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Potential roles of Nigella sativa oil in mitigating cortico-hippocampal injury and cognitive deficits associated with Permethrin-induced neurotoxicity

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ABSTRACT

Background: *Nigella sativa* is a medicinal plant with anti-oxidant and anti-inflammatory properties.

Objective: This study explored these properties in mitigating Permethrin-induced neurotoxicity of the prefrontal cortex and hippocampus of Wistar rats.

Methods: Sixteen male Wistar rats were divided into 4 groups: 1st Group (Control) received normal feed; 2nd Group received 1000 mg/kg of Permethrin (PER); 3rd Group received 14 ml/kg of *Nigella sativa* oil (NS); and the 4th Group received 1000 mg/kg of PER and 14 ml/kg of *Nigella sativa* oil (NS+PER). Treatment was for 14 days, following which behavioural tests were conducted to assess spatial learning and memory, locomotion, exploratory behaviour and anxiety, before the rats were sacrificed. The prefrontal cortex and hippocampus were excised and processed for histological examination using haematoxylin and eosin stains, and for astroglial activity using anti-glial fibrillary acidic protein as marker. The oxidative status was assessed through assessment of activities of superoxide dismutase and glutathione peroxidase.

Results: Permethrin treatment caused behavioural deficits, histopathological abnormalities such as poor staining intensity, cellular fragmentation and poor cellular architecture, reduction in activities of anti-oxidative enzymes and increased expression of activated astrocytes.

Conclusion: Administration of NS oil ameliorated the neurotoxic effects of Permethrin on the morphology and functions of the hippocampus and prefrontal cortex in Wistar rats.

Keywords: Permethrin, neurotoxicity, *Nigella sativa* oil, oxidative stress, behavioral deficits

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INTRODUCTION

Nigella sativa plant is a commonly used traditional remedy for a variety of clinical disorders and ailments (1). Nigella sativa is from the Ranunculaceae family of plants, and all parts of the plant are believed to be of medicinal benefits (2). Such clinical conditions in which studies have been conducted include inflammatory disorders, infectious diseases, cardiovascular disorders, diabetes mellitus, cancer and chronic illnesses, to mention but a few (3). The whole plant, seed and oil are at the forefront of these health benefits. The seeds or its oil are also used as a carminative, lactagogue, vermifuge, and as food spices and condiments (4, 5). Specifically, the seeds contain fixed and essential oils, nutrients like carbohydrates and proteins and other phytochemical constituents such as alkaloids, coumarins, flavonoids, glycoside, phenolics, saponins and sterols (2, 3). Thymoquinone is the major bioactive compound of the essential oil responsible for most of its medicinal benefits (6).

Having noted the anti-inflammatory and antioxidative activities of *Nigella sativa* oil, the current study explored these potentials in an insecticide-based neurotoxicity state, using Permethrin. Permethrin belongs to the synthetic pyrethroids family of chemicals which are more stable than the natural pyrethroids and are chemically manufactured to be more toxic (7). They are widely used as a pesticide in agriculture, houses, and gardens, as well as for the treatment of human and animal ectoparasites (8). There is a widespread use of pyrethroids in homes, with increasing risk of human exposure (9). Exposure to Permethrin could be topical, through inhalation or oral and it can be readily absorbed by the gastrointestinal tract (10).

Human exposure to Permethrin insecticides is toxic to the body and certain studies have used it to model different kinds of pathological conditions, including neurodegeneration (11-13). Cytotoxicity associated with Permethrin use has been linked to its ability to induce oxidative stress and generate reactive oxygen species (14). We have earlier demonstrated various degrees of cytoarchitectural alterations, behavioural deficits and biochemical derangements associated with Permethrin exposure in rodents (15, 16). The current study, however, sought to determine the roles Nigella sativa oil plays in correcting some of these abnormalities in the brain's structure and functions.

MATERIALS AND METHODS

Ethical consideration

This research work was conducted at the Faculty of Basic Medical Sciences' Animal House, University of Ilorin, Nigeria, and was approved by the Ethical Review Committee (UIL/UERC/13/46KA058).

Experimental design

Sixteen male Wistar rats with weight range of 58 –126 g were grouped into 4, with equal number of rats and were allowed to acclimatise for 7 days. The first group received standard rat feeds; the second group received 1,000 mg/kg of 0.6% Permethrin insecticide (Gongoni, Kano, Nigeria) (16); the third group received 14 ml/kg of Nigella sativa oil (Hemani International KEPZ, Karachi, Pakistan) orally, while the fourth group was co-treated with Permethrin and Nigella sativa oil at doses mentioned above. The dose of Nigella sativa oil used in this was lower than the LD50 of Nigella sativa in rats (17). Treatment was for 14 consecutive days.

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Behavioural study

After the treatment, the rats were assessed for learning, memory, locomotion, exploratory activities and anxiety. Morris water maze was used to assess the cognitive performance of the rats where spatial learning and the retention of memory was evaluated (18). The Y maze test examined spatial learning and cognitive memory in rats. The rats were evaluated according to the method by Kim et al (19). The open field test (OFT) was used to assess locomotion and exploratory behaviour of the rats. The parameters used included the number of line crossed (for locomotion) and rearing frequency for exploratory activity (20). The Elevated Plus Maze was described by Handley and Mithani (21) and validated by Pellow and colleagues (22) and was used to measure anxiety in rats. The assessment of anxiety behaviour of rodents was described by using the ratio of time spent in the open arms to the time spent in the closed arms of this four-armed maze, so that an increase in open arm activity (duration and/or entries) reflects anti-anxiety behaviour. The white and black box was also used to measure anxiety-related behaviour in the rats. The model used in this study was first described by Belzung et al. (23) and was based on the observation that nocturnal rodents such as rats naturally tend to explore a novel environment while the dark chamber was covered and not illuminated.

Animal sacrifice and Tissue collection

The rats were sacrificed by cervical dislocation. The whole brain was excised and divided into the right and left hemispheres. The right hemisphere was fixed in 4% neutral buffered saline for histological and histochemical analysis while the prefrontal cortex and hippocampus of the left hemisphere were homogenized in 30% sucrose solution for biochemical studies (glutathione peroxidase and superoxide dismutase assay).

Assay for biochemical analysis

Rats meant for enzymatic studies were sacrificed by cervical dislocation. Assay kits for glutathione peroxidase (GPx MBS-744364) and superoxide dismutase (SOD KT-60703) (Kamiya Biomedical Company, U.S.A.) were used to assess the hippocampus using the spectrophotometric technique (24). All reagents were set to room temperature before starting the procedures. Hippocampal tissues were homogenized in ice cold 30% sucrose solution with an automated homogenizer (Teflon homogenizer, GR3Z08). The homogenate was centrifuged (IEC Central GPSR Centrifuge, USA) at 2,500 revolutions per minute for 10 minutes and the supernatants were aspirated into an empty plain 5 ml specimen bottle and cooled to -20ºC (T Haier Thermocool, modnot HF-250 TA).

Tissue Processing for histology

Tissues preserved in fixative were processed according to the methods by Erukainure et al. (25) and stained with haematoxylin and eosin and/or cresyl fast violet techniques.

Data analysis

Data obtained were presented as mean ± standard error of mean (SEM). Data from the behaviour and biochemical evaluation were analyzed using one-way analysis of variance (ANOVA). P value of < 0.05 was considered statistically significant in all cases. The software package GraphPad prism version 7 was used for analysis and graphical representation of data.

RESULTS

Nigella sativa oil mitigated memory deficits associated with Permethrin exposure

Permethrin-treated rats showed a higher escape latency period (ELP) during the Morris water maze test compared to the control (Figure 1A). The ELP in rats exposed to Permethrin (PER) and those administered with *Nigella sativa* oil (NS) were similar; though this was unexpected, the rats co-treated simultaneously with NS and PER had lower ELP ($P > 0.05$). In the percentage correct alternation (PCA) test of the Y-maze paradigm, PER-treated rats had a lower PCA when compared with the control group (Figure 2B). The NS and NS+PER groups had comparable percentages which were higher than the PER group but lower than the control ($P > 0.05$).

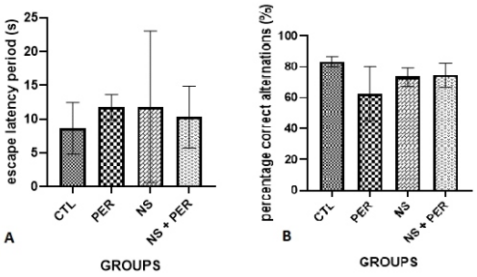


Figure 1 Graphical representation of findings of the escape latency period in Morris water maze (A) and percentage correct alternation of Y maze (B) across the groups. These revealed an increase in the escape latency in all the groups (especially the Permethrin group) compared to the Control group, with the least percentage correct alternation occurring in permethrin-treated rats. There was no statistical significance difference between the four groups ($P > 0.05$).

Nigella sativa oil enhanced locomotion and exploratory activities

Using the Open Field Test apparatus, locomotion indices and exploratory activities of the rats were observed. Observations from this behavioural paradigm showed that permethrin decreased number of lines crossed and rearing frequency of rats (Fig. 2) ($p>0.05$), whereas *Nigella sativa* oil increased the number of lines crossed and rearing frequency compared to the PER-treated rats. The rats that were co-treated with permethrin and *Nigella sativa* oil also had an increased number of lines crossed and rearing frequency compared to the permethrin-treated rats, though less than what was observed in NS group; however, there was no statistically significant difference between the groups ($P > 0.05$).

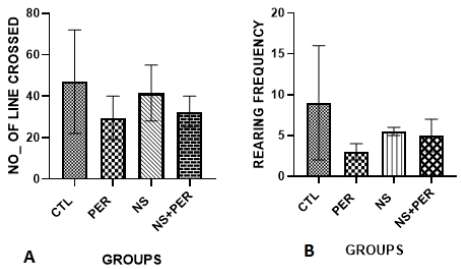


Figure 2 Behavioural paradigm for locomotion and exploratory activities using the Open Field Test, showing reduction in number of lines crossed (A) and rearing frequency (B) in rats exposed to Permethrin (PER) compared to others; rats given *Nigella sativa* oil (NS) had higher values compared with PER-treated ones, but lower than the Control (CTL).

Nigella sativa oil counterbalanced Permethrin-induced anxiety-related behaviours

This test utilized two separate tests to measure anxiety-related behaviours. These were closed arm entry and duration from

Elevated Plus Maze (Fig. 3A and B) and white box entry and duration from the White and Black Box (Fig. 3C and D). Rats treated with permethrin had increased closed arm entry and duration compared with the control and other groups, though the difference was more remarkable for closed arm duration. Rats that received only NS had the least closed arm entry value, while those co-treated with NS and PER was higher than the NS group but lower than the PER group. The closed arm duration of NS rats was lower than the PER group and comparable with the Control, while rats co-treated with NS and PER had higher closed arm duration compared with NS rats but lower than the PER-treated rats ($P>0.05$). White box entry and duration reduced in permethrin-treated rats compared with the Control, while rats treated with *Nigella sativa* oil had increased white box entry and duration compared with the Permethrin-treated rats and increased white box duration compared with the Control. Rats co-treated with *Nigella sativa* oil and Permethrin had higher white box entry and duration compared with all the groups (Fig. 3C and D).

Nigella sativa mitigates Permethrin-induced oxidative stress in the hippocampus and prefrontal cortex of Wistar rats

The activity of glutathione peroxidase (GPx) in the hippocampal tissues of Permethrin-treated rats was low compared with the Control (Fig. 4A). Rats that received *Nigella sativa* oil had the highest GPx level, while the rats that received NS alongside Permethrin had GPx level higher than the Permethrin-treated rats, though lower than the Control. Exposure to Permethrin caused a low level of superoxide dismutase (SOD) compared to the Control (Fig. 5B); SOD level was elevated in rats administered with Nigella sativa oil and those co-administered with *Nigella sativa* oil and Permethrin, although it was higher in the former group. The prefrontal cortex of rats in the control group (Fig. 4C) showed a higher SOD activity compared to the Permethrin-treated rats, while PFC of rats treated with *Nigella sativa* oil had a higher SOD activity compared to those co-treated with Permethrin and Nigella sativa oil.

Nigella sativa oil protects against Permethrin-induced cortico-hippocampal microarchitectural damage

The prefrontal cortex of rats in the control revealed normal pyramidal neurons, blood vessels, well organized cortical layers and distinctive Nissl staining intensity (Fig. 5). The PFC of Permethrin-treated rats showed pyramidal cell shrinkage and degeneration, as well as congestion of the blood vessels with marked changes in the neuronal nuclei in the form of pyknosis with increased intracellular spaces, reduced cell density and numerous cells with chromatolysis. The PFC of rats treated with *Nigella sativa* oil showed normal cytoplasmic organelles, normal pyramidal neurons and numerous cells with deeply stained neurons and normal staining intensity similar to the control group. Degenerative changes in the prefrontal cortex of rats co-treated with *Nigella sativa* oil and Permethrin were slightly reduced. This can be seen in the slightly improved appearance of pyramidal neurons, cell nuclei and fewer cells with chromatolysis. However, intracellular spaces and reduced cellular density can still be observed. Also observed were numerous pyknotic cells in the dentate gyrus of rats treated with Permethrin (Fig. 6), while those that were co-treated with Permethrin and NS presented with milder alterations, with layout similar to the NS-treated rats.

In both hippocampus (Fig. 6B) and prefrontal cortex (Fig. 6C), the presence of glial cells (astrocytes) were observed in all the groups. However, Permethrin treatment was associated with increased expression of activated astrocytes, while the occurrence of activated glial cells was a rare feature in brain tissues of rats that were co-administered with Permethrin and NS; tissues stained with anti-GFAP antibody showed minimal activated astroglial cells.

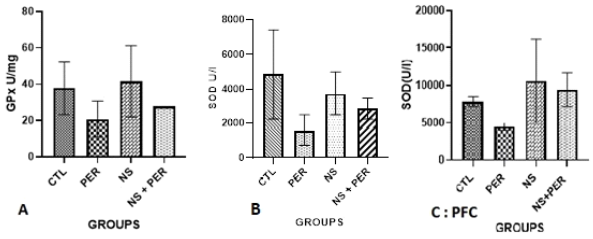


Figure 4 Activity of glutathione peroxidase (GPx) was least in the hippocampus (A) of permethrin-treated rats (PER) and highest in rats that received *Nigella sativa* oil (NS), compared with the control (CTL). Activity of superoxide dismutase (SOD) in the hippocampus (B) and prefrontal cortex (PFC) (C) was reduced in permethrin-treated rats (PER), but elevated in rats that received *Nigella sativa* oil (NS), compared with the control ($P > 0.05$)

DISCUSSION

Nigella sativa is one of the most studied medicinal plants and continues to be of relevance in traditional medicine around the globe in the management of a variety of clinical conditions (26). Of unique importance is the oil-component of the plant which has been acclaimed to be beneficial in cases of infection, inflammation, diabetes, pain and many other conditions (2). As

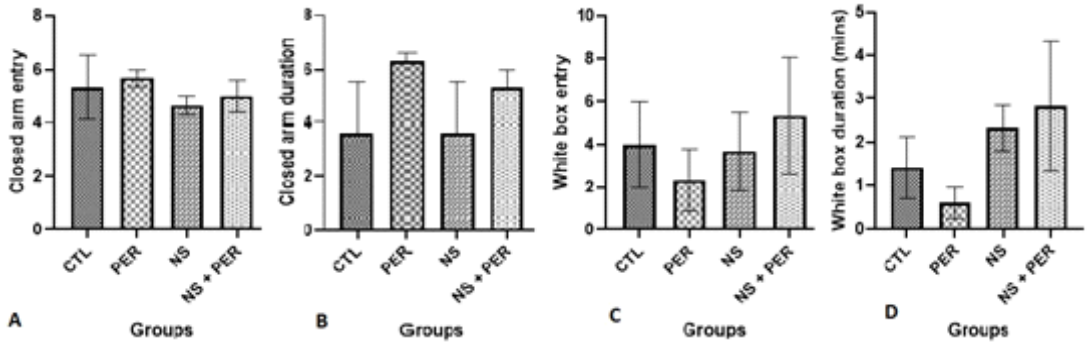


Figure 3 Assessment of anxiety using elevated plus maze (A and B) and the white and black box (C and D), showing increase in closed arm entry (A) and closed arm duration (B), but reduction in white box entry (C) and duration spent in the white box (D) by rats exposed to Permethrin (PER) compared to other rats, and those co-treated with *Nigella sativa* oil (NS) and Permethrin recorded the highest entry and duration (C&D)

presented in this study, Permethrin was used to induce neurotoxicity that resulted in cortico-hippocampal pathology similar to features of neurodegeneration.

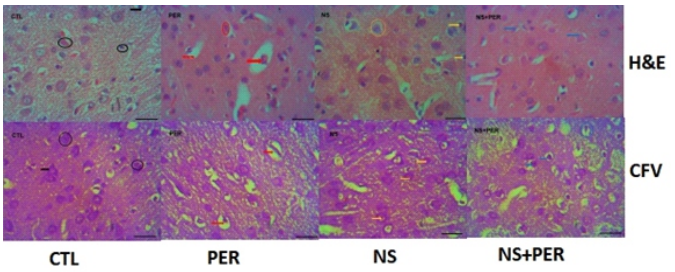


Figure 5: Representative photomicrographs of the prefrontal cortex of Wistar rats stained with haematoxylin & eosin (H&E) and cresyl fast violet (CFV), showing control (CTL) with clear cytoplasm, multiple pyramidal cells (black circle) with properly stained nuclei (black arrow); Permethrin-treated (PER) rats had shrunken pyramidal neurons (red circle), increased perinuclear space (red arrow) and chromatolysis (dotted red arrow). Rats treated with *Nigella sativa* oil (NS) presented with normal pyramidal cells (dotted yellow circle) as well as clear cytoplasm with finely placed nuclei and deeply stained Nissl substances (yellow arrow). Rats co-treated with *Nigella sativa* oil and permethrin (NS+PER) presented with normal pyramidal neurons similar to those in the control group (blue arrow) and cells with increased perinuclear space (dotted blue circles). Scale bar =25µ

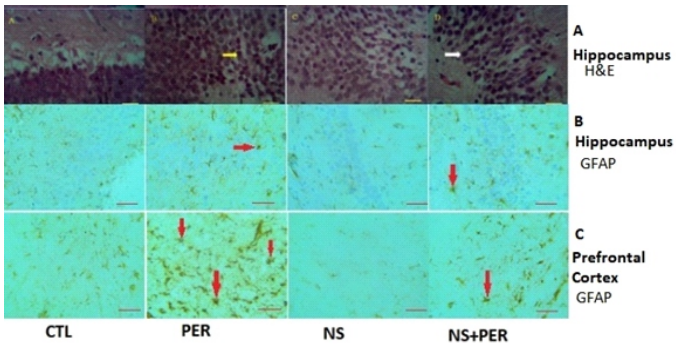


Figure 6: Representative photomicrographs of the cytoarchitecure of the hippocampus of Wistar rats, showing (Fig. 6A) normal histological architecture in the Control (CTL), densely packed and numerous pyknotic cells (yellow arrow) in Permethrin-treated (PER) rats, adequate staining intensity and an apparently normal histological architecture in rats administered with *Nigella sativa* oil (NS), and more adequately stained cells and a mixture of normal cells (white arrow), more pyramidal cells and some pyknotic cells in rats co-treated with NS and Permethrin (NS+PER). Figures 6B and C of the hippocampus and prefrontal cortex respectively were stained with anti-GFAP antibody, and showed increased expression of astrocytes (red arrows) in permethrin-treated (PER) rats, but reduced in rats co-treated with NS and Permethrin. Scale bar =25µ

Neuroinflammation is a common feature of neurodegenerative conditions (27). CNS inflammation is chiefly driven by microglia and astrocytes (28). Activation of these cells and subsequent production of other inflammatory cells and cytotoxic substances affect neuronal survival and induce neurodegeneration (29). The presence of activated astrocytes following permethrin treatment is indicative of neuroinflammation in the hippocampus and PFC. Astrocytes are involved in the CNS defense mechanism and repair of damaged neural tissue; these cells (including microglia) are key regulators of inflammatory responses in the central nervous system (30, 31). They function as inflammatory cells and produce many inflammatory molecules like cytokines and chemokines (29), which in turn mediate the recruitment of microglial cells to the sites of CNS injury (32). The rats exposed to Permethrin diet demonstrated significant astroglial activation in the hippocampus and PFC of the brain compared to the brain of rats in the Control group and those treated with *Nigella sativa* oil. The reduction in the presence of activated glial cells in the brain tissues of rats co-treated with PER and *Nigella sativa* oil compared with those treated with Permethrin only is an indication that NS oil has anti-inflammatory properties and could mitigate the inflammatory effects and consequences of Permethrin-induced neuroinflammation.

Oxidative stress and increased generation of ROS are central markers associated with several progressive pathological disorders (3). Oxidative stress is part of the pathophysiology of neurological disorders associated with neurotoxicity (33). Reactive oxygen species are part of the signaling molecules that induce microglial activation (34), as part of glial response to alterations in the internal mileu of the brain. Superoxide dismutase and glutathione peroxidase were the enzyme markers used to assess the oxidative status of rats in the current study. As expected, permethrin treatment led to reduction in the activities of these enzymes, signifying its potential to cause oxidative stress-induced neurotoxicity. Numerous studies in human and experimental animals have reported the antioxidant activities of *Nigella sativa* (35, 36). Findings from the current study further strengthen this position, as rats that were co-treated with NS oil reported reduced levels of activities of these enzymes.

We have earlier highlighted some of the behavioural deficits associated with permethrin toxicity (16). Unlike the parameters previously used to assess anxiety-like behaviour in permethrin-exposed rats (16), the current study used the closed arm entry and closed arm duration of the elevated plus maze test and the white box entry and duration spent in the white box of the white and black box test; the former was increased while the latter decreased. Perveen and colleagues (37) reported that *Nigella sativa* increased brain levels of serotonin and decreased serotonin turn-over with a consequent anti-anxiety effects. Other studies have reported similar positive effects on mood and anxiety as well as its effects in improving learning and memory (38-40). It was suggested that one possible mechanism for the anxiolytic effect of NS is by decreasing the metabolism of serotonin (39). In the current study, although statistically significant difference was not observed among the treatment groups, most of the permethrin-induced behavioural deficits were ameliorated with the use of Nigella sativa oil. Its co-treatment with Permethrin enhanced locomotion and exploratory activities and mitigated memory deficits caused by permethrin toxicity in the rats.

Permethrin exposure caused adverse morphological changes in the brain of experimental rats, as previously studied (16). Observations from the current study was not too far from this. In both hippocampal and PFC regions of the brain, evidence of degenerative and chromatolytic changes and compromised structural integrity was present. These changes adversely affect the normal functions of the hippocampus and PFC and ultimately alter normal behavioral pattern.

CONCLUSION

Permethrin insecticide induces oxidative stress with consequent inflammatory responses in the hippocampus and prefrontal cortex resulting in adverse neurochemical and behavioural alterations. The antioxidative and anti-inflammatory properties of *Nigella sativa* oil make it a candidate for consideration in mitigating pyrethroid-related neurotoxicity and neurobehavioural deficits.

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