Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Volume 2, Issue 1, January to March 2026, pp - 24-36

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Research Paper

Neuroprotective Effects of *Nigella sativa* Oil on Ciprofloxacin-Induced Neurotoxicity in Prefrontal Cortex of Wistar Rats

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Received : 24 August 2025
Revised : 19 September 2025
Accepted : 27 September 2025

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ABSTRACT

Ciprofloxacin is a widely prescribed fluoroquinolone antibiotic; however, accumulating evidence indicates its potential to induce oxidative stress–mediated neurotoxicity. Nigella sativa oil (NSO) possesses potent antioxidant and neuroprotective properties. This study investigated the protective effects of NSO against Ciprofloxacin-induced oxidative, inflammatory, and histological alterations in the brain of adult Wistar rats. Twenty-four adult male Wistar rats were randomly assigned into four groups (n = 6): control, NSO (1 ml/kg), Ciprofloxacin (500 mg/kg), and Ciprofloxacin + NSO. Treatments were administered orally for 14 days. Following treatment, brain tissues were harvested for biochemical assessment of oxidative stress and inflammatory markers, including nitric oxide (NO), glutathione peroxidase (GPx), and interleukin-1β (IL-1β). Histopathological evaluation of cortical tissues was also performed using standard staining techniques. Ciprofloxacin administration significantly increased nitric oxide and IL-1β levels and significantly reduced glutathione peroxidase activity compared to the control group ($p < 0.05$), indicating elevated oxidative stress, neuroinflammation, and impaired antioxidant defense. Histological examination revealed neuronal degeneration, cellular distortion, and disrupted cortical architecture in the Ciprofloxacin-treated group. Conversely, co-administration of NSO significantly attenuated the elevation in NO and IL-1β levels and restored GPx activity toward normal values ($p < 0.05$). Histologically, the combined treatment group showed marked improvement in neuronal integrity and cortical organization compared to the Ciprofloxacin-only group. NSO alone did not produce significant adverse changes compared to control. Nigella sativa oil mitigates Ciprofloxacin-induced oxidative stress, neuroinflammation, and neuro-histological damage in adult Wistar rats, likely through its antioxidant and anti-inflammatory mechanisms. These findings suggest that NSO may serve as a potential adjunct therapeutic agent to reduce fluoroquinolone-associated neurotoxicity.

KEYWORDS: Ciprofloxacin, *Nigella sativa* oil, Prefrontal cortex, Neurotoxicity, Oxidative stress

INTRODUCTION

Fluoroquinolones are a class of broad-spectrum antibiotics extensively used in clinical practice for the treatment of bacterial infections in both adult and pediatric populations[1]. Their widespread prescription has increased concerns regarding potential adverse effects beyond their antimicrobial action, particularly on the central nervous system. Ciprofloxacin, one of the most commonly used fluoroquinolones, readily penetrates biological tissues and crosses the blood–brain barrier, increasing the risk of neurological complications[2]. Emerging evidence indicates that Ciprofloxacin may interfere with normal brain development by inducing oxidative stress, disrupting neurotransmitter balance, and altering neuronal homeostasis[3].

Experimental studies have demonstrated that Ciprofloxacin exposure during critical developmental periods can impair neural function and behavior through mechanisms involving mitochondrial dysfunction, excessive generation of reactive oxygen species, and neuroinflammation[3,4]. Behavioral deficits such as impaired learning, memory dysfunction, anxiety-like behavior, and altered locomotor activity have been reported in Ciprofloxacin-treated rodents[5,6]. Histological findings further reveal neuronal degeneration, cortical disorganization, and reduced neuronal density in vulnerable brain regions, including the prefrontal cortex, a region essential for executive function and emotional regulation[7]. Despite these findings, the effects of Ciprofloxacin-induced neurotoxicity on higher-order cortical regions during early development remain inadequately explored.

In parallel, increasing attention has been directed toward the use of natural antioxidants as protective agents against drug-induced neural injury. *Nigella sativa* oil (NSO), derived from black seed, has gained considerable scientific interest due to its bioactive constituents, particularly Thymoquinone, which exhibits potent antioxidant, anti-inflammatory, and neuroprotective properties[8,9]. Experimental evidence indicates that *Nigella sativa* oil can attenuate oxidative stress, reduce inflammatory cytokine expression, and preserve neuronal integrity in models of chemically induced neurotoxicity[8]. Additionally, NSO has been shown to improve behavioral performance and protect against drug- and toxin-induced neurodegeneration by maintaining normal neuronal architecture and function[9].

Despite growing evidence that Ciprofloxacin poses risks to neural development, there is still limited understanding of its specific impact on higher-order brain regions such as the prefrontal cortex, particularly during early postnatal life. At the same time, while *Nigella sativa* oil has demonstrated promising neuroprotective effects, its role in mitigating Ciprofloxacin-induced cortical neurotoxicity remains relatively underexplored. This study was therefore designed to investigate the effects of Ciprofloxacin exposure on growth parameters, behavior, oxidative balance, and neuronal integrity in the prefrontal cortex of Wistar rat offspring, while also evaluating the potential protective role of *Nigella sativa* oil. By addressing this gap, the study aims to provide deeper insight into antibiotic-induced neurodevelopmental injury and the therapeutic potential of plant-derived antioxidants.

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-25-0039. Approval was obtained from the Animal Care and Handling Unit Review Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, in accordance with the Institutional Animal Care and Use Committee (IACUC). The principles of the 3Rs (Replacement, Reduction, and Refinement) were strictly adhered to during the handling, housing, and care of experimental animals to ensure animal welfare and scientific validity.

Chemicals and Reagents

Ciprofloxacin was purchased from One Step Pharmacy, Ilorin, Nigeria, and manufactured by Aurobindo Pharma, Neuland Laboratories, and Kesar Pharma. *Nigella sativa* oil (NSO) was obtained from Al-Ameen Store, Ilorin, and manufactured by Hemani Herbals Trading. Normal saline solution was manufactured by Fidson Healthcare Plc (Nigeria). Other reagents used included sucrose solution, formalin, ethanol, cresyl fast violet, Mayer's hematoxylin, eosin Y, distilled water, xylene, and DPX mounting medium. All reagents were of analytical grade.

Experimental Animals

Twenty unmated adult female Wistar rats and twelve adult male Wistar rats weighing between 220–280 g were purchased from Fulcrum Innovation Limited, Ilorin, Kwara State. The animals were acclimatized for two weeks before the commencement of the study and housed in plastic cages under standard laboratory conditions of 12-hour light/dark cycle, ambient temperature, and adequate ventilation at the animal facility of the Department of Human Anatomy, Al-Hikmah University, Ilorin. Standard rat chow and water were provided ad libitum. Mating was carried out using a ratio of one male to two females. Pregnant rats were separated prior to parturition. Eight pups per group were selected from different litters for subsequent analyses.

Experimental Design and Grouping

The experimental animals (Wistar rat pups) were randomly divided into four groups of six pups each. Group A served as the control and received normal saline (10 mL/kg body weight, orally). Group B received *Nigella sativa* oil (1 mL/kg body weight, orally). Group C received Ciprofloxacin (60 mg/kg body weight, orally). Group D received a combination of Ciprofloxacin (60 mg/kg body weight) and *Nigella sativa* oil (1 mL/kg body weight, orally). All treatments were administered orally using a silver cannula.

Neurobehavioral Assessment

Neurobehavioral performance was evaluated using standard behavioral tests conducted one day prior to sacrifice. The tests were carried out in a quiet and controlled environment. Cognitive and exploratory behaviors were assessed using the Open Field Test, Y-Maze, and Morris Water Maze.

Termination of Experiment and Tissue Collection

At the end of the experiment, animals were humanely euthanized by cervical dislocation. Brains were immediately excised, and the prefrontal cortex was carefully dissected. Portions of the tissues were fixed in formalin for histological analysis, while others were homogenized in sucrose solution for biochemical investigations.

Biochemical Analysis

For biochemical assays, prefrontal cortex tissues were homogenized in ice-cold sucrose solution and centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant was collected and used for the assessment of nitric oxide, glutathione peroxidase, and interleukin-1 using standard spectrophotometric and ELISA methods.

Histological Analysis

Prefrontal cortex samples were fixed in formalin, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Tissue sections of 5 µm thickness were obtained using a rotary microtome and stained with Hematoxylin and Eosin as well as Cresyl Fast Violet to demonstrate general cytoarchitecture and Nissl substance integrity.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism version 8.0. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple comparisons. Statistical significance was set at $p < 0.05$.

RESULTS

(a) Effect of Ciprofloxacin and *Nigella sativa* Oil on Body Weight

There was a progressive increase in body weight across all experimental groups over the study period. However, pups administered Ciprofloxacin alone showed a noticeable reduction in body weight gain compared with the control group. Co-administration of *Nigella sativa* oil with Ciprofloxacin improved body weight gain relative to the Ciprofloxacin-treated group. No marked difference was observed between the control and NSO-only groups. These observations are illustrated in Figure 1.

(b) Effect of Ciprofloxacin and *Nigella sativa* Oil on Litter Size

There was a significant and observable difference in litter size among the experimental groups at birth. The number of pups delivered by dams exposed to Ciprofloxacin were significantly lower while compared to CIPRO + NSO group, while control and NSO-only group had more pups. No cases of stillbirth or gross developmental abnormalities were observed across the groups. These observations indicate that the treatments significantly affected litter size under the experimental conditions illustrated in Figure 2.

(c) Effect of Ciprofloxacin and *Nigella sativa* Oil on Fur Appearance

Physical examination of the pups revealed normal fur development across all experimental groups, characterized by smooth, dense, and uniformly distributed fur. No significant difference was seen across the group for fur appearance but increased fur density was noticed in NSO only group. Co-administration of *Nigella sativa* oil with Ciprofloxacin markedly improved fur appearance, with pups showing fur characteristics comparable to those of the control group. Representative images of fur appearance are shown in Figure 3.

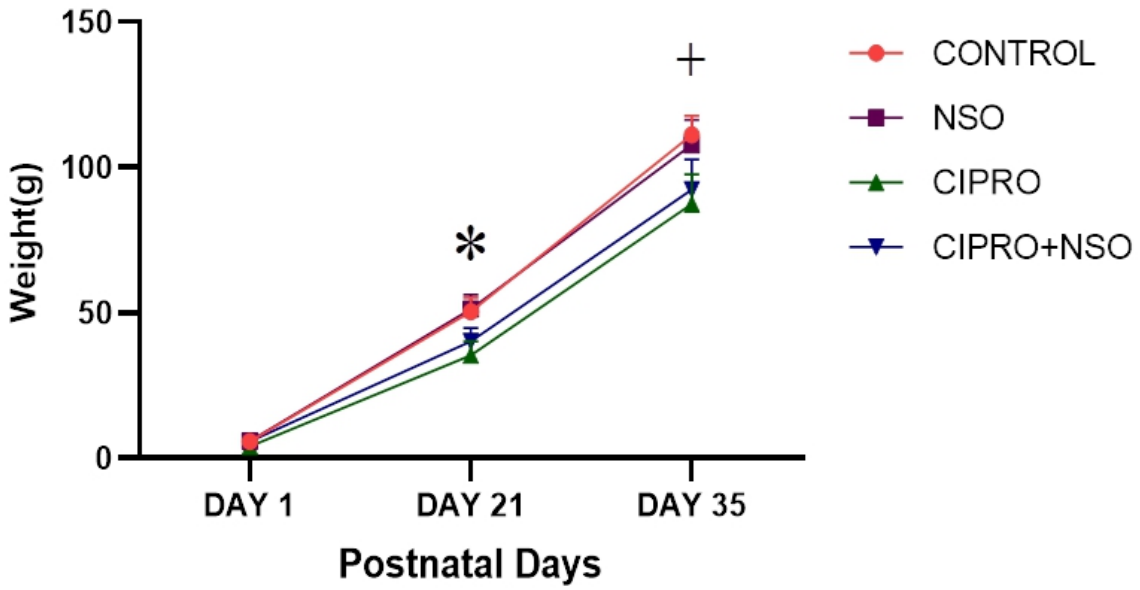

Figure 1: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on body weight of experimental animals. (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, while CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, + P<0.05 compare with CIPRO only

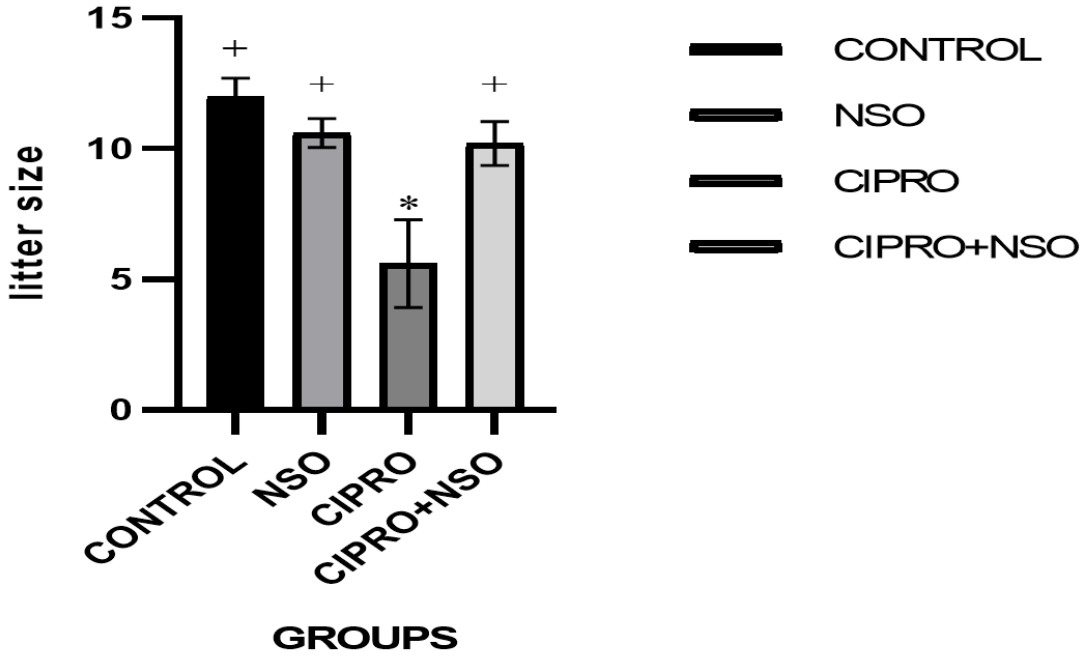

Figure 2: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on litter size of experimental animals. (+) indicates statistical significance compared to the NSO group, while CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, + P<0.05 compare with CIPRO only

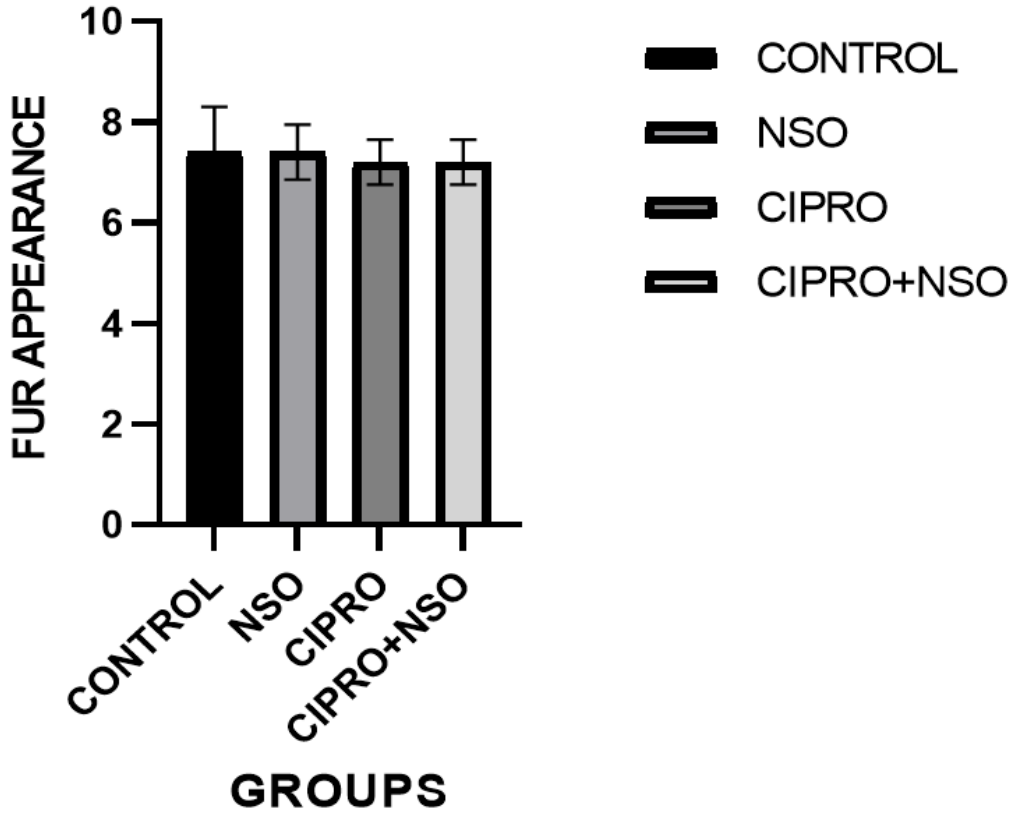

Figure 3: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on fur appearance of experimental animals. CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.)

(d) Effect of Ciprofloxacin and NSO Administration on Neurobehavioral Parameters

Open Field Test Performance

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

Rearing Frequency and Duration

The rearing frequency and duration varied significantly among the experimental groups. The control group exhibited the lowest rearing activity, followed by the *Nigella sativa* oil–treated group. Rats co-treated with Ciprofloxacin and *Nigella sativa* oil showed a moderate increase in rearing frequency and duration compared with the control and NSO groups. In contrast, the Ciprofloxacin-treated group displayed the highest rearing frequency and duration, indicating heightened behavioral activity. These observations are illustrated in Figures 4A and 4B.

The frequency of stretch-attend posture varied among the experimental groups. Rats treated with *Nigella sativa* oil exhibited the lowest frequency of stretching attempts, closely followed by the control group. In contrast, the Ciprofloxacin-treated group showed the highest frequency of stretch-attend posture. Co-administration of Ciprofloxacin with *Nigella sativa* oil reduced the frequency of stretching attempts compared with Ciprofloxacin alone, although values remained higher than those observed in the control and NSO groups. These findings are illustrated in Figure 4C.

Central Entry Frequency and Duration

The graph indicates that Ciprofloxacin markedly reduced both central entry frequency and duration compared to the control group, suggesting increased anxiety-like behavior or reduced exploratory activity in the open field test. NSO alone shows values relatively close to the control, implying no strong anxiogenic effect and possibly normal locomotor/exploratory behavior.

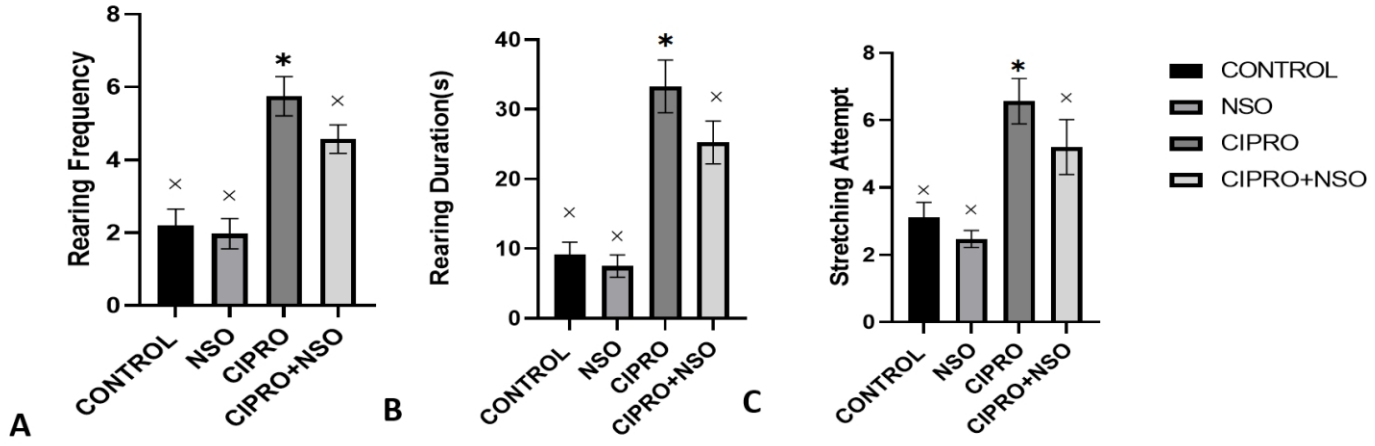

Figure 4: Graph showing the rearing frequency (A), rearing duration (B), stretching attempt (C) in the open field test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group. CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

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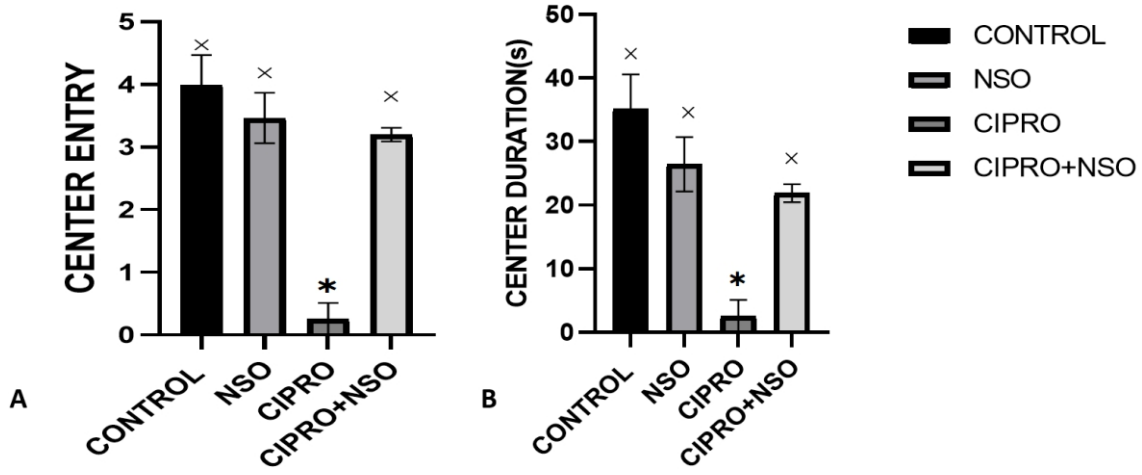

Figure 5: Graph showing the central entry frequency (A), central entry duration (B) in the open field test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group. CTR = Control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

Importantly, the CIPRO + NSO group shows a clear improvement compared to CIPRO alone, with values approaching the control/NSO levels, indicating that NSO may attenuate or counteract the behavioral effects induced by Ciprofloxacin.

I. Y-maze Test Performance

The Y-maze results show that Ciprofloxacin (CIPRO) significantly reduced spontaneous alternation percentage compared to the control group, indicating impaired spatial working memory and reduced cognitive performance. The NSO-treated group also showed a reduction compared to control, although the impairment was less severe than that observed with Ciprofloxacin alone.

Notably, the CIPRO + NSO group demonstrated improvement relative to the CIPRO group (x), suggesting that *Nigella sativa* oil partially ameliorated Ciprofloxacin-induced memory deficits, though performance did not fully return to control levels.

II. Morris-Water maze Test Performance

The Morris water maze results demonstrate clear differences in spatial learning and memory among the experimental groups across training and probe phases. On Day 1, the Cipro group shows impaired performance compared to the control, indicating delayed acquisition of spatial learning. NSO alone performs comparably to control, suggesting no detrimental effect on cognition. Notably, the Cipro + NSO group performs better than the Cipro group, indicating early attenuation of Ciprofloxacin-induced learning deficits. By Day 2, control and NSO groups exhibit improved performance consistent with learning consolidation, whereas the Cipro group continues to show poorer performance, reflecting sustained cognitive impairment [Figure 7].

Importantly, the Cipro + NSO group demonstrates improved performance relative to Cipro alone, suggesting partial recovery of learning ability. During the probe test, which assesses memory retention, the Cipro group shows reduced performance compared to control, indicating impaired spatial memory retention. In contrast, the Cipro + NSO group shows improved retention compared to Cipro alone, approaching control levels.

Histological Assessment

These observations in Figure 8 indicate that Ciprofloxacin induces notable neuronal damage in the PFC, while NSO co-administration attenuates the toxic effects and promotes structural restoration.

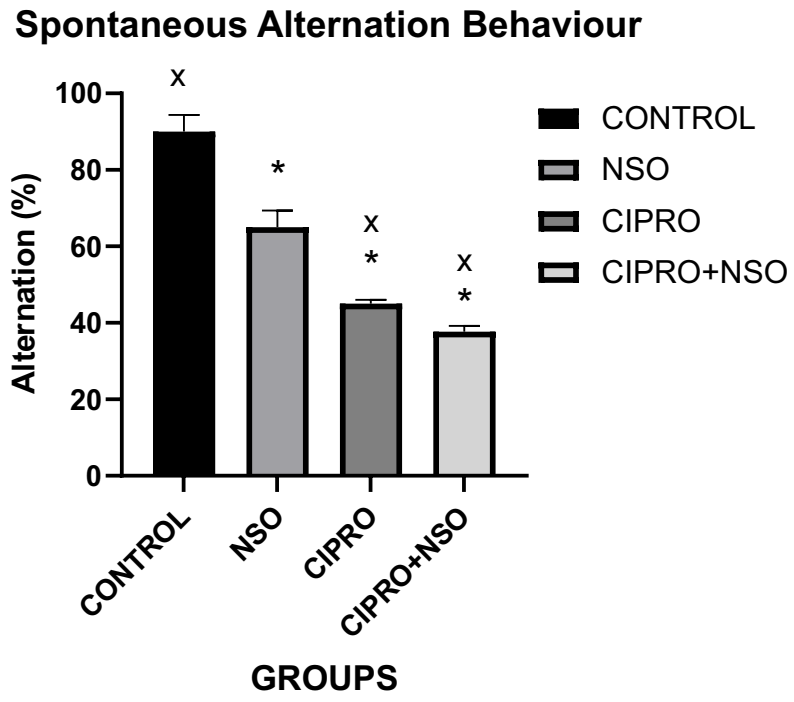

Figure 6: Graph showing the showing the percentage alternative behaviour in Y-maze test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

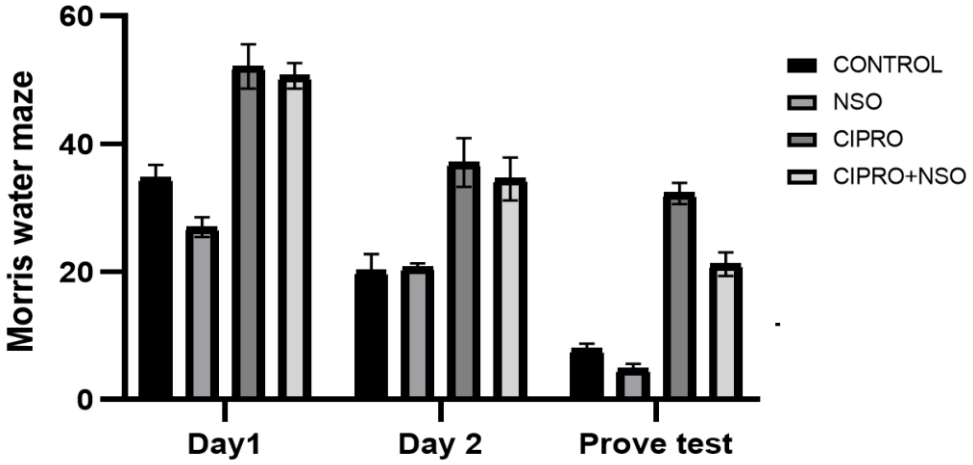

Figure 7: Comparative analysis of measured parameter across experimental groups
Grouped bar chart showing the mean values of the assessed variable across three experimental conditions within each group. Each cluster represents a distinct biological or treatment group, while individual bars within a cluster correspond to different treatment levels or exposure conditions. Data are presented as mean ± SEM (error bars)

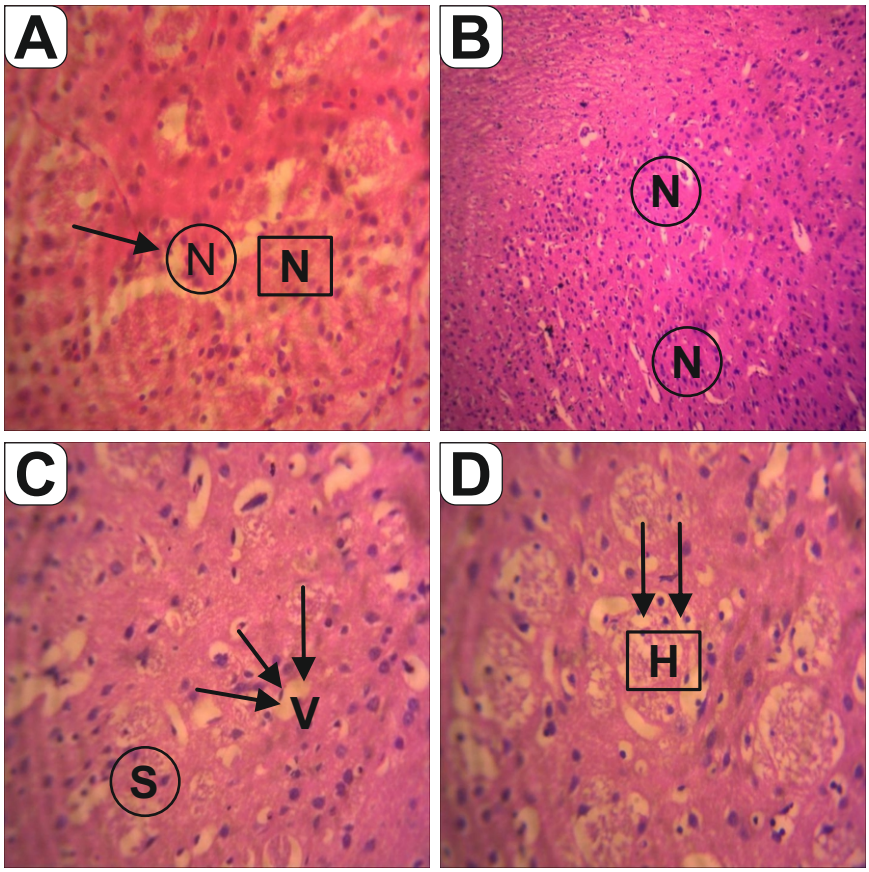

Figure 8: Photomicrographs of the prefrontal cortex (PFC) across experimental groups (H&E stain, ×400). (A) Control group showing well-preserved cortical architecture with intact nuclei (N) and normal neuronal arrangement. (B) NSO-treated group demonstrating well-preserved neurons (N) with maintained cytoarchitectural integrity comparable to control. (C) Toxic (Cipro) group revealing marked degenerative changes characterized by neuronal cell shrinkage consistent with chromatolysis (S) and prominent cytoplasmic vacuolation (V), indicative of neuronal injury. (D) Cipro + NSO group showing evidence of structural recovery with reduced degenerative features and areas suggestive of healing marked by scar deposition (H), indicating partial neuroprotection and reparative response

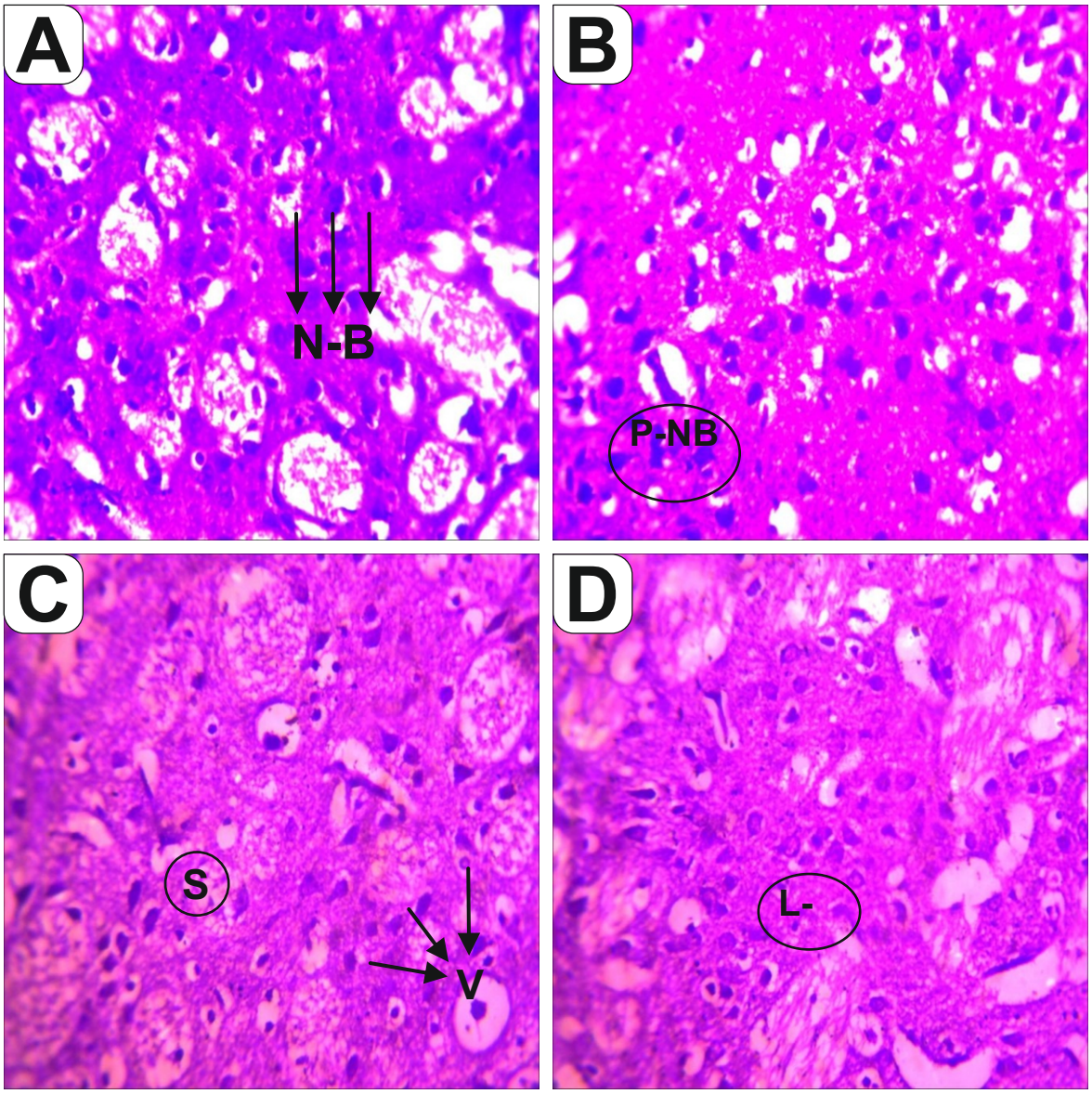

Figure 9: Photomicrograph showing the histology of the prefrontal cortex (PFC) of experimental groups (CFV ×400). (A) Control group showing well-preserved cortical architecture with intact neurons and prominent Nissl bodies (NB), indicating normal neuronal integrity; (B) NSO-treated group demonstrating densely packed and well-preserved Nissl bodies (PNB), comparable to the control group, suggesting maintained neuronal structural stability; (C) Ciprofloxacin-treated group revealing marked neurodegenerative alterations characterized by neuronal cell shrinkage due to chromatolysis (S) and prominent cytoplasmic vacuolation (V), indicative of neuronal injury and toxic insult; (D) Ciprofloxacin + NSO group showing reduced degenerative features with fewer Nissl bodies (LNB) compared to control but improved architecture relative to the Ciprofloxacin-only group, suggesting partial recovery and ongoing healing processes, possibly associated with reparative scar deposition

The photomicrographs demonstrate that Ciprofloxacin induces significant neuronal degeneration in the PFC, while co-administration of NSO attenuates the histopathological damage and promotes structural restoration.

(a) Effect of Ciprofloxacin and NSO administration on Biochemical Parameters

i. Pro-inflammatory Cytokine

The results show a clear alteration in IL-1β levels across the experimental groups. The Ciprofloxacin-treated group exhibited a marked and statistically significant increase in IL-1β concentration compared to the control group, indicating a pronounced pro-inflammatory response following Ciprofloxacin administration. The NSO-only group demonstrated IL-1β levels comparable to the control, suggesting that NSO does not independently induce inflammatory activation.

Co-administration of NSO with Ciprofloxacin resulted in a reduction in IL-1β levels relative to the Ciprofloxacin-only group, although levels remained slightly elevated compared to control. This pattern indicates that Ciprofloxacin triggers neuroinflammatory processes, while NSO exerts a modulatory or anti-inflammatory effect, attenuating Ciprofloxacin-induced elevation of IL-1β.

ii. Oxidative Stress Markers

Figure 11 shows that Ciprofloxacin significantly increased nitric oxide (NO) levels and significantly decreased glutathione peroxidase (GPx) activity compared to the control group, indicating increased oxidative stress and reduced antioxidant defense. Treatment with *Nigella sativa* oil (NSO) alone did not markedly alter these parameters compared to control. However, co-administration of NSO with Ciprofloxacin reduced the elevated NO levels and improved GPx activity compared to the Ciprofloxacin-only group. This suggests that *Nigella sativa* oil exerts a protective antioxidant effect against Ciprofloxacin-induced oxidative stress.

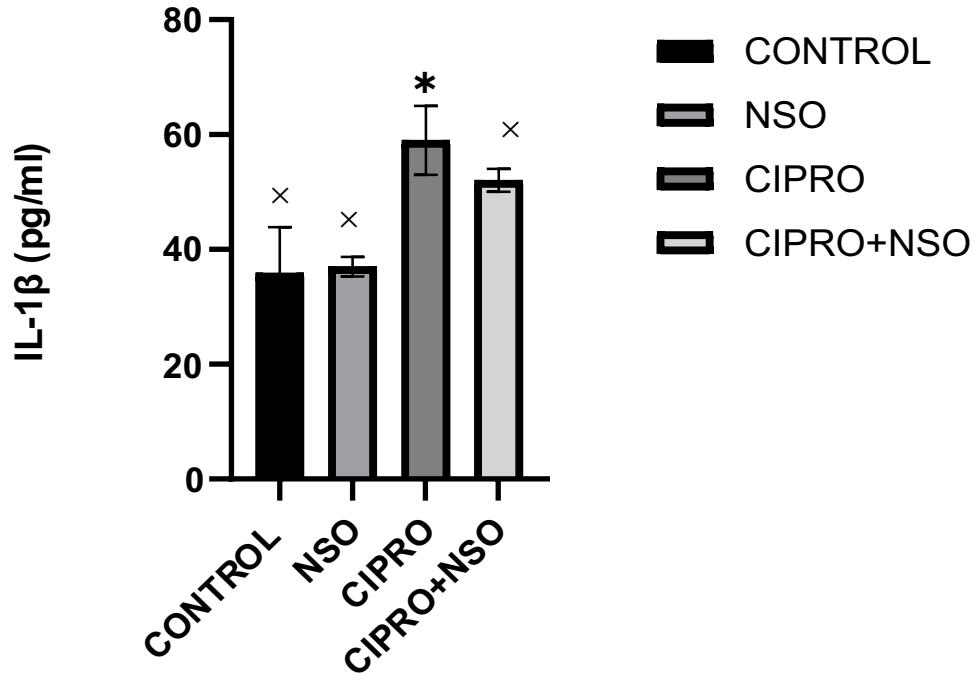

Figure 10: Graph showing the mean ± SEM levels of IL-1β (pg/ml) in control, NSO, Ciprofloxacin, and combined treatment groups. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

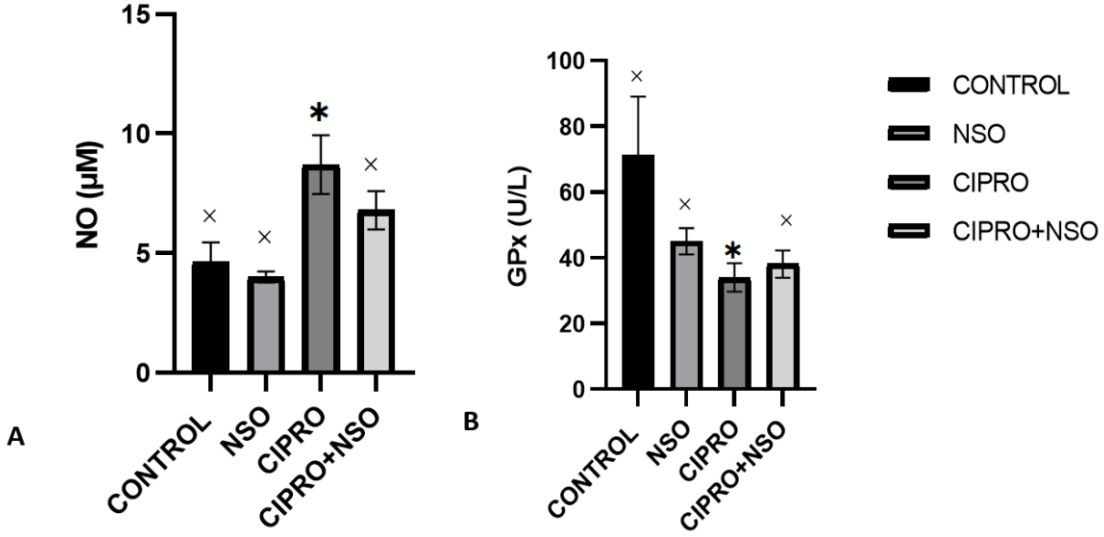

Figure 11: Graph showing the mean ± SEM levels of Nitric Oxide-NO (µM) (A) and Glutathione Peroxidase- GPx (U/L) (B) in control, NSO, Ciprofloxacin, and combined treatment groups. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

DISCUSSION

The present study demonstrates that early postnatal exposure to Ciprofloxacin induces significant neurobehavioral impairment, oxidative imbalance, neuroinflammation, growth suppression, and structural degeneration within the prefrontal cortex (PFC) of Wistar rat offspring. Importantly, co-administration of *Nigella sativa* oil (NSO) markedly attenuated these alterations, suggesting a protective role mediated primarily through antioxidant and anti-inflammatory mechanisms. When examined collectively, the behavioral, biochemical, and histomorphological findings converge to establish oxidative stress–driven neuroinflammation as a central mechanism underlying Ciprofloxacin-induced cortical toxicity.

Fluoroquinolones, including Ciprofloxacin, are known to penetrate the blood–brain barrier and have been associated with central nervous system adverse effects such as agitation, anxiety, seizures, and cognitive disturbances[10,11]. Mechanistically, fluoroquinolones can antagonize GABA-A receptors and disrupt excitatory-inhibitory neurotransmission balance, leading to neuronal hyperexcitability[12]. During early postnatal development, the prefrontal cortex undergoes intense synaptogenesis, dendritic remodeling, and myelination, rendering it particularly vulnerable to pharmacological insults[13]. The behavioral alterations observed in this study including increased rearing activity, elevated stretch-attend posture, reduced central exploration, impaired spontaneous alternation in the Y-maze, and delayed acquisition in the Morris water maze reflect dysfunction of prefrontal-dependent executive processing and working memory networks[14,15].

The reduction in spontaneous alternation percentage indicates compromised spatial working memory, a function critically dependent on intact prefrontal–hippocampal circuitry[16]. Similarly, impaired performance in the Morris water maze reflects disruption in cortical integration of spatial learning and memory consolidation processes[17]. Anxiety-like behavior observed in the open field test further suggests altered cortical regulation of emotional processing. These findings are consistent with previous reports demonstrating behavioral hyperactivity and cognitive disturbances following fluoroquinolone exposure in experimental models[12,18]. Thus, the neurobehavioral phenotype observed in this study provides functional evidence of cortical disruption.

Ciprofloxacin administration also significantly elevated nitric oxide (NO) levels while suppressing glutathione peroxidase (GPx) activity in the prefrontal cortex. This indicates a pronounced shift toward oxidative stress. Fluoroquinolones have been reported to induce mitochondrial dysfunction, resulting in excessive production of reactive oxygen species (ROS)[19]. Increased NO can react with superoxide radicals to generate Peroxynitrite, a potent cytotoxic oxidant capable of inducing lipid peroxidation, protein nitration, and DNA damage[20]. Concurrent reduction in GPx activity further compromises endogenous antioxidant defense, amplifying neuronal susceptibility to oxidative injury. Oxidative stress is widely recognized as a fundamental mediator of drug-induced neurotoxicity and developmental cortical damage[21]. The present biochemical findings therefore provide mechanistic support for the observed structural degeneration.

In parallel with oxidative imbalance, Ciprofloxacin markedly

elevated interleukin-1β (IL-1β) levels, indicating activation of neuroinflammatory pathways. IL-1β is a pro-inflammatory cytokine released by activated microglia and plays a pivotal role in synaptic dysfunction, impaired long-term potentiation, and neuronal apoptosis[22]. Sustained neuroinflammation during critical developmental windows can alter cortical circuitry and compromise executive functioning later in life[23]. Oxidative stress and inflammation are closely interconnected; ROS-mediated activation of NF-κB signaling promotes cytokine production, including IL-1β, establishing a self-propagating inflammatory cascade[24]. The concurrent elevation of NO and IL-1β observed in this study therefore suggests that Ciprofloxacin-induced oxidative stress acts upstream of neuroinflammatory activation, culminating in cortical injury.

Histological findings further corroborate the biochemical and behavioral data. Ciprofloxacin-treated offspring exhibited neuronal shrinkage, chromatolysis, cytoplasmic vacuolation, and reduced Nissl substance within the PFC. Chromatolysis reflects dissolution of rough endoplasmic reticulum and impaired protein synthesis, typically occurring in response to axonal or metabolic injury[25]. Cytoplasmic vacuolation suggests mitochondrial and membrane disruption, consistent with oxidative damage[21]. The reduction in Nissl bodies observed with Cresyl Fast Violet staining indicates compromised neuronal metabolic integrity. Collectively, these structural alterations confirm significant neurodegeneration within the prefrontal cortex and align with the functional deficits observed in behavioral assays.

Beyond neural parameters, Ciprofloxacin exposure was associated with reduced body weight gain and decreased litter size. Fluoroquinolone-induced mitochondrial impairment may disrupt cellular proliferation and metabolic regulation during development[19]. Early-life oxidative stress is known to interfere with growth signaling pathways and neurodevelopmental maturation[26]. The developmental impact observed in this study underscores the broader systemic consequences of early antibiotic exposure and reinforces concerns regarding fluoroquinolone use during vulnerable periods. So, co-administration of *Nigella sativa* oil significantly mitigated Ciprofloxacin-induced alterations across behavioral, biochemical, and histological domains. NSO reduced NO levels, restored GPx activity, and attenuated IL-1β elevation, suggesting effective modulation of oxidative and inflammatory pathways. The principal bioactive compound of NSO, thymoquinone, has been shown to possess potent free radical scavenging properties, enhance endogenous antioxidant enzyme activity, and inhibit NF-κB–mediated inflammatory signaling[27,28]. Previous studies have demonstrated that *Nigella sativa* and thymoquinone protect against chemically induced neurotoxicity and improve cognitive performance in oxidative stress models[29,30]. The behavioral improvement observed in the CIPRO+NSO group particularly in working memory and spatial learning indicates functional preservation of cortical circuitry.

Moreover, NSO co-treatment reduced neuronal degeneration and preserved cortical cytoarchitecture, with improved Nissl body integrity compared to Ciprofloxacin alone. This structural recovery likely reflects reduced oxidative damage and suppression of inflammatory-mediated neuronal apoptosis. The partial normalization of IL-1β levels further supports NSO's immunomodulatory role. Together, these findings suggest that NSO interrupts the pathogenic cascade initiated by Ciprofloxacin at multiple levels, thereby preserving neuronal viability and cortical function.

CONCLUSION

This study supports a mechanistic sequence in which Ciprofloxacin exposure induces mitochondrial dysfunction and ROS overproduction, leading to nitric oxide elevation, antioxidant enzyme suppression, activation of inflammatory cytokines, neuronal degeneration, and subsequent executive and cognitive dysfunction. *Nigella sativa* oil appears to counteract this cascade through antioxidant reinforcement and anti-inflammatory modulation, resulting in structural and functional neuroprotection. The significance of this study lies in its integrated demonstration that Ciprofloxacin can compromise higher-order cortical development during early life, and that plant-derived antioxidants such as *Nigella sativa* oil may offer a viable adjunctive neuroprotective strategy. These findings contribute to the growing body of literature cautioning against indiscriminate fluoroquinolone exposure during neurodevelopmental windows and provide a scientific basis for further translational investigation of *Nigella sativa* oil in mitigating antibiotic-induced neurotoxicity.

AUTHORS' CONTRIBUTION

Sample collection and data analysis were conducted by Atoyebi A.T., Nasirudeen A.E., and Adebiyi A. O. The manuscript draft was prepared by Nasirudeen A.E., Adebiyi A. O., and Alasirin K. The final manuscript draft and result interpretation was facilitated by Adebiyi A. O., Abdulrauf A., Lawal I. A., Hamzat F. O., Ibrahim S. O., Eze S. M., and Dare B. J.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

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