Ameliorative Effects of Nigella Sativa Oil on Permethrin-Induced Neurotoxicity in the Cerebellum of Male Wistar Rats

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Abstract

Background: Permethrin, a pyrethroid insecticide commonly used for indoor residential treatment and outdoor applications can cause neuroinflammation and oxidative stress in rodent brains, resulting in a variety of neurodegenerative alterations. *Nigella sativa oil* (NSO) and its phytochemical components have been reported to possess anti-inflammatory, anti-hyperlipidemic, anti-oxidative and other beneficial properties. The goal of this study is to examine how NSO affects permethrin-induced neurotoxicity in the cerebellum of adult male Wistar rats.

Materials and Methods: Sixteen (16) young male rats were divided into four groups, with each group containing four rats. Group A (control) was fed on a regular rat diet, Group B was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin, Group C was fed a regular diet with 14 ml/kg of NSO, Group D was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin and 14 ml/kg of NSO. All administration lasted for 14 days. The experimental animals were sacrificed and their cerebelli were excised for post-mortem studies and biochemical analysis.

Results: Permethrin-treated animals presented with a reduction in the cerebellar level of glutathione peroxidase (GPx) with a corresponding increase in tumour necrosis factor alpha (TNF-α). Histological observations showed impaired cellular characterization in the cerebellum of permethrin-treated rats. *Nigella sativa* oil administration mitigated permethrin effects by increasing GPx level, decreasing TNF-α, and limiting cerebellar histoarchitectural alterations.

Conclusion/Recommendations: In conclusion, our study demonstrated that Nigella sativa oil significantly enhanced the activity of GPx (glutathione peroxidase) and reduced levels of TNF-α, suggesting its potential neuroprotective effects. These results underscore the therapeutic potential of Nigella sativa oil in mitigating oxidative stress and inflammation caused by neurotoxins like permethrin.

Keywords: Permethrin, *Nigella sativa* oil, neuroinflammation, oxidative stress, toxicity

Introduction

The nervous system's normal histoarchitecture and functions can be disrupted by exposure to harmful substances (neurotoxins), which can cause neurons and other brain cells to malfunction. Exposure to heavy metals like lead and mercury, substances used in chemotherapy, radiation treatment, drug therapies, organ transplants, certain foods and food additives, pesticides, industrial or cleaning solvents, cosmetics, and some naturally occurring chemicals can cause neurotoxicity.[1]

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Exposure to environmental toxins, such as pesticides, has been linked to an increased risk of developing neurological dysfunction. [2] Although the cause of neurodegenerative conditions is unknown, evidence regarding energy metabolism, excitotoxicity, and oxidative damage is becoming more persuasive. [3] These processes interact in a sophisticated way. Neuronal depolarization, activation of N-Methyl-D-aspartate excitatory amino acid receptors, and increases in intracellular calcium buffered by mitochondria may result from a failure in energy metabolism. Mitochondria are the main intracellular source of free radicals; higher mitochondrial calcium concentrations enhance free radical production. However, mitochondrial DNA is particularly vulnerable to oxidative stress. [4] Oxidative stress and mitochondrial damage have been implicated in the pathogenesis of many neurodegenerative conditions, such as Alzheimer's disease, amyotrophic lateral

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sclerosis, and Parkinson's disease.[5]

Due to its strong insecticidal action and minimal mammalian toxicity, permethrin, the most commonly used synthetic Type I pyrethroid insecticide, is widely utilized worldwide. Initially, permethrin was thought to have low toxicity in non-target animals. However, as its usage became more widespread globally, evidence emerged that it might cause neurotoxicity, immunotoxicity, hepatotoxicity, cytotoxicity, digestive system toxicity, reproductive effects, effects in the body.[6] It affects the neurological systems of insects, disrupting neuronal functions by interfering with sodium channels, resulting in muscular spasms, paralysis, and death.[7] Permethrin, a synthetic pyrethyroid insecticide can be absorbed through various routes such as dermal, inhalation and oral routes with the oral route constituting the most effective route through which it enters systemic circulation (60-70 %).[8]

Once absorbed, it is distributed throughout the body as a result of its lipophilic nature. It also has the ability to accumulate in tissues including fats and brain tissues, crossing biological membrane as well as blood-brain barrier.[8] It is metabolized in the liver and plasma through metabolic pathways such as hydrolysis and oxidation process leading to metabolites such as 3-phenoxybenzyl alcohol glucuronide in urine and others such as cis- and trans-dichlorovinyl chrysanthemic acids (DCVA) and phenoxybenzoic acid derivatives.[8] The primary route of elimination is through excretion via urinary excretion which peaks within 12 to 24 hours post exposure.[8]

Permethrin has been shown to cause neuroinflammation in brain areas involved in memory creation, processing, and consolidation. Permethrin acts on the axons in the peripheral and central nervous systems, causing prolonged opening of sodium channels.[8] Since neuroinflammation and cognitive impairment are inextricably linked, this clarifies one of the possible mechanisms by which permethrin influences behavior.[9] The harmful effects of permethrin are increasingly being linked to oxidative stress, according to mounting data. In the newborn rat brain, permethrin causes neurotoxicity and oxidative stress. Prenatal permethrin exposure may impair brain development due to vascular alterations. [10] More importantly, permethrin treatment throughout childhood may have long-term consequences in adulthood, affecting homeostatic processes, physiological indices, and oxidative status.[11]

Phytochemical elements of certain medicinal plants, such as NSO, could be used in the treatment of neurodegenerative conditions. [12] For millennia, NSO (Ranunculaceae) has been used to treat various animal and human maladies in diverse cultures worldwide. NS is consumed in different forms which includes seeds, oils and extracts. Previous studies have shown that Nigella sativa seeds contain both fixed and essential oils, proteins, alkaloids, and saponin. However, most of the biological activities of Nigella sativa are attributed to its main active constituent, thymoquinone. [13] Thymoquinone is absorbed through the gastrointestinal tract when ingested with the oils through its lipophilic nature permits better solubility and uptake in the intestinal mucosa. [14] Once absorbed, they are distributed throughout the body where they bind to plasma protein and cross biological and blood brain barrier to accumulate in various tissues. [15] The active component of NSO is metabolized in the liver. Thymoquinone undergoes biotransformation through oxidation and conjugation through which therapeutic properties and inactive forms are prepared for excretion. [16] Elimination of thymoquinone and other metabolites occurs through elimination in the urine and feces with a half-life which spans from a few hours to several days. [17]

NSO is medically effective against a wide range of clinical conditions, such as inflammatory conditions, infectious diseases, infertility, and neurological and mental illnesses, among others. [12] The goal of this study was to ascertain the role of NSO in alleviating neurochemical and histochemical alterations associated with permethrin-induced cerebellar injury in male Wistar rats.

Materials and Methods

Sixteen (16) young male Wistar rats (Rattus norvegicus), averaging 89.5 g were used. They were housed in plastic rectangular cages with gauze nets and fed standard rat feed on a daily basis containing 15% crude protein, 7% fat, 10% crude fiber, 1% calcium, 0.35% phosphorus, and a caloric measurement of 2.55 kcal/kg, purchased from Ogo-Oluwa Feeds and Flour Mill Limited, Ilorin, Kwara State. They had unlimited access to clean water and clean surroundings, with bedding changed everyday. Before the trial began, the rats were allowed to acclimatize for seven days.

Treatment of animals

A Rambo bug powder (RamboR®; Gongoni Company Limited, Kano, Nigeria), containing permethrin 0.6% and inert carriers 99.40%, was used. The animals were divided into four groups, each with four animals, labeled A-D respectively, with n=4/group. Group A (Control) had a normal diet. Group B had 0.6% permethrin (1000 mg/kg body weight) mixed with their diet. [9] Group C received Nigella sativa oil (NSO)

(Hemani International KEPZ, Karachi, Pakistan) at 14 ml/kg via oral cannula. Group D was given a combination of a 0.6% permethrin diet and NSO at similar doses. The treatment lasted for 14 days to investigate the prolonged effects of NSO on permethrin toxicity in the cerebellum of male Wistar rats.

Tissue processing for histological, histochemical and immunohistochemical demonstration

The animals for qualitative analysis (4 rats per group) were sacrificed via cervical dislocation once the study was completed. On ice, the cerebellum was removed, and the cerebellar hemispheres were separated. For biochemical tests, the left cerebellar hemispheres were immediately placed in a 30% cold sucrose solution, while the right cerebellar hemispheres were fixed in 10% formal saline for histological and immunohistochemical examinations. The tissues were prepared for histology, embedded in paraffin, and sectioned using a rotary microtome at a thickness of 5 µm. Hematoxylin and eosin (H&E) staining was used for general histology, and cresyl fast violet (CFV) staining was used for the histochemical demonstration of Nissl substances.

Colorimetric assay for biochemical studies

Enzymatic experiments were conducted on right cerebellar hemispheres maintained in a 30% cold sucrose solution. The cerebellum of rats was assessed using a spectrophotometric approach employing a glutathione peroxidase assay kit (ELISA reader Diatech DR200BS, UK)[13]. Before beginning the procedures, all reagents and samples were brought to room temperature. Cerebellar tissues were weighed and homogenized with an automated homogenizer (Teflon homogenizer GR3200, China) at 4°C in an ice-cold 30% sucrose solution. The homogenate was poured into a 5 mL specimen container, which was then placed in an ice-filled centrifuge tube (IEC central GPSR, USA). The homogenate was centrifuged at 3,000 rpm for 15 minutes. The supernatants were aspirated into labeled glass cuvettes that were kept in the freezer. The assay was conducted according to the instructions on the assay pack provided by the manufacturer. A pro-inflammatory cytokine, termed tumor necrosis factor alpha, was also used to assess the degree of neuroinflammation

Light microscopy

Histological sections were captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA).

Data analysis

This research was conducted at the Animal facility situated at the College of Health Science, University of Ilorin, Nigeria, and was approved by the University of Ilorin Ethical Review Committee (UERC) following the recommendation of the College of Health Sciences Ethical Review Committee, in compliance with the Institutional Animal Care and Use Committee (IACUC).

The numerical data obtained were analyzed statistically using one-way ANOVA, followed by Tukey's post hoc test with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA; version 8 for Windows). Results were expressed as mean ± SEM (standard error of the mean). Statistical differences between control and treated groups were determined at $p<0.05$ and $p<0.01$, indicating statistical significance.

Results

The body weights were measured after 14 days of treatment. The control group gained the most weight (Table 1), followed by permethrin, NSO, and the group co-treated with NSO and permethrin (0.002). The permethrin group had a reduced weight difference compared with the Control, but higher than the NSO group. The group co-treated with permethrin and NSO had the lowest weight difference.

Table 1: Mean of the initial and final weights for each group, mean weight difference, Mean cerebellar weight, and cerebellar/body weight ratio

Groups	Mean initial weight (g)	Mean final weight (g)	Mean weight difference (g)	Mean cerebellar weight (g)	Mean cerebellar/ body weight ratio	p value
A: Control	85.25± 11.20	120.0± 2.79	34.75± 9.52	0.185 ± 0.015	0.001	p<0.05
B: Permethrin (1000 mg/kg)	87.25± 9.49	113.0± 11.60	25.75± 2.98	0.240 ± 0.051	0.002	p<0.05
C: *Nigella sativa* oil (14ml/kg)	89.20± 8.92	99.5± 9.36	10.25± 3.30	0.202 ± 0.026	0.002	p<0.05
D: *Nigella sativa* (14 ml/kg) oil + Permethrin (1000 mg/kg)	90.50± 7.41	101.0± 10.25	10.50± 3.50	0.157 ± 0.023	0.002	p<0.05

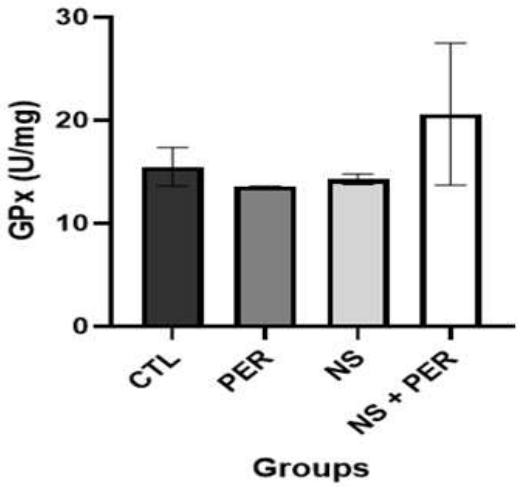

Figure 2. Graphical representation of cerebellar glutathione peroxidase (GPx) activity assayed across the four groups.

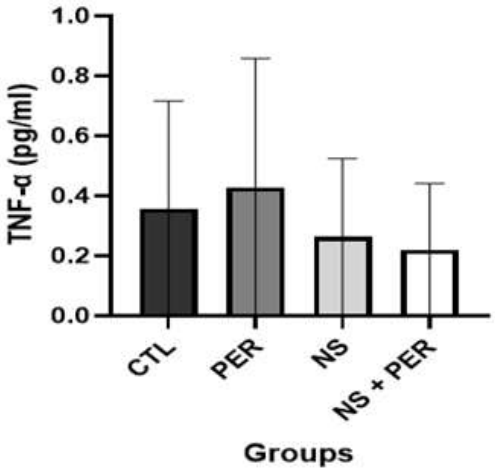

Figure 3. Graphical representation of cerebellar tumor necrosis factor (TNF-α) activity assayed across the four groups.

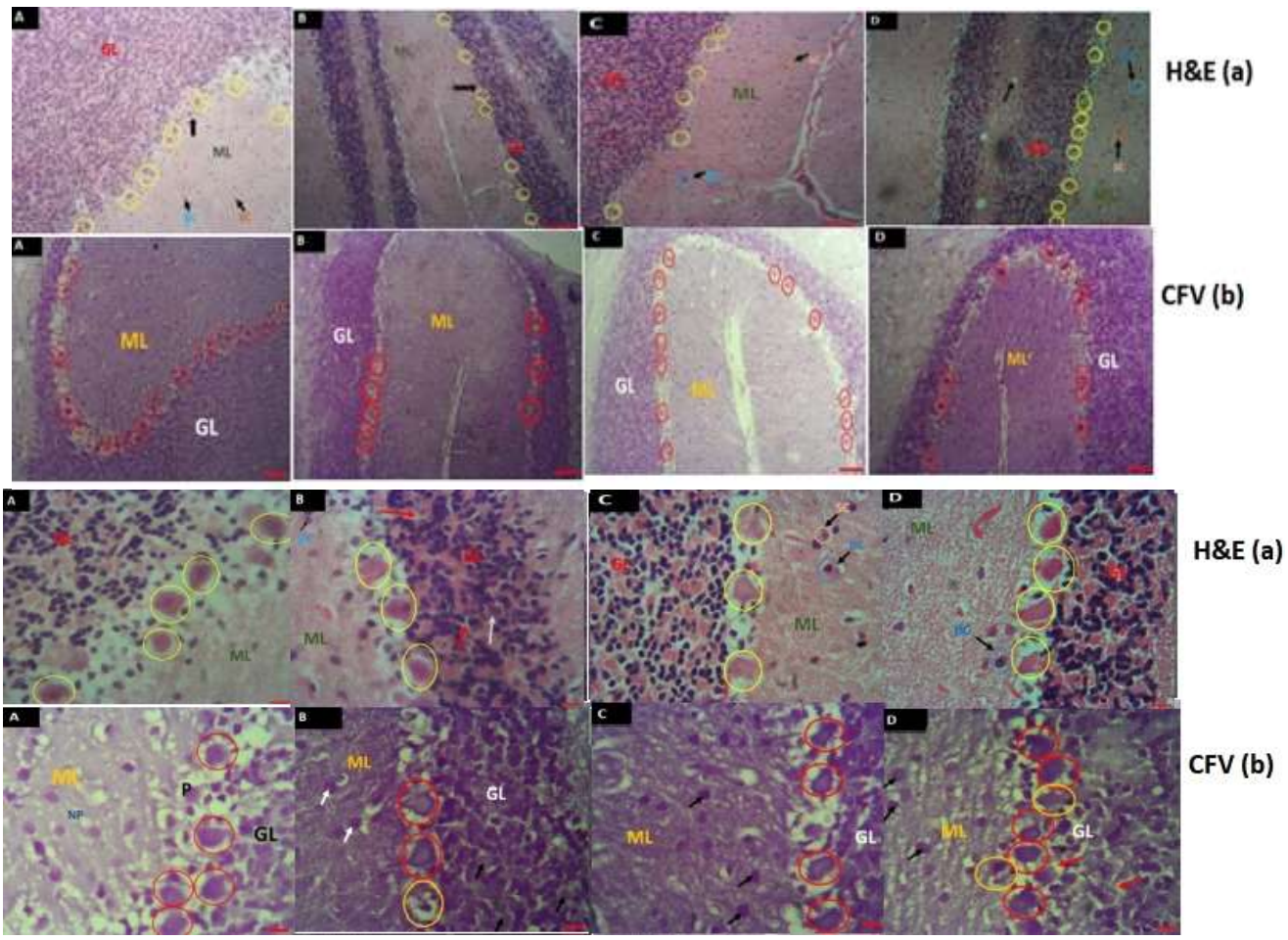

Figure 5. Representative photomicrographs of the cerebellum of the experimental animals stained with hematoxylene and eosin (a) and cresyl fast violet (b). (25 µm scale bar).

***Nigella sativa* oil counterweighs permethrin-induced oxidative stress in the cerebellum**

Cerebellar glutathione peroxidase (GPx) activity was assayed for in the four groups. Permethrin caused a decrease in GPx activities in the cerebellum (Fig. 2). *Nigella sativa* oil administration caused an increase in the level of GPx compared with the permethrin-treated rats, while rats that were co-treated with NSO and permethrin had elevated levels of GPx relative to those treated with permethrin only. Permethrin caused a

decrease in GPx activities in the cerebellum (Fig. 2). *Nigella sativa* oil administration caused an increase in the level of GPx compared with the permethrin-treated rats, while rats that were co-treated with NSO and permethrin had elevated levels of GPx relative to those treated with permethrin only.

***Nigella sativa* oil reverses permethrin-induced cerebellar inflammation**

Permethrin treatment was associated with elevated level of tumour necrosis factor alpha (TNF-α) in the cerebellum of Wistar rats ($p>0.05$) in comparison with other groups (Fig. 3). The group treated with NSO had a lower TNF-α level compared with the permethrin-treated group, while rats co-treated with NSO and permethrin had lower TNF-α levels compared to the permethrin-treated group. Permethrin treatment was associated with elevated level of tumour necrosis factor alpha (TNF-α) in the cerebellum of Wistar rats ($p>0.05$). This was in comparison with other groups (Fig. 3). The group treated with NSO had a lower TNF-α level compared with the permethrin-treated group, while rats co-treated with NSO and permethrin had lower TNF-α levels compared to the permethrin-treated group. Representative photomicrographs of the cerebellum of the experimental animals. (a) showed a panoramic view of the cerebellar histomorphology stained with Hematoxylin and Eosin (100 µm scale bar). The control (A) and NSO (C) groups presented with granular (GL) and molecular layers (ML) well delineated by Purkinje cells (yellow rings). The Permethrin only group (B) presented with reduced cellular density. Permethrin plus NSO (D) group presented with moderately thicker granular cell layers compared to 'B'. 'D' presented with deeply stained basket (BC) and stellate cells (SC). (b) showed the Nissl profiling of the cells in the cerebellar cortex of the experimental animals stained with cresyl fast violet (100 µm scale bar). The control (A) and NSO (C) groups presented with typical Nissl staining intensity. The neurons were lightly stained and well situated in their respective neuropils. The outer molecular layer (ML) and the inner granular layer (GL) were clearly delineated by the Purkinje layer (red rings). Permethrin plus NSO group presented with deep Nissl staining intensity of the Purkinje cells (red rings) and granule cells. The outer molecular layer and the inner granular layer were clearly delineated by the Purkinje layer.

Effect of permethrin exposure on histoarchitecture and Nissl profile of the cerebellum

The neuronal morphology and cytoarchitectural arrangements of the cerebellum were studied using Haematoxylin and Eosin and Cresyl fast violet stains (Figures 4 & 5). Observation of the cerebellar cortex showed apparently normal histomorphology in the control groups. The cerebellar cortex of the control animals and animals treated with NSO showed moderately stained cells with normal morphological presentation, cellular layering and cellular density, displayed a single layer of well outlined Purkinje cells with prominent soma and axonal projections plunging deep into the molecular layers. The granular layers of these two groups consist of small moderately stained granule cells with dense organization. Neuronal morphology of Permethrin-treated rats demonstrated fragmented cerebellar layers with cryptic granules, degenerating Purkinje cells with pyknotic cell bodies, and short dendritic processes. However, the rats treated with Permethrin and NSO indicated cerebellar layers and neuronal morphology, similar to that of the control with the exception of deeply stained granule cells which were moderately stained in control and NSO groups.

Figure 4 showed representative photomicrographs of the cerebellum of the experimental animals. (a) showed a panoramic view of the cerebellar histomorphology stained with Hematoxylin and Eosin (100 µm scale bar). The control (A) and NSO (C) groups presented with granular (GL) and molecular layers (ML) well delineated by Purkinje cells (yellow rings). The Permethrin only group (B) presented with reduced cellular density. Permethrin plus NSO (D) group presented with moderately thicker granular cell layers compared to 'B'. 'D' presented with deeply stained basket (BC) and stellate cells (SC). (b) showed the Nissl profiling of the cells in the cerebellar cortex of the experimental animals stained with cresyl fast violet (100 µm scale bar). The control (A) and NSO (C) groups presented with typical Nissl staining intensity. The neurons were lightly stained and well situated in their respective neuropils. The outer molecular layer (ML) and the inner granular layer (GL) were clearly delineated by the Purkinje layer (red rings). Permethrin plus NSO group presented with deep Nissl staining intensity of the Purkinje cells (red rings) and granule cells. The outer molecular layer and the inner granular layer were clearly delineated by the Purkinje layer.

Figure 5 showed representative photomicrographs of the cerebellum of the experimental animals. The control (A) presented with scanty granule cells, and moderately stained Purkinje cells (yellow rings). NSO (C) groups presented with lightly stained molecular layer, moderately stained granular layer with moderate cell population and moderately stained Basket (BC) and Stellate cells (SC). The Permethrin only (B) group presented with lightly stained Purkinje cells with chromatolytic changes, fragmented Purkinje cells (yellow rings) (b), cellular fragmentation (red arrow) and granule cell chromatolysis (white arrows) (a), and deeply stained basket cells (white arrows) in the molecular layer (ML) (b). Permethrin plus NSO (D)

group presented with well stained Purkinje cells, Basket cells, Stellate cells and Granule cells. (25 µm scale bar).

Discussion

The use of Permethrin as a pesticide to control domestic insects that are a nuisance and a health risk is on the rise [19]. Despite being a highly effective pesticide, there is mounting evidence of Permethrin toxicity [20]. Permethrin has been linked to a variety of health problems according to several studies [11]. The effects of Permethrin on body weight fluctuations, oxidative stress, and cerebellar histomorphology in adult male Wistar rats were investigated in this study, as well as the effect of NSO on ameliorating these effects.

In the current study, permethrin caused a reduction in body weight of rats, but increased cerebellar weight, similar to what we have previously noted [9]. *Nigella sativa* oil administration was associated with significant weight loss in the current study. This is consistent with the work of Mahmoudi *et al* [21] who studied the effects of *Nigella sativa* extracts on the lipid profile and uncoupling protein-1 gene expression in mice. The capacity of NSO to boost UCP-1 expression, an uncoupling protein that reduces the proton gradient created during oxidative phosphorylation in the mitochondria, could be linked to its potential to improve metabolic energy expenditure and hence, weight loss [21]. *Nigella sativa oil* improves lipid profile by decreasing plasma levels of low density lipoprotein and increasing plasma levels of the high density lipoprotein, making it ideal for the treatment of hypertension caused by hypercholesterolemia and coronary heart disease.[22,23]

Glutathione peroxidase (GPx) is an oxido-reductase enzyme important in maintaining the homeostasis of reactive oxygen species.[24] Its level was assessed in the current work to determine the oxidative status of the cerebellum. Permethrin is an exogenous source of free radicals [25], which are capable of adversely affecting biological molecules, thereby altering the normal redox status. Permethrin causes oxidative stress by producing reactive oxygen species (ROS) such as hydrogen peroxide and reactive nitrogen species (RNS) which deplete the intrinsic antioxidant system, as seen in this study. Permethrin also increases induced nitric oxide synthase (iNOS) mRNA expression, which contributes to oxidative stress. Oxidative stress causes lipid peroxidation, with a consequent compromise of plasma membrane integrity and associated degenerative cellular changes [26]. Introduction of NSO improved the oxidative status of rat's cerebellar tissue with a resultant cytoprotective action on the integrity of the cell membrane. This was deduced from the elevated levels of GPx in the cerebellum of rats co-treated with *Nigella sativa* and permethrin relative to those treated with permethrin only. These findings suggests that *Nigella sativa* was able to deplete the excessively generated reactive oxygen species (ROS) by the actions of permethrin. Hence, *Nigella sativa* prevents oxidative stress induced by permethrin. This observation is consistent with previous studies that observed increased intrinsic anti-oxidative enzymes following NSO administration [27].

Innate immune responses in the central nervous system (CNS) can be activated in a variety of ways to eliminate foreign molecules, infectious pathogens, dying and apoptotic neurons, or changed proteins that develop as a result of stress, aging, injury, or other pathologies. TNF-α, alongside other neuroinflammatory markers are produced by macrophages and microglia as an initial response to injury to the body and (CNS). Chronic microglia activation, while not immediately harmful to neurons and may even be protective, may cause neuronal damage through signaling to the blood brain barrier. As a result, cells from the adaptive immune system are recruited into the CNS.[28] The innate immune system increased the production of TNF-α a pro-inflammatory marker, after brain impairment caused by permethrin exposure, according to this study. The neuroinflammation generated by permethrin was reversed by NSO, as demonstrated by a decrease in cerebellar TNF-α levels in the rats co-treated with NSO and permethrin. This finding underlines the anti-inflammatory effects of *Nigella sativa.*

Despite the short duration of permethrin exposure, morphological changes in the cerebellar cortices of the experimental rats were identified. Some of these changes such as degenerative and chromatolytic changes, were caused by assaults on cerebellar cells and tissues as a result of neurochemical and oxidative perturbations. The cellular components and the normal architectural structure of the cerebellum were damaged to varying degrees by permethrin-induced cerebellar injury. These changes have a negative impact on signal processing and synaptic complex's functionality, as seen in patients with neurodegenerative disorders. The pathogenesis of major neurodegenerative diseases has been linked to oxidative stress and lipid peroxidation. The findings above were consistent with the decreased endogenous oxidative status that followed permethrin toxicity, and oxidative stress has been proposed as a component of the pathogenesis of neurodegenerative disorders linked to neurotoxicity. This study found that administering NSO reverses and corrects the effects of permethrin on cerebellar morphology, consistent with previous observations.[29]

Conclusions

Permethrin toxicity significantly alters cerebellar morphology and neurochemistry by causing oxidative damage and neuroinflammation. Therefore, caution is

advised during use, and body contact should be avoided. Administration of Nigella sativa oil for 14 days demonstrated therapeutic capabilities and cytoprotective potential in reducing the morphological and oxidative damage associated with permethrin-induced neurotoxicity. While considered safe in appropriate doses, long-term benefits may depend on duration, sex differences, and dosage.

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