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Moringa oleifera oil modulates cerebellar neuroinflammation and oxidative stress associated with permethrin neurotoxicity

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Abstract

Permethrin is a commonly used domestic insecticide with varying degrees of neurotoxicity in both insects and mammals. The phytochemical components of *Moringa oleifera* oil (MOR) have several health benefits including antioxidative and anti-inflammatory properties. This study investigated the role of MOR in ameliorating cerebellar damage associated with permethrin neurotoxicity. Sixteen (16) male Wistar rats with an average weight of 89.6 g were randomly divided into four (4) groups: Group A (control) was fed on a normal rat diet, Group B was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin, Group C was fed a normal diet with 5 ml/kg of MOR, Group D was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin and 5 ml/kg of MOR. The rats were treated for 14 days. The rats were euthanised thereafter and the cerebellum was removed and processed for both biochemical and histochemical examinations using appropriate solution, to assess oxidative status, neuroinflammation (tumour necrosis factor-alpha, TNF-α), Nissl distribution and microarchitecture of the cerebellum. Permethrin-treated rats recorded a reduction in glutathione peroxidase activity, but significant increase in TNF-α; cerebellar histoarchitecture was disrupted with varying degrees of degenerative and chromatolytic changes, which were mitigated in rats that received MOR intervention. The action of MOR on permethrin-induced cerebellar damage is by enhancing antioxidant defence mechanism and modulating neuroinflammation.

Keywords: Permethrin, *Moringa oleifera* oil, cerebellum, neurotoxicity

Introduction

Multiple studies have looked into the potential dangers of being exposed to various insecticides. Physiological consequences have been described in several investigations, which could lead to the development of degenerative disorders with epidemiological implications [1]. Food, water, sea life, birds, and biological substrates like tissues and breast milk have all been found to contain residues from these chemicals [2].

Permethrin is a synthetic pyrethroid widely used in agriculture, veterinary medicine, and pest control in the home [3]. Although it is thought to be safe for domestic usage, studies have shown that permethrin has detrimental effects on the brain [4, 5]. In mammals and insects, permethrin causes neurotoxicity by causing a mild protraction of sodium channel permeability in nerve membrane channels during excitation. Hundreds to thousands of repeated nerve impulses in the sense organs can be caused by these very short trains. Pyrethroid damage to the voltage-dependent sodium channel causes sodium channels to stay open far longer than normal, resulting in this repeated activity [6]. Repetitive nerve impulses may induce oxidative stress, hence there is possibility that permethrin exposure will result in an excess of reactive oxygen species (ROS) [7]. Acute pyrethroid poisoning is associated with altered nerve function, primarily including neuroexcitatory effects in the brain, spinal cord, and peripheral nervous system, according to physiological and neurochemical studies of pyrethroid intoxicated animals [8].

Moringa oleifera has generated a lot of interest in nutrition and medicinal research because of its

excellent nutritional and ethno-medical benefits. Its leaves, roots, seeds, bark, fruits, flowers and stem have all been shown to have pharmacological effects [9-13]. Many of its properties, such as antihypertensive, analgesic, anti-cancer, neurodepressant, antibacterial, anti-inflammatory, and antiepileptic characteristics, have been scientifically demonstrated [14, 15].

With a possibility of cerebellar damage following permethrin intoxication, the current study investigated the cytoprotective, anti-inflammatory, and anti-oxidative properties of *Moringa oleifera* seed oil in restoring the resultant histomorphological changes in neuronal cells of the cerebellum.

Materials and Methods

Experimental animals and care

Sixteen male Wistar rats (*Rattus norvegicus*) with weight range of 85.25 ±11.12 g to 94.00±8.43 g were used for the study. The animals were housed in the Animal Facility of the Faculty of Basic Medical Sciences, University of Ilorin, under standard conditions. They were fed with standard rat feeds and water *ad libitum* and allowed to acclimatize for 7 days before the commencement of the study.

Treatment of animals

The study used Rambo insect powder (Rambo®, Nigeria), which contains 0.6% Permethrin and 99.4% inert carriers, as well as *Moringa oleifera* seed oil (MOR). The animals were divided into four groups (A - D) each consisting of four rats. Group A received a standard rat diet; Group B received a standard rat diet mixed with 1,000 mg/kg of 0.6% Permethrin [16]; Group C received 5 ml/kg of MOR; Group D received standard rat diet mixed with 1,000 mg/kg Permethrin insecticide and 5 ml/kg of MOR. Treatment was for 14 consecutive days. The weight of the rats was monitored and recorded throughout the treatment period.

Tissue processing for the histological and histochemical demonstration

A day after the termination of the study, the rats were euthanized and the brain was removed from the cranium. The cerebellum was excised and divided at the mid-sagittal line. The right half was fixed in 4% buffered saline while the left hemisphere was put in cold sucrose solution for biochemical studies. The tissues were processed for histology, embedded in paraffin, and sectioned with a Rotary microtome at a thickness of 5 µm. Hematoxylin and eosin (H&E) stain was used for general histology, and cresyl fast violet (CFV) stain was used for histochemical demonstration of Nissl substances [17].

Biochemical studies

The left hemisphere of each cerebellum excised was weighed and stored in cold sucrose solution four times the weight of the cerebellar hemisphere. At 4°C, equal weights of brain tissues were pulverized with an automated homogenizer in an ice-cold 30% sucrose solution. The homogenate was centrifuged at 2,500 rpm for 10 min. The supernatants were aspirated into clear glass cuvettes that were placed on ice. Appropriate biochemical assay kit for glutathione peroxidase (MBS744364) was used to assess the cerebellum for oxidative stress. The level of pro-inflammatory cytokine, tumour necrosis factor-alpha was also assessed. The assay was carried out according to the manufacturer's instructions on the assay pack.

Light microscopy and Data analysis

An Olympus binocular research microscope (Olympus, New Jersey, USA) was used to capture histological and histochemical sections, which was connected to a 5.0 MP Amscope camera (Amscope Inc, USA). The GraphPad application 10.0 was used to analyze the data obtained. The level of significance was determined at p values less than 0.05, and the data were presented as mean±SEM.

Results

Morphological observations

Observation of the body weights of the rats revealed a significant increase in weight between day 1 and day 14 of administration for all the groups ($P<0.01$). However, rats treated with permethrin diet recorded the least weight gain compared to control and the group treated with MOR only (Table 1). The opposite was observed in organ weights as animals fed with a diet containing permethrin recorded an increase in cerebellar weight compared to the control and *Moringa* group.

Table 1: Weight changes in experimental rats

Groups	Mean initial weights (g)	Mean final weights (g)	Weight difference (g)	Organ weights (g)	Organ/Body weight ratio
A: Control	86.75±10.66	120.00±2.80	33.25	0.189	0.0015
B: Permethrin (1000 mg/kg)	89.75±9.73	113.00±11.60	23.25	0.242	0.0021
C: *Moringa oleifera* oil (5 ml/kg)	98.00±8.68	119.00±11.92	21.00	0.190	0.0016
D: *Moringa oleifera* oil (5 ml/kg) + Permethrin (1000 mg/kg)	93.50±10.04	112.75±10.64	19.25	0.205	0.0018

Moringa oleifera oil mitigated permethrin-induced cerebellar oxidative stress

Glutathione peroxidase (GPx) activity in the cerebellum was assessed (Fig. 1). Glutathione peroxidase level was least in the permethrin (PER) group compared with the control and other treated groups (Fig. 1). The level was highest in *Moringa oleifera* oil (MOR) group, while GPx level in the group co-treated with PER and MOR was lower than the MOR group but higher than the PER group and control (p>0.05).

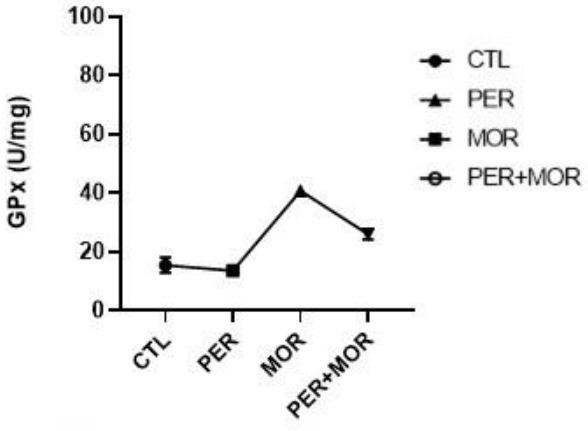

Figure 1: The activity of glutathione peroxidase (GPx) in the cerebellum Wistar rats was least in permethrin (PER) treated group and highest in *Moringa oleifera* oil (MOR) treated group compared to other groups. The activity was lower in the group co-treated with permethrin and *Moringa oleifera* oil (PER+MOR) compared with the MOR group (p>0.05).

Moringa oleifera oil prevented cerebellar neuroinflammation associated with permethrin

The level of pro-inflammatory cytokine- tumour necrosis factor-alpha (TNF-α) was assessed (Fig. 2). Rats that consumed the permethrin diet had an elevated level of TNF-α in their cerebellar tissues when compared with the Control. Administration of *Moringa oleifera* oil orally ordinarily resulted in a reduced level of TNF-α compared to the Control and the permethrin-treated rats, and the level was further reduced in the rats co-treated with permethrin and *Moringa*. However, these changes were not statistically significant so (p>0.05).

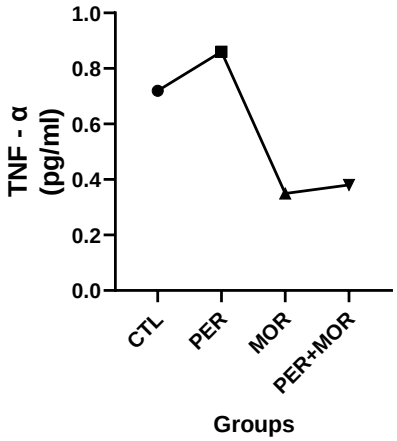

Figure 2: The activity of tumour necrosis factor (TNF-α), an inflammatory marker, showed an elevated level in the permethrin (PER) group compared to control (CTL) and *Moringa oleifera* oil (MOR) treated groups. The activity was least in the group co-treated with permethrin and *Moringa oleifera* oil (PER+MOR) (p>0.05).

Histomorphology and Nissl demonstration in the cerebellar cortex

The cytoarchitecture of the cerebellar cortex in control and MOR-treated rats comprised of the molecular and granular layers with the Purkinje cell layer in between them (Fig. 3). The cellular arrangement and population of cells appeared normal. The granular layer was made up of small granule neurons that were densely distributed. They had highly positive expression of Nissl bodies and deeply stained neurons also (Fig. 4). Permethrin-treated rats had distorted cortical layers, Purkinje cell layer showed sparsely visible cell bodies and dendrites, while the granule cells in the granular layer were also sparsely distributed, when compared to other groups (Fig. 3). Nissl staining was poor with the presence of chromatolytic Purkinje cells (Fig. 4). Rats co-treated with permethrin and MOR had normal cerebellar architecture. Their Purkinje cells showed visible cell bodies and dendrites deeply projecting into the molecular layers and the granular layer comprised of densely distributed small granule cells (Fig. 3). They also had Nissl positive cells (Fig. 4).

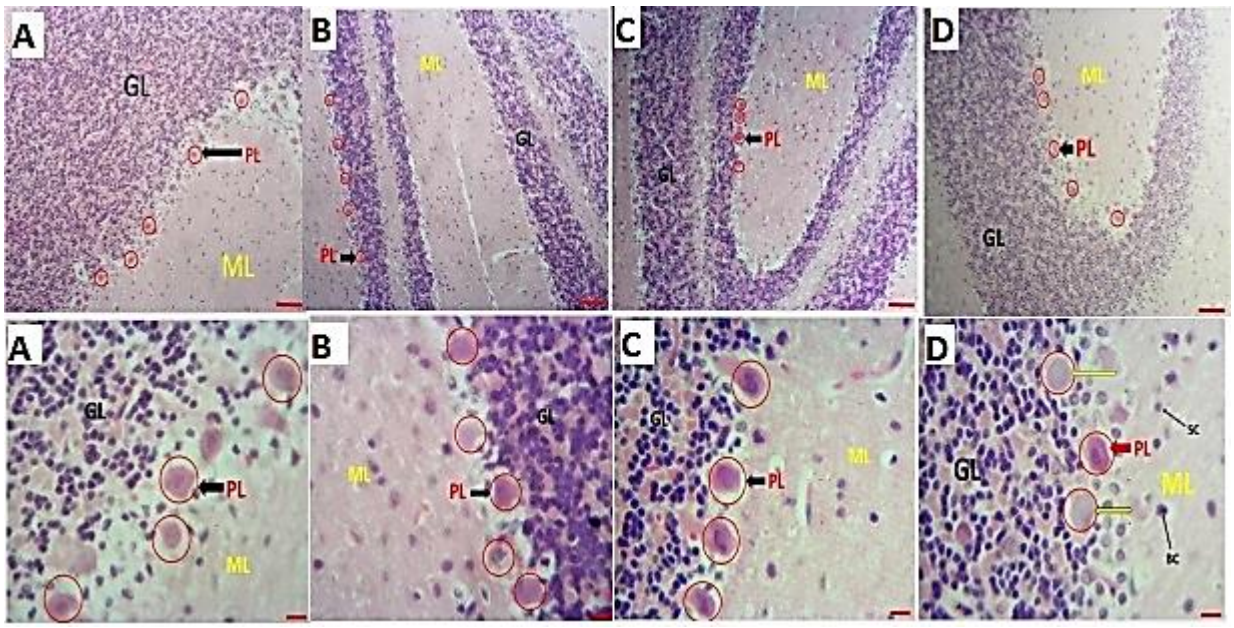

Figure 3: Representative photomicrographs of the cerebellar cortex of Wistar rats stained with Haematoxylin & Eosin at low and high magnifications (scale bar: 100 µm and 25 µm respectively) administered with normal rat diet (A), permethrin diet (PER), *Moringa oleifera* oil (MOR), and a combination of permethrin and *Moringa oleifera* oil (PER+MOR). Slides A & C presented with normal histomorphology of the cerebellum; the cerebellar cortex showed densely packed internal granular layer (GL) separated from the external molecular layer (ML) by a single-celled layer of Purkinje cells (red circles; PL). B and D revealed decreased cellular density in the granular layer (GL) with deeply stained granule cells, thin granular cell layers and deeply stained granular cells.

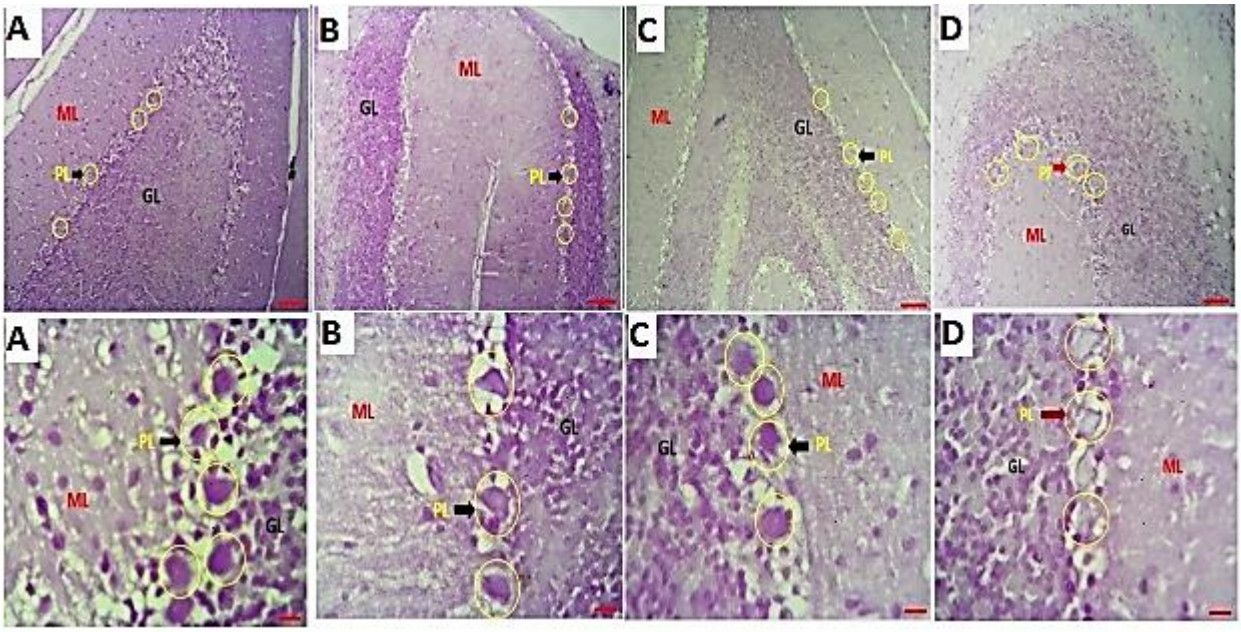

Figure 4: Representative photomicrograph of the cerebellar cortex of Wistar rats following Nissl staining at low and high magnifications (scale bar: 100 µm and 25 µm respectively) administered with normal rat diet (A), permethrin diet (PER), *Moringa oleifera* oil (MOR), and combination of permethrin and *Moringa oleifera* oil (PER+MOR). The panoramic view of the cerebellar cortex were shown at low magnification A to D. At a higher magnification, 'A' revealed highly positive expression of Nissl bodies, deeply stained neurons in their respective neutrophils, satisfactory cellular density in the molecular (ML) and granular layers (GL). Nissl staining was reduced in 'B' with reduced cell population in the Purkinje cells and molecular layers. Features in 'C' were comparable with the control (A); 'D' presented with deep Nissl staining intensity with a higher Purkinje cell population compared to 'B' (cresyl fast violet).

Discussion

Permethrin is a synthetic pyrethroid that is widely used to control pests and disease vectors in agriculture. Because of its low environmental toxicity in mammals, it was once thought to be harmless for humans and other animals. However, its neurotoxicity at large dosages was quickly discovered, and other adverse effects at chronic low levels more recently [18]. The neurotoxicity of permethrin at high doses has been extensively investigated [19]. After a single stimulus, permethrin alters sodium channels in insects and mammals, resulting in persistent depolarization and recurrent discharges in presynaptic nerve fibres[20]. Permethrin raises the chances of neuronal deterioration and neurodegeneration [21]. Permethrin-related neurodegenerative processes have been investigated, and one of the most common of these is a negative impact on the redox system. A pro-oxidant condition can promote posttranscriptional modifications in the electron transport system, which is a primary factor of neurodegeneration, and this enhances the formation of reactive oxygen species while inhibiting mitochondrial complex I. As a result, a lack of antioxidant enzymes in many organs causes protein, lipid, and DNA damage [22].

Observation from the assay of the activity of GPx which revealed a depletion of endogenous antioxidant levels is similar to what has initially been reported in some brain regions. As we had earlier documented, exposure to permethrin causes a dose-dependent depletion of endogenous oxidative enzymes across different brain regions of rats [5]. Weidinger and Kozlov [23] also submitted that permethrin could decrease the antioxidant defence system resulting in damage to cellular macromolecules such as DNA, lipids and protein. However, according to Chargui *et al*. [24], permethrin activity on antioxidants is influenced most importantly by rat sex as their research revealed a decrease in superoxide dismutase activity in females after permethrin exposure and an increase in males. The introduction of *Moringa oleifera* oil, a potent exogenous antioxidant, restored the cerebellar oxidative status and redox balance in the current study. This antioxidative property of *Moringa oleifera* has been explored in many experimental models of clinical disorders with positive outcomes [9, 10, 11, 25, 26, 27]. The plant enhances the antioxidant defence mechanisms, thereby alleviating permethrin-induced oxidative stress and its associated neurodegenerative changes.

Increased levels of reactive oxygen species can set off a chain reaction of molecular and transcriptional events. Pro-inflammatory cytokines are produced by local microglia as well as infiltrative neutrophils, monocytes, and lymphocytes [28]. Although other brain cells can produce cytokines, microglia are the primary source of TNF-α and other cytokines such as interleukin-1 and interleukin-6 during neuroinflammation [29, 30] . The expression of TNF-α was found to be significantly higher in our study. TNF-α participates in a variety of processes, including neurogenesis, neurotransmission, cell proliferation, and neuronal excitability, as a pro-inflammatory cytokine [31]. Depending on the concentration, experimental model, and cell-derived regions, TNF-α has both pro- and anti-neurogenic properties [32]. In this regard, our findings showed that a high permethrin dose increased TNF-α expression in the cerebellum of rats. As a result of its interaction with voltage-gated sodium channels, permethrin may directly activate microglial cells, contributing to an excessive accumulation of intracellular Na+, which depolarizes the cells and causes them to release TNF-α [33]. This proinflammatory cytokine has the ability to change the balance of excitatory to inhibitory neurotransmission, resulting in a higher synaptic excitatory/inhibitory ratio [34]. Moringa oleifera oil's ability to reduce TNF-α expression indicates that it has the potential to modulate the neuroinflammatory process linked to permethrin-induced neurodegeneration..

Permethrin exposure altered the cytoarchitecture of the cerebellar cortex to varying degrees, including severe cellular chromatolysis. Permethrin-induced neurotoxicity causes oxidative and inflammatory insults, which result in these changes. In the cerebellar cortices of rats treated with only permethrin, chromatolytic and apoptotic changes were more pronounced in the Purkinje cell layer, where a reduction in cell population was observed. Furthermore, giving *Moringa oleifera* oil with permethrin at the same time prevented severe chromatolysis and apoptosis in the cerebellar cortex, as well as the locomotive deficits seen in rats given permethrin alone. This could be due to *Moringa oleifera* oil's antioxidant properties, as previous research suggested that the presence of phytoconstituents that scavenge free radicals and activate endogenous antioxidant enzymes could be responsible for its protective effects. Similarly, *Moringa oleifera* oil boosts the antioxidant status of neuronal cells at the right dose, preventing possible permethrin-induced neuronal cell damage [10]. The antioxidant capacity of MOR in this study is due to the presence of various antioxidant compounds in the plant, including ascorbic acid, flavonoids, phenolics, and carotenoids [35].

Conclusion

The phytochemical constituents of *Moringa oleifera* oil have anti-oxidant and anti-inflammatory properties. These two mechanisms give MOR a two-pronged approach to combating neurodegenerative changes caused by permethrin exposure, boosting the brain's endogenous antioxidant defence mechanism, counteracting permethrin neurotoxicity and preserving the cerebellar structure and functions.

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