural injury. *Nigella sativa* oil (NSO) has gained prominence due to its bioactive constituents, particularly thymoquinone and thymohydroquinone, which exhibit potent antioxidant, anti-inflammatory, and neuroprotective properties⁷⁻⁹. Thymoquinone has been shown to attenuate oxidative stress by reducing lipid peroxidation and enhancing endogenous antioxidant enzyme activity, including catalase and glutathione peroxidase⁹. These properties are especially relevant for counteracting DBP-induced oxidative damage and neuronal apoptosis within the developing cerebellum¹⁰.

Experimental evidence further supports the efficacy of NSO in preserving neural architecture and function against chemically induced neurotoxicity^{8,10}.

Despite regulatory efforts, DBP remains widely present in plastics, cosmetics, and other consumer products, resulting in persistent exposure throughout life, including during critical periods of prenatal development^{4,5}. Although DBP is rapidly metabolized to mono-butyl phthalate and excreted following glucuronidation, both DBP and its metabolites have been detected in various biological samples, including urine, serum, breast milk, and amniotic fluid^{3,5}. While the neurotoxic effects of DBP on brain development are increasingly recognized, limited data exist regarding protective strategies against DBP-induced cerebellar damage during gestation. Therefore, the present study aims to investigate the neuroprotective role of *Nigella sativa* oil in preventing DBP-induced cerebellar injury during prenatal exposure, with the goal of reducing developmental impairments in the offspring of exposed Wistar rats.

METHODOLOGY

Ethical Approval

All experimental procedures were conducted in strict compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC). Ethical approval for the study was obtained from the Ethical Review Committee of Al-Hikmah University, Ilorin, Nigeria (approval number: HUI-FHS-ERC-25-0041). The principles of the 3Rs (Replacement, Reduction, and Refinement) were strictly adhered to in the handling, housing, and care of the experimental animals to ensure animal welfare and scientific validity.

Chemicals and Reagents

Di-butyl phthalate (DBP) was obtained from Central Drug House (P) Ltd., New Delhi, India (Batch No: 171019) and purchased through Laotech Scientific Nigeria Company. *Nigella sativa* oil (NSO) was procured from Hemani Herbs Trading and purchased from Al-Alameen Store, Ilorin, Nigeria. Normal saline solution was manufactured by Fidson Healthcare Plc (Nigeria) and purchased from One Step Pharmacy. All other reagents, including sucrose,

formalin, ethanol, methylated spirit, cresyl fast violet, and ELISA kits, were of analytical grade and used as supplied.

Experimental Animals

The experimental animals used in this study were Wistar rats bred and housed at the Animal Holding Facility of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ilorin, Kwara State, Nigeria. The rats were originally procured from Fulcrum Innovation Limited, Ilorin, Kwara State. A total of twenty unmated female Wistar rats and twelve adult male albino rats, weighing between 220 g and 280 g, were utilized.

Animals were maintained under standard laboratory conditions of temperature (20–22 °C), adequate ventilation, and a 12-hour light/dark cycle. They were fed pelleted rat feed and provided with clean drinking water *ad libitum*. All animals were allowed to acclimatize for two weeks prior to the commencement of the experiment and were housed in plastic cages with sawdust bedding that was replaced regularly.

Experimental Design and Grouping

Mating was carried out during the third week of acclimatization using a 2:1 female-to-male ratio. Pregnancy was confirmed by vaginal smear analysis and progressive weight gain. Confirmed pregnant rats were randomly assigned into four groups (n = 5 per group):

- **Group A (Control):** Normal saline (10 ml/kg body weight, orally)
- **Group B (NSO):** *Nigella sativa* oil (1 ml/kg body weight, orally)
- **Group C (DBP):** Di-butyl phthalate (500 mg/kg body weight, orally)
- **Group D (DBP + NSO):** Di-butyl phthalate (500 mg/kg body weight) + *Nigella sativa* oil (1 ml/kg body weight, orally)

Treatments were administered once daily from gestational day 4 to gestational day 21 using an appropriately sized oral cannula. The dose of DBP was selected based on previous studies demonstrating consistent induction of neurotoxic effects¹¹, while the dose of NSO was chosen based on reports of antioxidant and neuroprotective efficacy without toxicity¹².

Neurobehavioral Assessment

Neurobehavioral assessment was conducted on postnatal day 35 (PND35) prior to sacrifice. Eight pups were randomly selected from each group for behavioral testing. Locomotor activity and anxiety-like behavior were evaluated using the open field test (OFT). Each rat was placed individually in the center of a 100 × 100 cm square arena divided into 16 equal

squares, and exploratory behavior was recorded for 5 minutes. Parameters assessed included total locomotion, number of center square entries, and time spent in the central square. The apparatus was cleaned with ethanol after each trial to eliminate olfactory cues.

Termination of Experiment and Tissue Collection

At the end of the experimental period, animals were humanely euthanized by cervical dislocation. Brains were immediately excised, and the cerebellum was carefully dissected and divided for histological and biochemical analyses.

Histological Analysis

Cerebellar tissues from all experimental groups were harvested and fixed immediately in 10% neutral buffered formalin at room temperature for at least 48 hours. The tissues were then trimmed into coronal sections, dehydrated through ascending grades of ethanol, cleared in xylene, and embedded in molten paraffin wax at approximately 55 °C. Serial sections of 5 µm thickness were obtained using a rotary microtome and mounted on clean glass slides for histological staining.

Sections were stained with Hematoxylin and Eosin (H&E) to evaluate general cerebellar histoarchitecture and Cresyl Fast Violet (CFV) to demonstrate Nissl substance integrity. Staining procedures involved deparaffinization, rehydration, and staining using standard protocols, followed by dehydration, clearing, and mounting with DPX. Slides were examined under a compound light microscope using 4× and 10× objectives to assess neuronal integrity, Purkinje cell distribution, and overall cortical organization. Photomicrographs were captured using a calibrated digital imaging system, and all evaluations were performed in a blinded manner by independent observers to minimize bias and assess treatment-related morphological changes and neuroprotective effects.

Biochemical Analysis

Following sacrifice, cerebellar tissues were excised, rinsed, and homogenized in ice-cold 0.25 M sucrose. Homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the supernatant was used for oxidative stress and antioxidant enzyme analyses. Catalase (CAT) activity was measured spectrophotometrically at 410 nm based on hydrogen peroxide decomposition. Lipid peroxidation was assessed by malondialdehyde (MDA) levels using the TBARS assay at 532 nm, while glutathione peroxidase (GPx) activity was determined via reduced glutathione-dependent hydroperoxide reduction at 460 nm. These assays collectively evaluated oxidative stress status, antioxidant defense, and neuroprotective effects in cerebellar tissue.

Statistical Analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. Comparisons among groups were made using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Statistical significance was set at $p \leq 0.05$.

RESULTS

1. Physical Observations during Gestation (Maternal)

A. Maternal Weight Changes

Maternal weight changes during gestation varied across the experimental groups [Figure 1]. Dams in the control and *Nigella sativa* oil-treated groups maintained a progressive and physiologically appropriate pattern of weight gain throughout gestation. In contrast, exposure to Di-butyl phthalate was associated with disrupted gestational weight progression, suggestive of maternal stress and metabolic alteration. Notably, concurrent administration of *Nigella sativa* oil moderated these adverse effects with a gradual improvement in maternal weight trajectory.

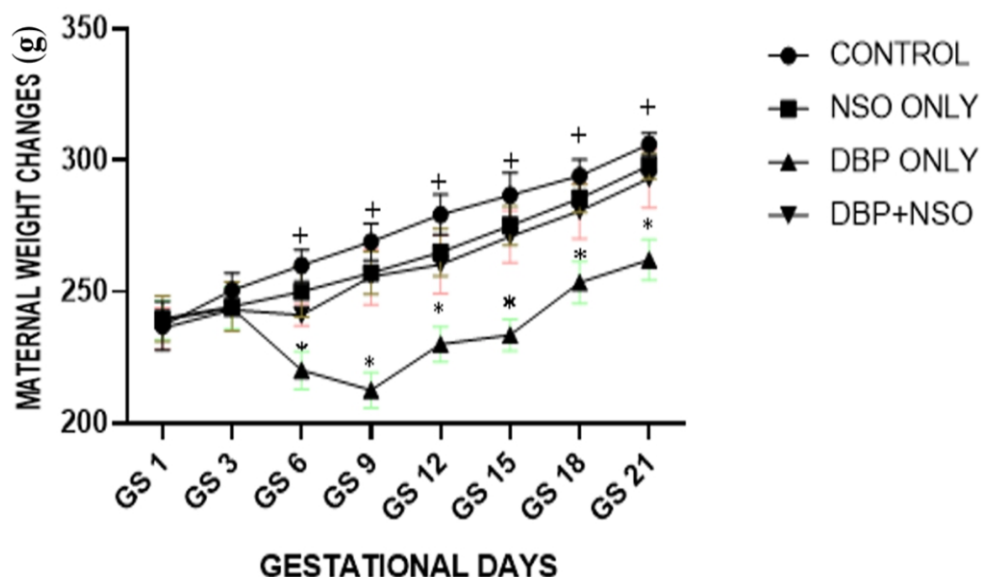


Figure 1: Graph showing the effect of DBP and NSO administration on maternal body weight changes of experimental animals. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

B. Duration of Gestation

Gestational duration varied across the experimental groups [Figure 2]. Dams in the control and *Nigella sativa* oil-treated groups showed gestation lengths within the expected physiological range. In contrast, Di-butyl phthalate exposure was associated with a prolongation of gestation, indicating disruption of normal parturition timing. Co-administration of *Nigella sativa* oil moderated this effect, suggesting a protective influence against DBP-induced gestational delay.

C. Litter Size

Litter size varied across the experimental groups [Figure 3]. Control and *Nigella sativa* oil-treated dams exhibited litter outcomes consistent with normal gestational performance. In contrast, prenatal exposure to Di-butyl phthalate was associated with a reduction in litter size, suggesting adverse effects on gestational outcome. Co-administration of *Nigella sativa* oil moderated this effect, indicating partial preservation of normal pregnancy outcome despite DBP exposure.

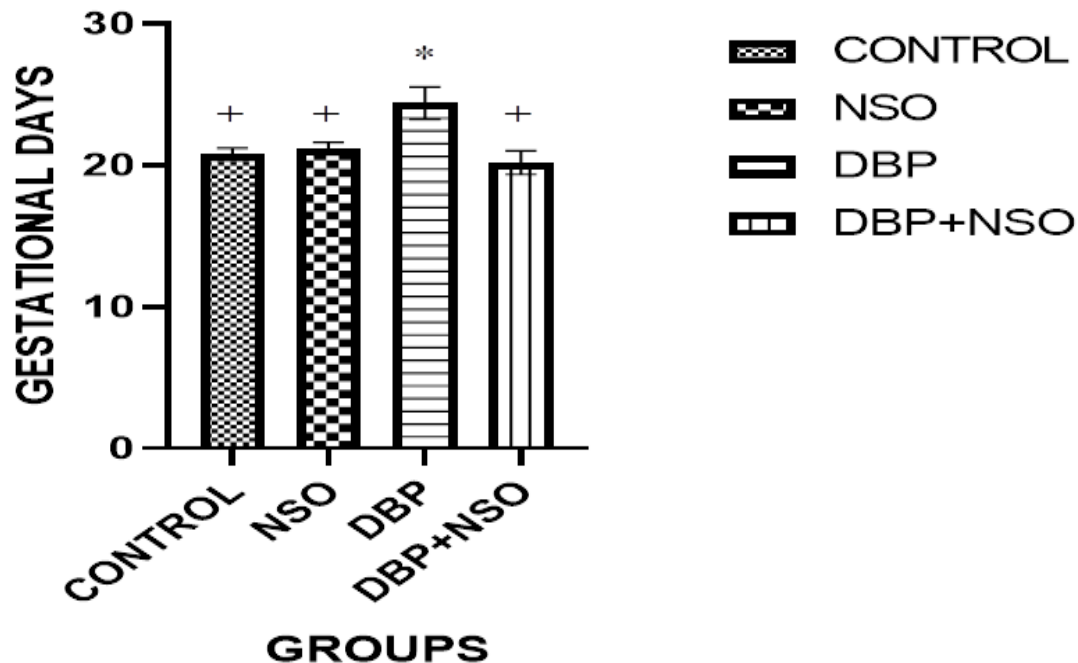


Figure 2: Graph showing the effect of DBP and NSO administration on the duration of gestation across experimental groups. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

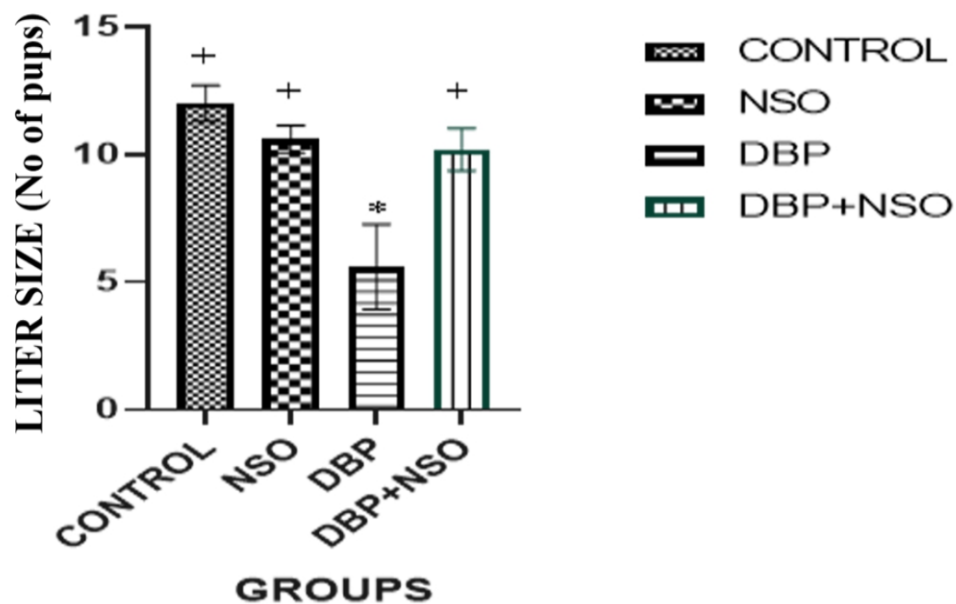


Figure 3: Graph showing the effect of DBP and NSO administration on litter size across experimental groups. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

1. Physical Observations during Gestation (Offspring)

A. Offspring Body Weight Changes

Postnatal body weight progression of offspring differed across the experimental groups [Figure 4]. Pups from the control and *Nigella sativa* oil-treated groups exhibited growth patterns consistent with normal postnatal development. In contrast, prenatal exposure to Di-butyl phthalate was associated with altered weight gain, reflecting impaired early growth. Co-administration of *Nigella sativa* oil attenuated these effects, suggesting a modulatory influence on DBP-induced growth alterations.

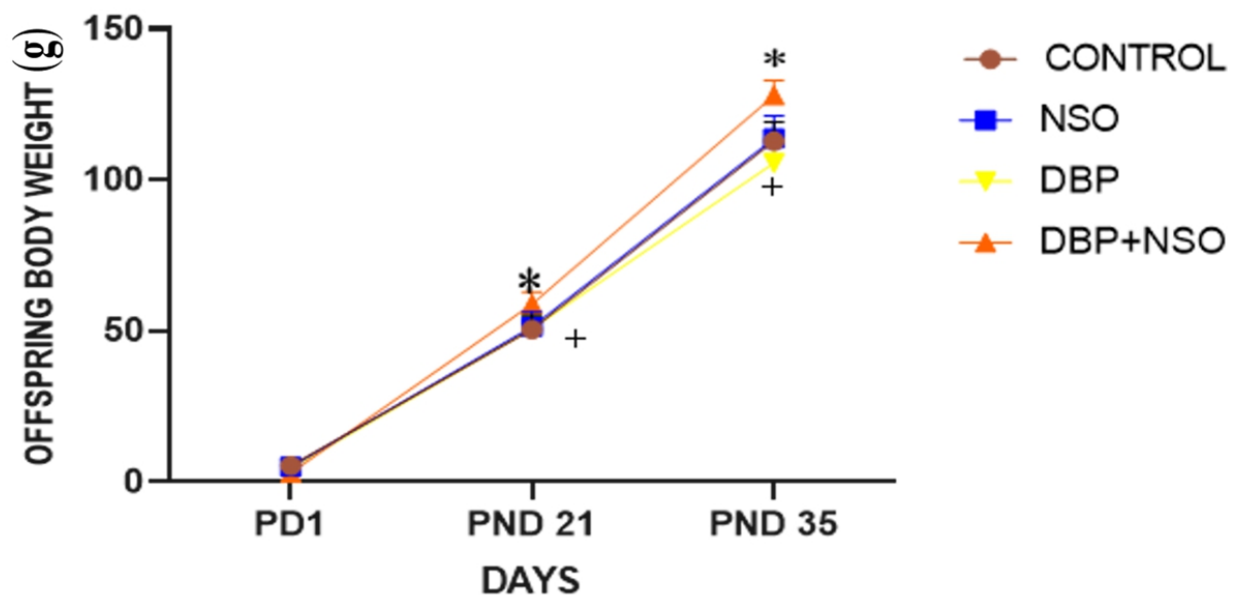


Figure 4: Graph showing the effect of DBP and NSO administration on offspring body weight changes across experimental groups. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

B. Fur Appearance

Assessment of fur appearance revealed no marked differences across the experimental groups [Figure 5]. Fur development followed a similar timeline in all groups; however, offspring in the *Nigella sativa* oil-treated group and Control group exhibited visibly increased fur density. Prenatal exposure to Di-butyl phthalate did not produce overt alterations in fur appearance, and co-administration of *Nigella sativa* oil did not markedly deviate from the general pattern observed.

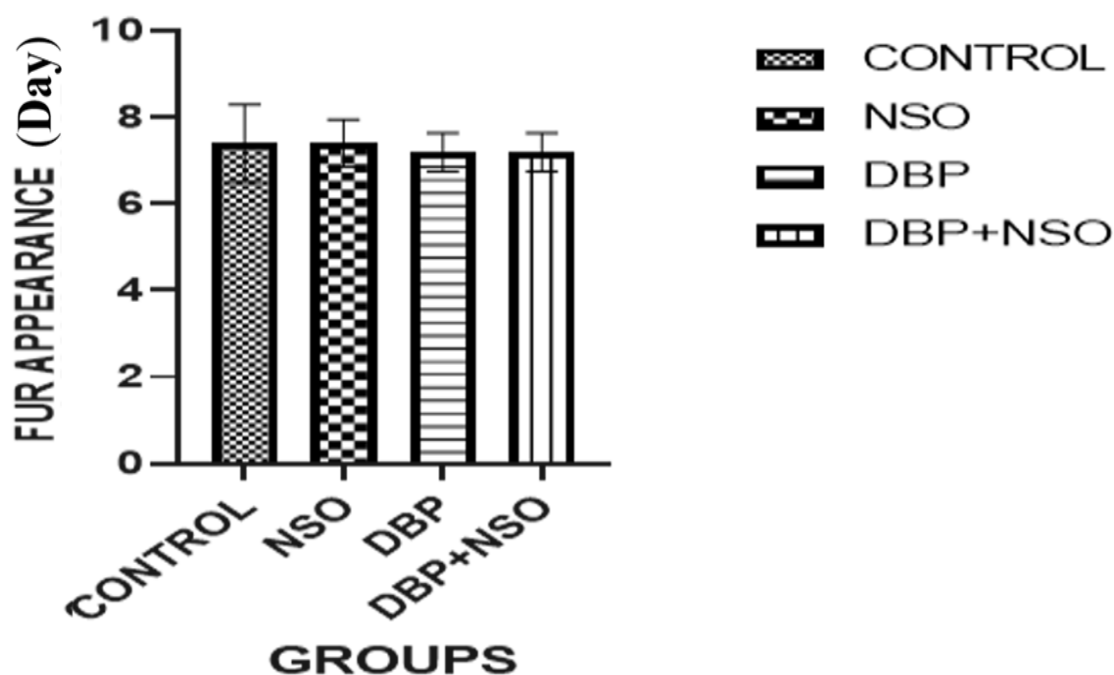


Figure 5: Graph showing fur appearance day across experimental groups. **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

No statistically significant difference was observed across the groups.

C. Eye Opening Assessment

Assessment of eye opening revealed no marked differences across the experimental groups [Figure 5]. Eye Opening followed a similar timeline in all groups. Prenatal exposure to Di-butyl phthalate did not produce overt alterations in eye opening of the offspring, and co-administration of *Nigella sativa* oil did not markedly deviate from the general pattern observed in control and *Nigella sativa* oil treated groups.

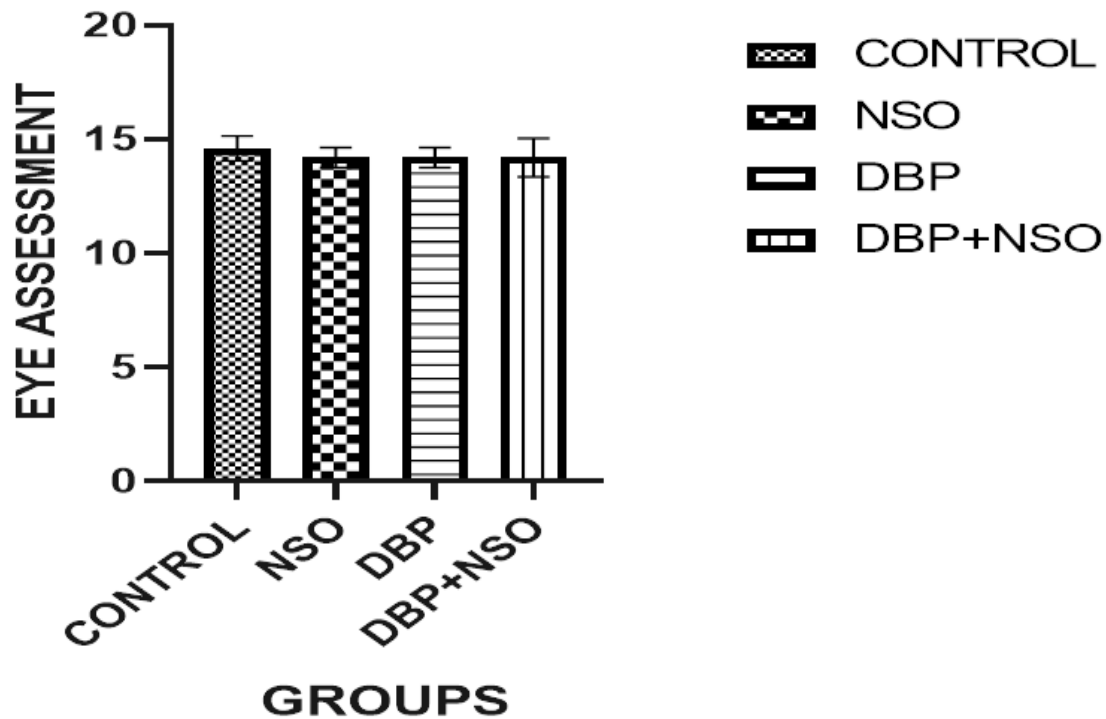


Figure 6: Graph showing eye opening assessment across experimental groups. **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

No statistically significant difference was observed across the groups.

3. Neurobehavioral Test (Open Field test)

Neurobehavioral performance assessed using the open field test differed across the experimental groups [Figure 7]. Offspring in the control and *Nigella sativa* oil-treated groups displayed normal locomotor and exploratory activity, as evidenced by a higher number of line crossings. In contrast, offspring prenatally exposed to Di-butyl phthalate showed a significant reduction in line crossings, indicating impaired locomotor activity and reduced exploratory behavior. Co-administration of *Nigella sativa* oil significantly ameliorated this deficit, with the DBP + NSO group demonstrating improved locomotor performance compared to the DBP-only group, suggesting a protective effect of NSO against DBP-induced neurobehavioral impairment.

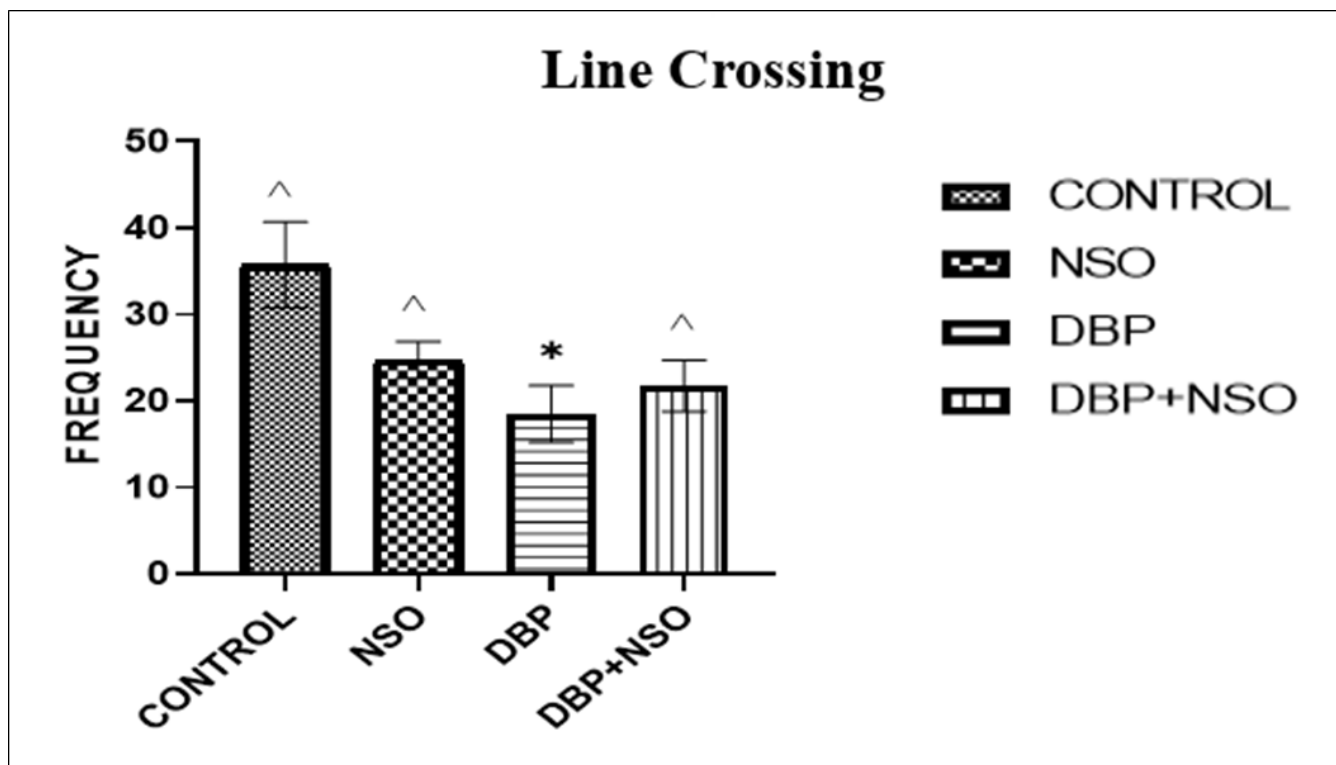


Figure 7: Graph showing the number of line crossings in the open field test across experimental groups. Asterisk (*) indicates statistical significance compared to the control group, (^) indicates statistical significance compared to the DBP-only group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

4. Biochemical Analysis

A. Levels of Malondialdehyde (MDA)

Lipid peroxidation, assessed by malondialdehyde levels, differed significantly among the groups [Figure 8]. Offspring from the Di-butyl phthalate group exhibited a significant elevation in MDA levels compared to control, indicating increased oxidative damage. In contrast, control and *Nigella sativa* oil-treated groups showed lower MDA levels consistent with normal oxidative status. Co-administration of *Nigella sativa* oil significantly reduced MDA levels compared to the DBP-only group, demonstrating attenuation of DBP-induced lipid peroxidation.

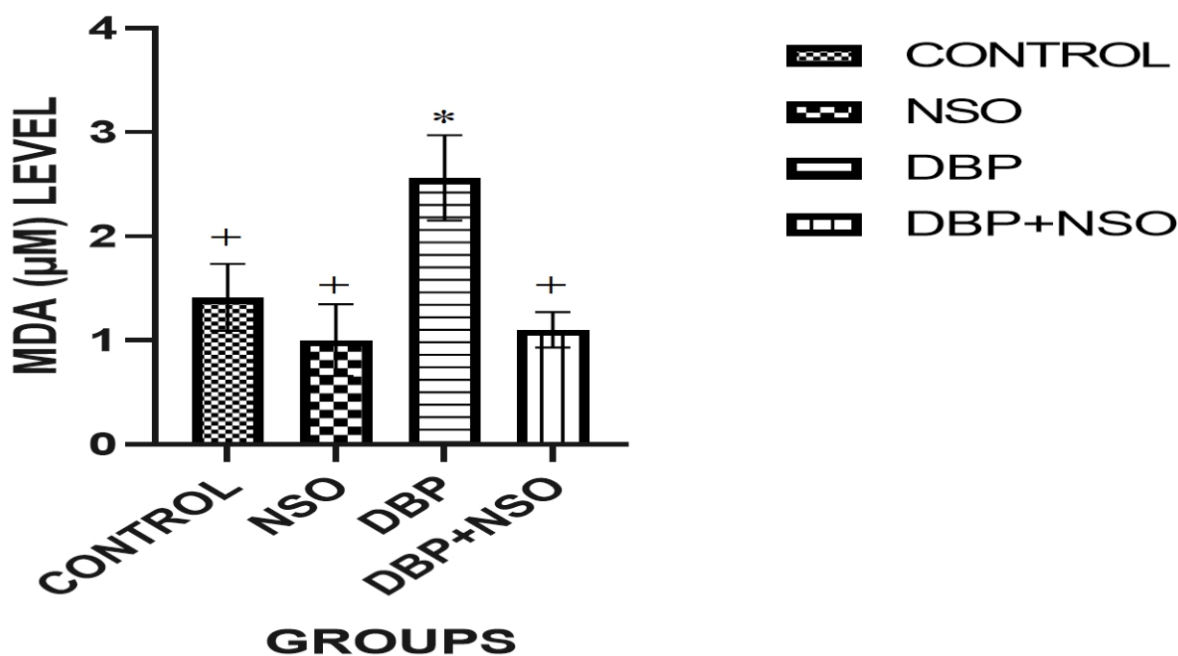


Figure 8: Graph showing malondialdehyde (MDA) levels in the cerebellum of experimental animals. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the DBP-only group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

A. Activities of Catalase

Catalase activity in the cerebellum varied significantly among the experimental groups [Figure 9]. Control and *Nigella sativa* oil-treated groups exhibited normal catalase activity levels. In contrast, Di-butyl phthalate exposure resulted in a significant reduction in catalase activity, reflecting compromised antioxidant defense. Co-administration of *Nigella sativa* oil significantly restored catalase activity compared to the DBP-only group, indicating attenuation of DBP-induced oxidative stress.

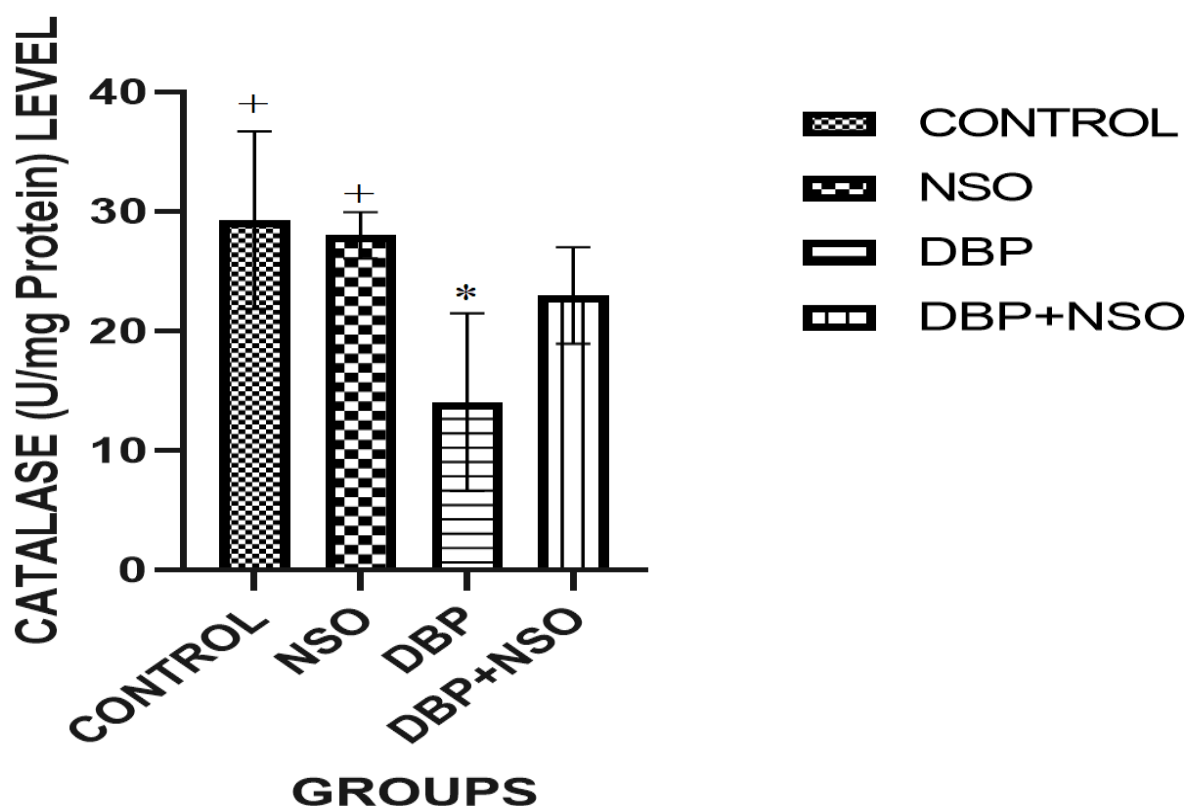


Figure 9: Graph showing catalase levels in the cerebellum of experimental animals. Asterisk (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the DBP-only group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.).

C. Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity showed marked differences across the experimental groups [Figure 10]. The control and *Nigella sativa* oil groups-maintained baseline GPx activity. However, a significant decrease in GPx activity was observed in the Di-butyl phthalate-exposed group, indicating impaired antioxidant capacity. Co-treatment with *Nigella sativa* oil significantly increased GPx activity relative to the DBP-only group, suggesting restoration of endogenous antioxidant defense mechanisms.

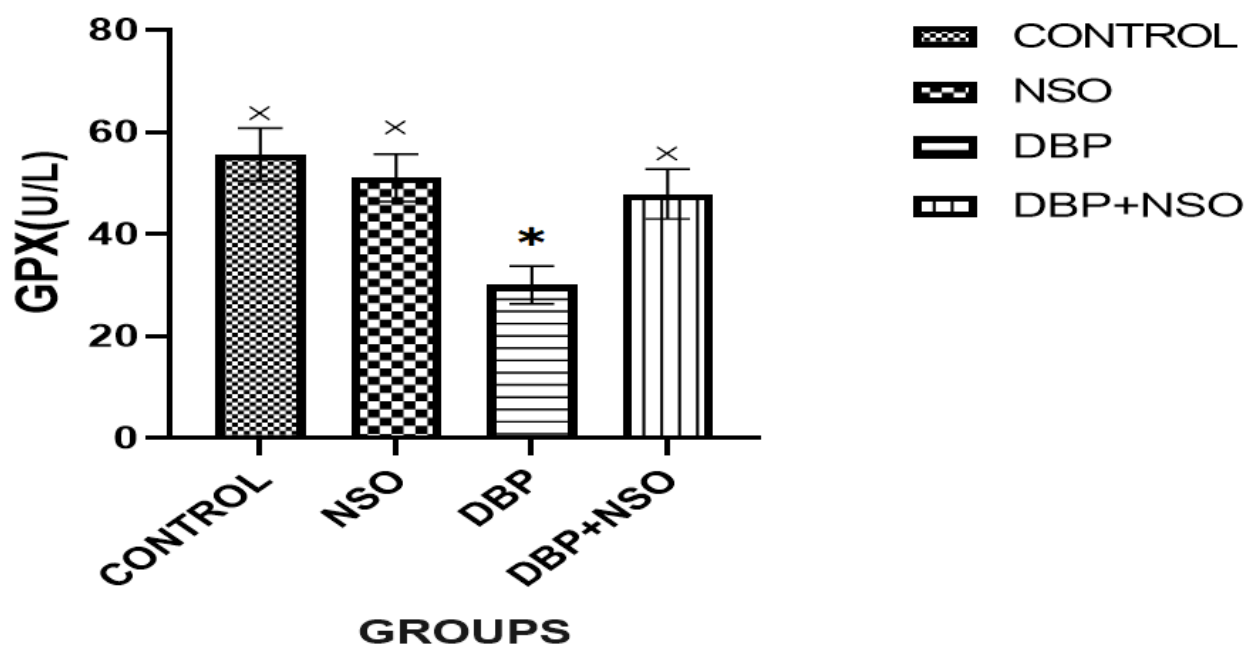


Figure 10: Chart showing glutathione peroxidase (GPx) levels in the cerebellum of experimental animals. Asterisk (*) indicates statistical significance compared to the control group, (x) indicates statistical significance compared to the DBP-only group, while **CONTROL** = Control (normal saline), **NSO** = *Nigella sativa* oil (1 ml/kg b.w.), **DBP** = Di-butyl phthalate (500 mg/kg b.w.), and **DBP+NSO** = Di-butyl phthalate (500 mg/kg b.w.) + *Nigella sativa* oil (1 ml/kg b.w.)

1. Histological Findings

A. Hematoxylin and Eosin Stain

Hematoxylin and eosin–stained cerebellar sections showed well-preserved cortical organization with intact molecular, Purkinje, and granular layers in the control and *Nigella sativa* oil–treated groups [Figure 11]. In contrast, the Di-butyl phthalate group exhibited marked histopathological alterations, including neuronal degeneration, shrinkage of cell bodies, vacuolation, pyknotic nuclei, and disrupted layering. Co-administration of *Nigella sativa* oil attenuated these changes, with the DBP + NSO group showing improved cellular morphology and relative preservation of cerebellar architecture compared to the DBP-only group.

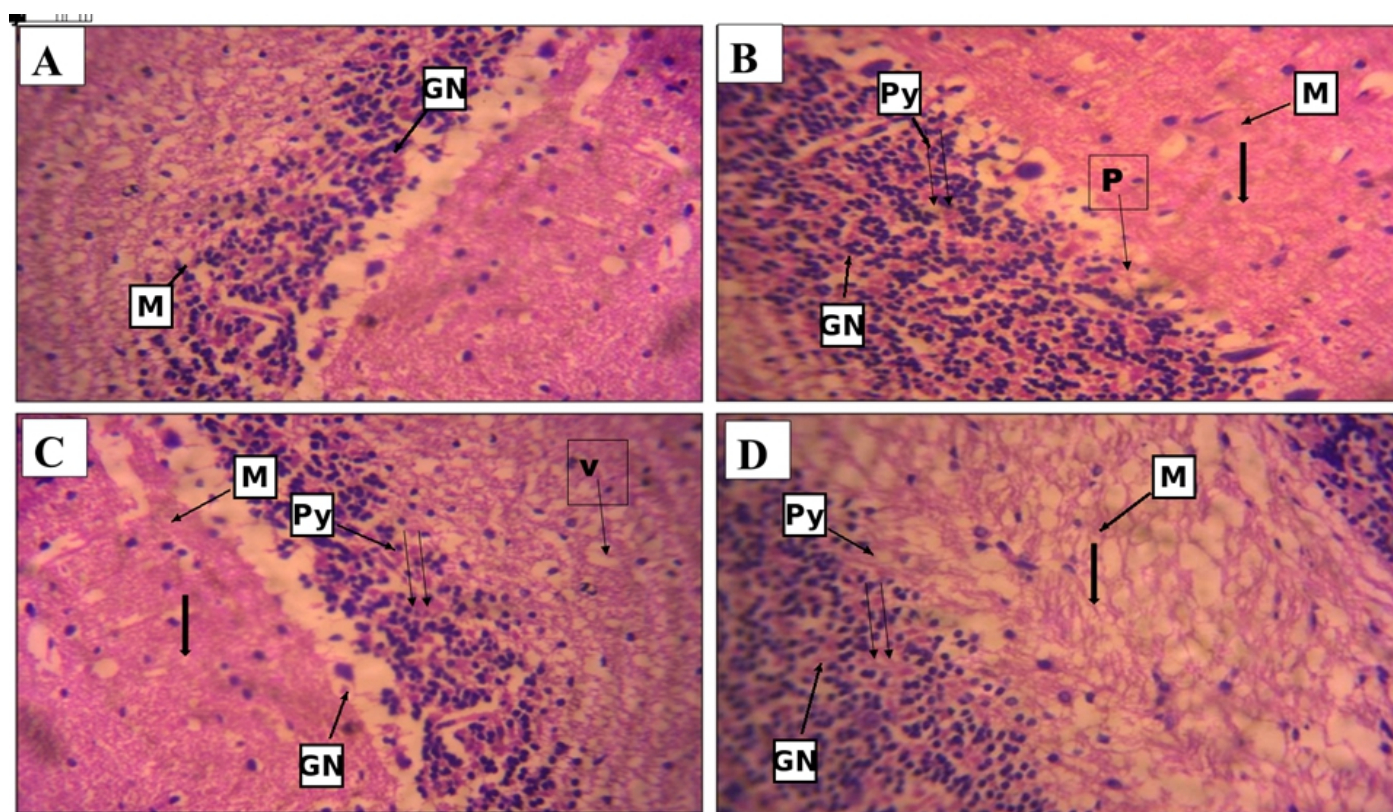


Figure 11: Photomicrograph showing cerebellar histoarchitecture stained with H&E (×400). **CONTROL (A)** and **NSO (B)** groups show normal layering and intact neurons, **DBP group (C)** shows neuronal degeneration and structural disruption, while **DBP+NSO group (D)** shows reduced histological alterations.

GN = Granular layer, P = Purkinje layer, M = Molecular layer, Py = Pyknotic nuclei, V = Vacuolation

B. Cresyl Fast Violet (Nissl Profile) Stain

Cresyl fast violet staining revealed dense and uniform Nissl substance distribution in the control and *Nigella sativa* oil-treated groups, indicating preserved neuronal integrity [Figure 12]. In contrast, cerebellar sections from the Di-butyl phthalate group showed reduced staining intensity, chromatolysis, dispersion of Nissl bodies, vacuolation, and pyknotic nuclei, suggestive of impaired neuronal metabolism. Co-treatment with *Nigella sativa* oil resulted in restoration of Nissl staining intensity and improved neuronal appearance relative to the DBP-only group.

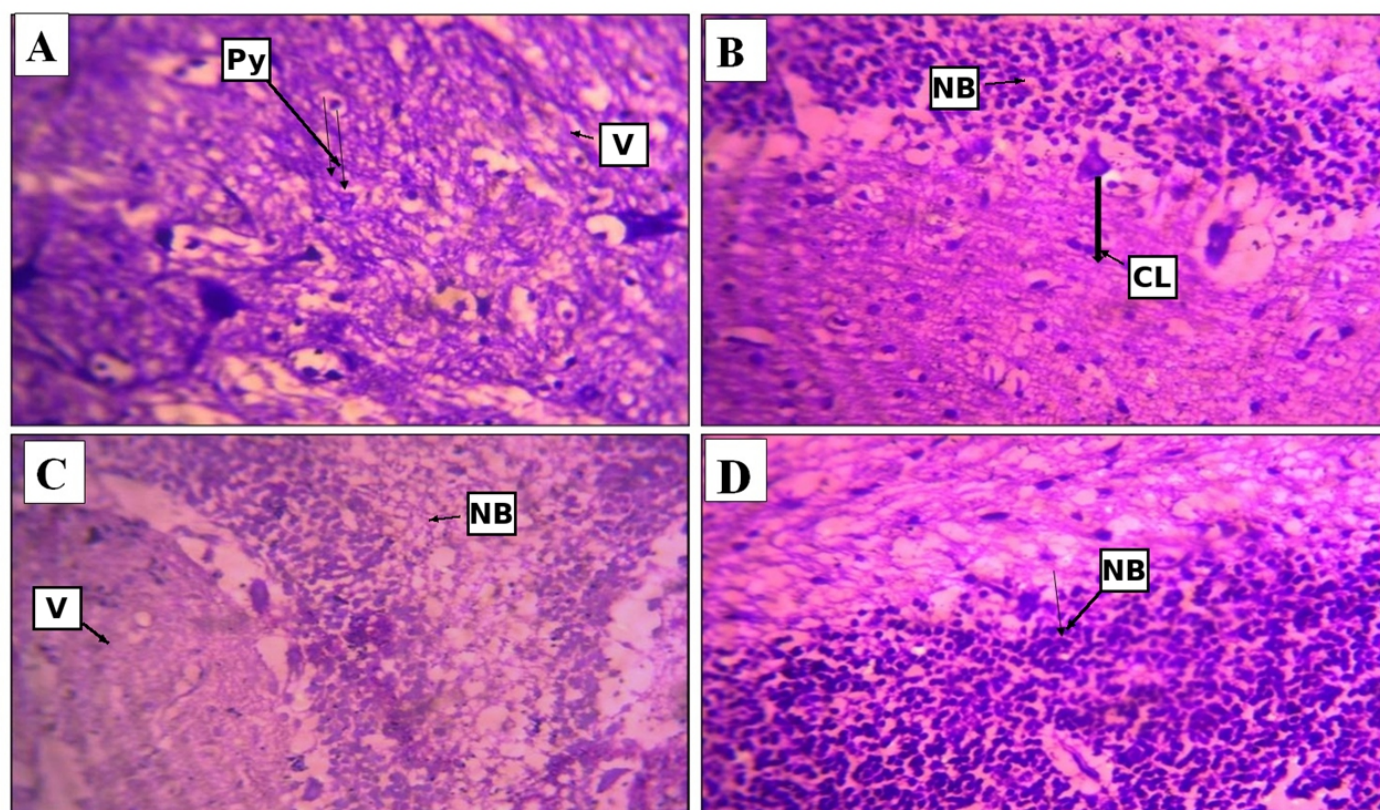


Figure 12: Photomicrograph showing Nissl substance distribution in cerebellar sections stained with CFV (×400). **CONTROL (A) and NSO groups (B)** show intense Nissl staining, **DBP group (C)** shows reduced staining and chromatolysis, while **DBP+NSO group (D)** shows restored Nissl bodies.

NB = Nissl bodies, **CL** = Chromatolysis, **Py** = Pyknotic nuclei, **V** = Vacuolation

DISCUSSION

Prenatal exposure to Di-butyl phthalate (DBP) in the present study produced pronounced adverse effects on maternal gestational indices, offspring development, neurobehavioral performance, oxidative status, and cerebellar histoarchitecture. DBP-exposed dams showed evidence of gestational stress, including altered weight gain patterns and prolonged gestation [Figures 1 and 2], which were accompanied by reduced litter size and impaired postnatal growth in offspring [Figures 3 and 4]. These observations align with earlier reports demonstrating that phthalates disrupt endocrine homeostasis, placental steroidogenesis, and nutrient transfer, resulting in intrauterine growth restriction and altered pregnancy outcomes¹³. Similar developmental impairments following prenatal phthalate exposure have been linked to long-term vulnerability to neurobehavioral and cognitive dysfunction, supporting the relevance of the present findings¹⁴.

Neurobehavioral assessment using the open field test revealed that offspring prenatally exposed to DBP exhibited significantly reduced locomotor activity and exploratory behavior [Figure 7]. Such hypoactivity and anxiety-like responses are indicative of dysfunction in brain regions governing motor coordination, emotional regulation, and executive control, particularly the cerebellum^{15,16}. These results are consistent with previous experimental studies reporting diminished exploratory behavior and increased anxiety following DBP exposure, effects attributed to disrupted neurotransmitter signaling, oxidative stress, and altered synaptic plasticity^{6,11}. Notably, co-administration of *Nigella sativa* oil (NSO) markedly improved locomotor performance and exploratory behavior [Figure 7], corroborating earlier studies that demonstrated the behavioral ameliorative effects of antioxidant-rich natural products in models of developmental neurotoxicity.

Biochemical analyses further substantiated the neurotoxic impact of prenatal DBP exposure. Cerebellar tissues from DBP-exposed offspring showed significantly elevated malondialdehyde (MDA) levels alongside reduced catalase (CAT) and glutathione peroxidase (GPx) activities [Figures 8–10], confirming the induction of oxidative stress. These findings are in agreement with prior reports identifying lipid peroxidation and impaired antioxidant defenses as central mechanisms in phthalate-induced neurotoxicity¹⁷. The heightened susceptibility of the developing brain to oxidative injury, owing to its high lipid content, rapid oxygen consumption, and immature antioxidant systems, has been well documented and provides a plausible explanation for the pronounced oxidative imbalance observed in this study¹⁶.

Conversely, NSO co-administration significantly attenuated DBP-induced oxidative stress, as evidenced by reduced MDA levels and restoration of CAT and GPx activities toward control values [Figures 8–10]. This protective effect is consistent with previous studies attributing the antioxidant and

neuroprotective properties of NSO primarily to thymoquinone, its major bioactive constituent, which enhances endogenous antioxidant enzyme activity and scavenges reactive oxygen species¹⁸. Earlier experimental models have similarly shown that *Nigella sativa* and thymoquinone mitigate oxidative damage, preserve mitochondrial integrity, and suppress neuroinflammatory pathways in chemically induced neurotoxicity and neurodegeneration¹⁹. The biochemical normalization observed in the DBP + NSO group therefore reinforces existing evidence supporting NSO as a potent modulator of redox homeostasis in neural tissues.

Histological findings provided structural confirmation of the biochemical and behavioral outcomes. Hematoxylin and eosin-stained cerebellar sections from DBP-exposed offspring revealed marked cytoarchitectural disruption, including Purkinje cell degeneration, neuronal vacuolation, pyknotic nuclei, and loss of normal cortical layering [Figure 11]. Cresyl fast violet staining further demonstrated chromatolysis, dispersion of Nissl substance, and reduced staining intensity, indicative of impaired protein synthesis and neuronal degeneration [Figure 12]. These alterations are consistent with earlier reports of DBP-induced developmental neurotoxicity characterized by neuronal loss and cerebellar dysfunction²⁰. In contrast, co-administration of NSO preserved cerebellar cortical organization, improved Purkinje cell integrity, and restored Nissl substance distribution [Figures 11 and 12]. These histological improvements are in agreement with previous studies demonstrating the ability of *Nigella sativa* oil and thymoquinone to protect neuronal structure and maintain synaptic integrity in experimental models of neurodegeneration⁸.

CONCLUSION

This study shows that chronic prenatal exposure to Di-butyl phthalate (DBP) induces significant neurodevelopmental toxicity in the cerebellum of Wistar rat offspring. DBP caused Purkinje cell degeneration, disrupted cortical layering, vacuolations, and neuronal chromatolysis in the cerebellum, accompanied by anxiety-like behavior and reduced exploratory activity. These impairments were associated with elevated oxidative stress, reflecting DBP's neurotoxic and endocrine-disrupting effects. Importantly, co-administration of *Nigella sativa* oil (NSO) mitigated these adverse outcomes by preserving neuronal integrity, restoring cortical architecture, reducing oxidative stress, and partially improving behavior, likely via its antioxidant, anti-inflammatory, and cytoprotective actions attributed to thymoquinone. These findings highlight that DBP exposure during critical developmental periods can cause significant cerebellar deficits, while NSO offers significant neuroprotection, supporting its potential as a natural intervention against environmental neurotoxins.

DECLARATIONS

Funding: None Declared.

Conflict of Interests Disclosure: The author(s) have declared that no competing interests exist.

Declaration of Generative AI and AI-Assisted Technologies: The author(s) declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

ARTICLE INFORMATION

Accepted for Publication: June 03, 2026

Published: June 27, 2026

Peer Review: Externally Peer Reviewed.

Open Access: This is an open access article distributed in accordance with the Creative Commons Attribution Non-Commercial (CC BY- NC 4.0) license, which permits others unrestricted use, distribution, and reproduction in any medium, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial.

Copyright: © 2026 By The Authors

REFERENCES

- Koch HM, Calafat AM. Human body burdens of chemicals used in plastic manufacture. *Philos Trans R Soc B Biol Sci.* 2009;364(1526):2063–2078.
- Mondal S, Bandyopadhyay A. Antioxidants in mitigating phthalate-induced male reproductive toxicity: a comprehensive review. *Chemosphere.* 2024; 364:143297. doi: 10.1016/j.chemosphere.2024.143297.
- Heidari T, Batavani RA, Malekinejad H, Hobbenaghi R. Evaluation of di-n-butyl phthalate reproductive toxicity in pregnant rats and their offspring and assessment of vitamin E administration in reducing toxicity. *Vet Res Forum.* 2022;13(2):201–208. doi:10.30466/vrf.2020.118147.2806.
- Hauser R, Calafat AM. Phthalates and human health. *Occup Environ Med.* 2005;62(11):806–818.
- Li X, Jiang L, Cheng L, Chen H. Dibutyl phthalate-induced neurotoxicity in the brain of immature and mature rat offspring. *Brain Dev.* 2014;36(8):653–660. doi:10.1016/j.braindev.2013.09.002.
- Nahla E, Arya P, Maneesha P, Chitra KC. Exposure to the plasticizer Di-butyl phthalate causes oxidative stress and neurotoxicity in brain tissue. *Environ Sci Pollut Res Int.* 2024;31(14):21399–21414. doi:10.1007/s11356-024-32604-7.
- Hamdy NM, Taha RA. Effects of *Nigella sativa* oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacology.* 2009;84(3):127–134.
- Hatipoglu D, Ates MB, Senturk G, Koca O, Bulut A, Donmez N. Neuroprotective role of *Nigella sativa* seed oil in mitigating bisphenol A-induced neurodegeneration. *Sci Rep.* 2025;15(1):22477. doi:10.1038/s41598-024-80654-1.
- Sedaghat R, Roghani M, Khalili M. Neuroprotective effect of thymoquinone, the *Nigella sativa* bioactive compound, in a 6-hydroxydopamine-induced hemi-parkinsonian rat model. *Iran J Pharm Res.* 2014;13(1):227–234.
- Fanoudi S, Alavi MS, Hosseini M, Sadeghnia HR. *Nigella sativa* and thymoquinone attenuate oxidative stress and cognitive impairment following cerebral hypoperfusion in rats. *Metab Brain Dis.* 2019;34(4):1001–1010. doi:10.1007/s11011-019-00394-4.
- Li XJ, Jiang L, Chen L, Chen HS, Li X. Neurotoxicity of Di-butyl phthalate in brain development following perinatal exposure: a study in rats. *Environ Toxicol Pharmacol.* 2013;36(2):392–402.
- Ojetunde AO, Alhassan A, Suleiman I, Isa AS. *Nigella sativa* oil protects against aluminum chloride-induced cognitive impairment via modulation of cholinergic activity, brain neurotransmitter, and oxidative stress. *Biol Med Nat Prod Chem.* 2025;14(2):739–746.
- Qian Y, Shao H, Ying X, Huang W, Hua Y. The endocrine disruption of prenatal phthalate exposure in mother and offspring. *Front Public Health.* 2020;8:366. doi:10.3389/fpubh.2020.00366.
- Miodovnik A, Edwards A, Bellinger DC, Hauser R. Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence. *Neurotoxicology.* 2014; 41:112–122. doi: 10.1016/j.neuro.2014.01.007.
- Gaur K, Siddique YH. Phthalates induced neurotoxicity: a mechanistic approach. *CNS Agents Med Chem.* 2024.

16. Enogieru AB, Momodu OI. The developing cerebellum as a target for toxic substances: protective role of antioxidants. *Cerebellum*. 2021;20(4):614–630. doi:10.1007/s12311-021-01231-0.
17. Rowdhwal SS, Chen J. Toxic effects of di-2-ethylhexyl phthalate: an overview. *Biomed Res Int*. 2018; 2018:1750368.
18. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation during global cerebral ischemia-reperfusion injury in rat hippocampus. *Phytomedicine*. 2007;14(9):621–627.
19. Radad K, Moldzio R, Taha M, Rausch WD. Thymoquinone protects dopaminergic neurons against MPP⁺ and rotenone. *Phytother Res*. 2009;23(5):696–700.
20. Fu Y, Dong J, Wang J, You M, Wei L, Fu H, Wang Y, Chen J. Developmental exposure to di-(2-ethylhexyl) phthalate induces cerebellar granule cell apoptosis via the PI3K/AKT signaling pathway. *Exp Neurobiol*. 2018;27(6):472–488. doi:10.5607/en.2018.27.6.472.