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Original Article

Protective effects of *Moringa oleifera* oil on permethrin-induced toxicity in the prefrontal cortex of young male Wistar rats

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Abstract

BACKGROUND AND AIM: Permethrin is a type I pyrethroid commonly used as an insecticide in pest control. Although it is intended for use in agricultural and textile settings, including residential areas, its extensive use poses a probable risk of human exposure. This study was conducted to determine the changes in the prefrontal cortex following permethrin exposure and the possible protective effects of *Moringa oleifera* oil.

METHODOLOGY: Sixteen young male Wistar rats were randomly selected and divided into four (4) groups (n=4). Group A (Control group) received a normal rat diet, while Group B received a permethrin diet (0.6% Permethrin) at the dose of 1000 mg/kg. Group C received a normal diet and was administered 0.5 ml/kg of *Moringa oleifera* oil, while Group D received both the permethrin diet and *Moringa oleifera* oil simultaneously. Animals underwent behavioural analysis on the final day of treatment. The rats were then anesthetized and sacrificed, followed by transcardial perfusion with normal saline and 4% paraformaldehyde. The prefrontal cortex was excised from the brain and processed for tissue histochemistry and biochemical analysis.

RESULTS: Results revealed reduced locomotory and exploratory activities and increase anxiety level in rats, as well as cytoarchitectural distortion, cell vacuolation, and redox imbalance in the prefrontal cortex. Administration of *Moringa oleifera* oil led to significant improvement in neurobehavioral deficits, a comparatively normal cytoarchitectural profile, and oxidative status caused by permethrin in prefrontal cortex of male rats.

CONCLUSION: Findings in this study indicated that *Moringa oleifera* could ameliorate the damage caused by permethrin in prefrontal cortex of male Wistar rats.

Keywords:
Permethrin; prefrontal cortex; *Moringa oleifera;* oxidative stress

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INTRODUCTION

Permethrin is a synthetic pyrethroid insecticide derived from natural pyrethrins, however, they are used in the treatment of certain disease conditions including scabies and pediculosis (Sunderkötter *et al.*, 2021). In spite of its therapeutic advantage, permethrin has toxic effects on the nervous system. Permethrin like other pyrethroids is capable of crossing the blood-brain barrier to exert their toxic effects on the brain with resultant motor deficits (Omotoso et al., 2020). A study carried out by Omotoso *et al.,* revealed that it affects normal working and spatial memory in exposed animals with presentation of anxiogenic properties and dose dependent alteration in their locomotive drive, indicating alteration in the normal physiology of key brain regions involved in processing and execution. Moreover, permethrin induces neuroinflammation which has a positive interplay with cognitive decline. It also mediates oxidative stress by overwhelming and ultimately compromising intrinsic antioxidant defense system via increased production of reactive oxygen species (Omotoso et al., 2020). There is increasing evidence of its association with neurological disorders. Recent studies in rats suggest that the neurotoxicity it causes is the most devastating *al.,* 2020). Moreover, in the early stages of development when the signaling pathways are formed. It has been reported to increase α-synuclein, decrease striatal dopamine levels, induce oxidative stress, and inhibit mitochondrial complex I of the electron transport chain (Agrawal *et al.,* 2015). All of these changes in the striatum are the hallmarks of Parkinson's

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disease. In fact, the Parkinson's disease model induced by permethrin is suitable for investigating the initial markers of the disease which may help to find new ways to manage it (Mohammadi *et al*., 2019).

Anti-diabetic (Wang et al., 2022), anti-cancer, anti-inflammatory (Ma et al., 2020), hypocholesterolemic, hypoglycemic, antioxidant and neuroprotective activities of *Moringa oleifera* has been reported (Ezeamuzie *et al*., 1996; Gbadamosi *et al,* 2016). The essential oil got from *Moringa oleifera has* been reported to contain flavonoids such as quercetin and luteolin (Pari *et al*., 2007). Quercetin contains phenolic hydroxyl groups with antioxidant action, and powerfully inhibits the production of both reactive oxygen and nitrogen species (Omotoso *et al,* 2018). Alongside anti-inflammatory properties, Moringa oleifera has mental health benefits, as exemplified by its anxiolytic and antidepressant effects (de Siqueira Patriota et al, 2024). Thus, it finds complementary use, not only in mood disorders but also in inflammatory diseases like rheumatoid arthritis (Ummah et al, 2023). *Moringa oleifera* leaf extract improves memory deterioration and gives significant antioxidants to counteract oxidative stress in rats (Ganguly *et al*., 2005). It is recognised as a nutritional intervention in physiological states like pregnancy and also in chronic illnesses like HIV (Hadju et al, 2020, Gambo et al, 2021). Therefore, the present study aimed to determine the roles of *Moringa oleifera* oil on permethrin – induced toxicity on the prefrontal cortex of young male Wistar rats through behavioural, biochemical and histological assessments.

MATERIALS AND METHODS

Animal Care and Use

The study was approved by the University Ethical Review Committee, University of Ilorin, Nigeria, and the protocols and treatment procedures were carried out according to the guidelines. Sixteen (16) adult male Wistar rats (85g to 110g) gotten from a private property in Ogbomosho, Oyo State and housed in the Animal Facility of the Faculty of Basic Medical Sciences, University of Ilorin. They were allowed to acclimatize period of one week before the commencement of the treatments. During the acclimatization period, the rats were fed with normal rat chow with access to water ad libitium and housed in well-ventilated cages in the University of Ilorin, College of Health Sciences' Animal House at room temperature.

The Normal rat's chow prepared from cereals grain, vegetable oil, premix (vitamins and minerals) essential amino acids and others was obtained from OgoOluwa Feeds and Flour Mill Limited, Sango Ilorin, Kwara State, Nigeria.

Experimental Design

Rambo insect powder used in this study was produced by Gongoni Co. Ltd, Kano, Nigeria and it contained 0.6% Permethrin and 99.4% inert carriers. Rats were separated into four (4) groups (A, B, C and D). Each group contained four (4) rats. Group A (Control group) was fed with normal rat diet while Group B was fed with Rambo powder insecticide at the dose of 1000 mg/kg of rat chow. Group C was given normal diet and administered 0.5 ml/kg body weight of *Moringa oleifera* oil, while Group D was treated with permethrin diet (1000 mg/kg) and *Moringa oleifera* oil simultaneously. These treatments continued for a period of two weeks. Administration of permethrin was carried out according to Omotoso et al., 2020.

Behavioural assessment

Open field test assessment

The open field test is designed to evaluate exploratory behavior, anxiety levels, and locomotor activity in a controlled environment. The behavioral parameters assessed were the number of lines crossed, center square entry and rearing frequency. The dimension for the open field test box was 100 cm × 100 cm × 50 cm with square grids on the floor measuring 25 cm in length. A center square of the same length was also drawn. The young male rats were randomly picked by their tails and dropped in the center square at the beginning of the assessment. The animals were allowed to explore for five minutes during this time. The number of lines crossed was the frequency with which the rats crossed one of the grid lines with all four paws; the center square entry was the frequency with which the rats entered the center square with all four paws and the central square duration was the amount of time spent within the center square. While the rearing frequency was the number of times the young rats stood on their hind limbs. The box was clean properly off feaces and urine that might have been deposited by the previous animals.

The light and dark box test

The light and dark box test is a behavioral tool that plays an important role in assessing anxiety. It is made up of wood which measures 75 cm × 30 cm × 30 cm and was divided into a light and dark chamber which measured 50 cm long 25 cm long respectively. As the name implies, the light chamber was illuminated with natural white light while the dark chamber was not illuminated. There was a link between both chambers through which the rats could move freely from one chamber to the other. Rats were placed to face the dark chamber in the center of the light chamber and were allowed an exploration time of 10 minutes. Both chambers were cleaned properly off feaces and urine that might have been deposited by the previous animals.

Y-maze test

The Y-maze model is designed to evaluate spatial working memory. It had an angle of 120 degrees, with arms measuring 75cm and 15 cm in length and breadth respectively. Animals were placed at the junction where the three arms meet and are allowed to move freely for 5 minutes. They were assessed based on exploratory activities and frequency of entry of arms they have not visited earlier. The manner of alternation was recorded while

the percentage correct alternation of each rat was assessed as a ratio of the correct alternation to the total alternation multiplied by 100.

Elevated plus maze

The elevated plus maze test was used to measure anxiety in experimental rats.

Weight changes

The initial weights were taken with a weighing scale prior to treatments while the final weights were recorded at the end of treatment before the animals were sacrificed.

Biochemical studies

Assay for superoxide dismutase levels were carried out in cerebellar homogenates using spectrophotometric techniques. The tissue was placed in 0.25 M sucrose solution and homogenized. Tissue homogenate was collected in a 5 ml serum bottle and centrifuged at 3000 rpm for 15 minutes using a centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China). The supernatant was collected with Pasteur pipettes and placed in a freezer at -4 ^{O}C, and thereafter assayed. Biochemical assay was carried out as described by Sun and Zigma (1978) for superoxide dismutase.

Tissue processing

After the termination of treatment, the rats were anesthetized intramuscularly with 0.5 mL/kg-ketamine and transcardially perfused first with normal saline then with 4% paraformaldehyde. The cerebellum was excised from the fixed brain and dehydrated in ascending grades of alcohol (50%, 70%, 90% and 100%). Tissues were then cleared in xylene for 2 times 15 minutes each. Infiltration and embedding were done with paraffin wax in Leica hot air oven at 56°C. Tissue sections were obtained serially using a rotary microtome (Leica RM2245) and then mounted on glass slides. Sections were taken at 5 µm for histology (Omotoso *et al*., 2020). The prefrontal cortex was processed for Hematoxylin and Eosin (H&E) staining according to Pearse (1975) and Fischer *et al*. (2005) to demonstrate the general cytoarchitecture of the prefrontal cortex, while the cresyl fast violet staining technique was adopted to demonstrate the integrity of the Nissl bodies.

Data analysis

Quantitative data was obtained from SPSS (version 20) software and presented using GraphPad® (version 6). The outcomes were compared using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Significance was set at $p<0.05$ (95% confidence interval). The results were represented in bar charts with error bars to show the mean and standard error of mean respectively.

RESULTS

Roles of *Moringa oleifera on body weight changes of young Wistar rats exposed to permethrin*

In this study the difference between the initial and final weights of the experimental animals were estimated to assess change in body weight, after the termination of experiment. It was observed that there was a slight decrease in the body weight of the control group animals when compared to the permethrin group (Figure 1) with no significant statistical difference while the weight observed in the moringa group was higher than the permethrin and permethrin + Moringa group. In the final weight analysis, there was similar weight increase between the control and the Moringa group and close weight gain seen between the permethrin and permethrin + Moringa group with no statistical significance. However, significant statistical difference ($p<0.05$). was observed in the weight difference between the control and Moringa and permethrin + Moringa- treated groups respectively.

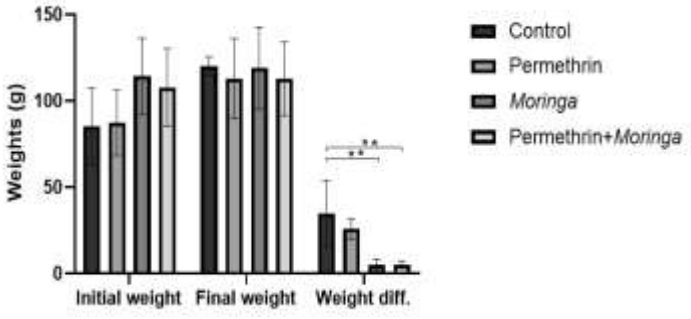

Figure 1. A bar chart showing the initial weight, final weight and weight differences of the rats. The p value < 0.05 was considered to be significant and a confidence interval of 95 % was used.

Moringa oleifera mitigates permethrin-induced memory decline

Figure 2a showed the Open field test model which was used to measure exploratory activity and anxiety in experimental rats used in this study. The number of line crossed of the open field test was statistically significantly lower ($p\leq0.05$) in the permethrin group when compared to the control. Although, the permethrin + Moringa group was higher compared to the permethrin only group, it was not statistically significant. This elevation deduced an increase in exploratory activity. The rearing frequency of the open field test as seen in **figure 2b** was highest in the control group and lowest in the permethrin group. There was a decrease in the permethrin only group when compared to the control group however, it was not statistical significant. A slight increase was observed in the Moringa + permethrin group compared with the permethrin only group. No statistical significance was recorded between the group. There was an increase in the anxiety level of animals in permethrin only group when compared to control, however this observation was not statistically significant. The center square entry of the open field test was accessed in **figure 2c** and it was observed that this change was lowest in the permethrin group when compared to the control. No statistical significance was recorded. Center square entry was found to be higher in the Moringa + permethrin group compared to the

permethrin group; no significant statistical difference was seen. This revealed the ability of Moringa to increase the exploratory activity of animals, although this was not statistically significant.

Figure 2d showed results for the Y-maze test which was used to measure short term memory in the experimental rats used in this study. Permethrin reduced the percentage correct alternation relative to the control group however, this decrease was not statistically significant. The percentage correct alternation of the Y-maze test was higher in the Moringa + permethrin group compared to the permethrin only group. This observation was not statistically significant, although it showed an increase in short term memory in moringa + permethrin group compared to permethrin only group.

Figure 3a shows graphical representation of the elevated plus maze test used to measure anxiety in the experimental rats in this study. The number of closed arm entry and duration in the permethrin only group was higher compared to control group, however there was no significant statistical difference between them. This activity was decreased in the moringa + permethrin group compared to permethrin only group. This showed reduced anxiety level in animals administered moringa + permethrin however, the decrease was not statistically significant.

The light and dark box test outcome as represented in figure 3b and 3c was used to measure anxiety in the experimental rats used in this study. The number of light box entry and duration in the permethrin only group was lower compared to the control group. However, this activity was found to be reduced in the Moringa + treatment group when compared to the permethrin only group. This also shows reduced anxiety level in animals administered moringa + permethrin when compared to the permethrin only group. The reduction was not statistically significant.

Figure 3d shows a graphical representation of the closed arm duration used to measure anxiety in the experimental rats. The duration of time spent by the permethrin group in the closed arm was longer compared to the control, however there was no significant statistical difference between them. This activity was slightly decreased in the moringa + permethrin group compared to permethrin only group. The outcome showed reduced anxiety level in animals administered moringa + permethrin however, the decrease was not statistically significant.

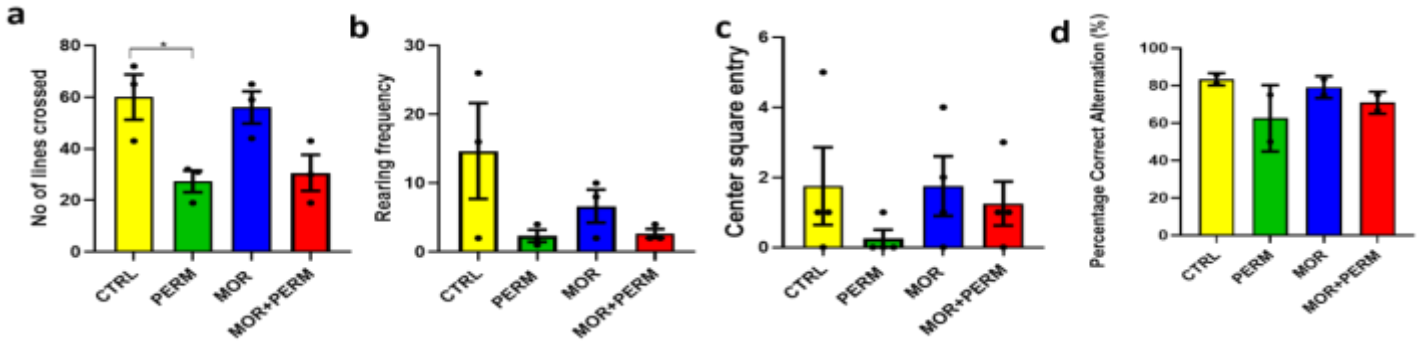

Figure 2: Behaviour (locomotion, exploratory and memory). CTL= Control group, PERM = Permethrin group, MOR = Moringa, MOR+PERM = Moringa and permethrin. In figure a, number of lines crossed in the Moringa only group is significantly lower compared to the control group and also Moringa + Permethrin group, in figure b, c the rearing frequency and center square entry is lower in permethrin group compared to other group. In figure d, the percentage correct alternation is lower in permethrin group compared to other groups.

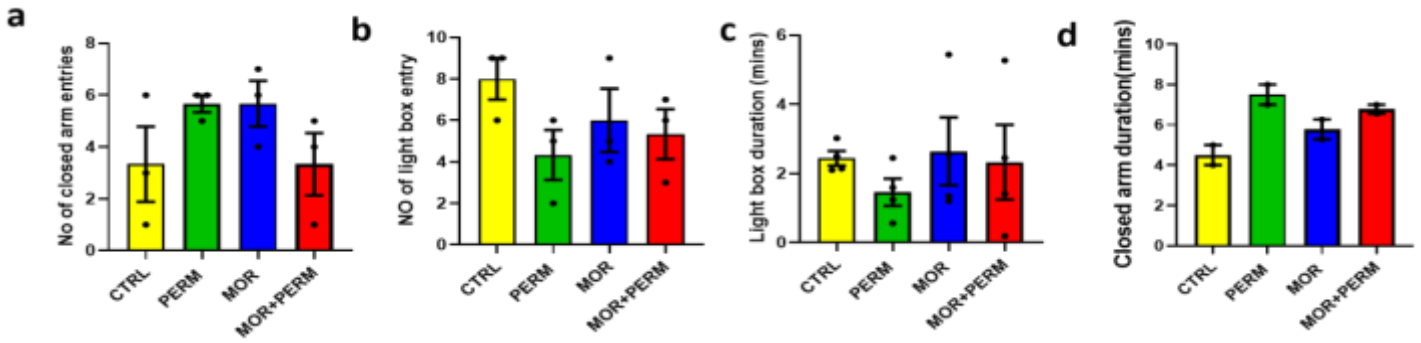

Figure 3: Behaviour (anxiety). CTL= Control group, PERM = Permethrin group, MOR = Moringa, MOR+PERM = Moringa and permethrin. In figure b, c, the number of light box entry and duration is lower in permethrin group compared to Nigella sativa + permethrin group. In figure a, d, the closed arm entry and duration is higher the permethrin group compared to Nigella sativa + permethrin group.

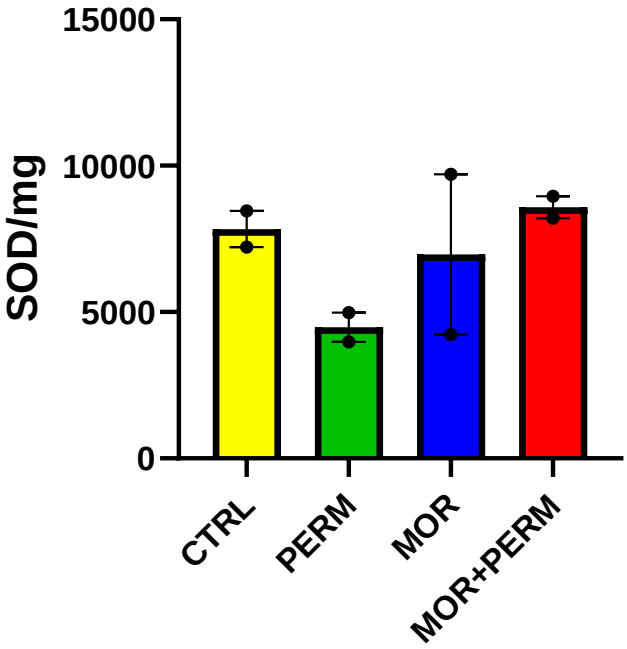

Figure 4: Activities of superoxide dismutase (SOD) in the prefrontal cortex of rats. There was a decrease in SOD activities in rats administered with permethrin compared to rats Permethrin+Moringa group.

Moringa oleifera counterbalances Permethrin-induced oxidative stress

Superoxide dismutase activity

There was a reduced level of superoxide dismutase (SOD) enzyme in the prefrontal cortex of the permethrin group when compared to the control group. However, a combination of Moringa + permethrin led to an increase in this enzyme when compared to the permethrin only group. There was no statistically significant difference across the groups.

Histological and histochemical observations

The general histomorphology of the prefrontal cortex was demonstrated to reveal the layers; molecular layer (L1), external granular and pyramidal layers (L2 & L3) and internal granular and pyramidal layers (L4 & L5). The pyramidal layer was presented at x400. In the Haematoxylin and Eosin stain, there appear to be no changes across all groups. However, in the Nissl profile demonstration by cresyl fast violet stain, there appeared to be minimal staining intensity in the prefrontal cortex of permethrin only group when compared with the control group. There was an improvement in the staining intensity of Nissl granules in the moringa + permethrin group as they appear more deeply stained when compared to Nissl granules in the prefrontal cortex of the permethrin only group.

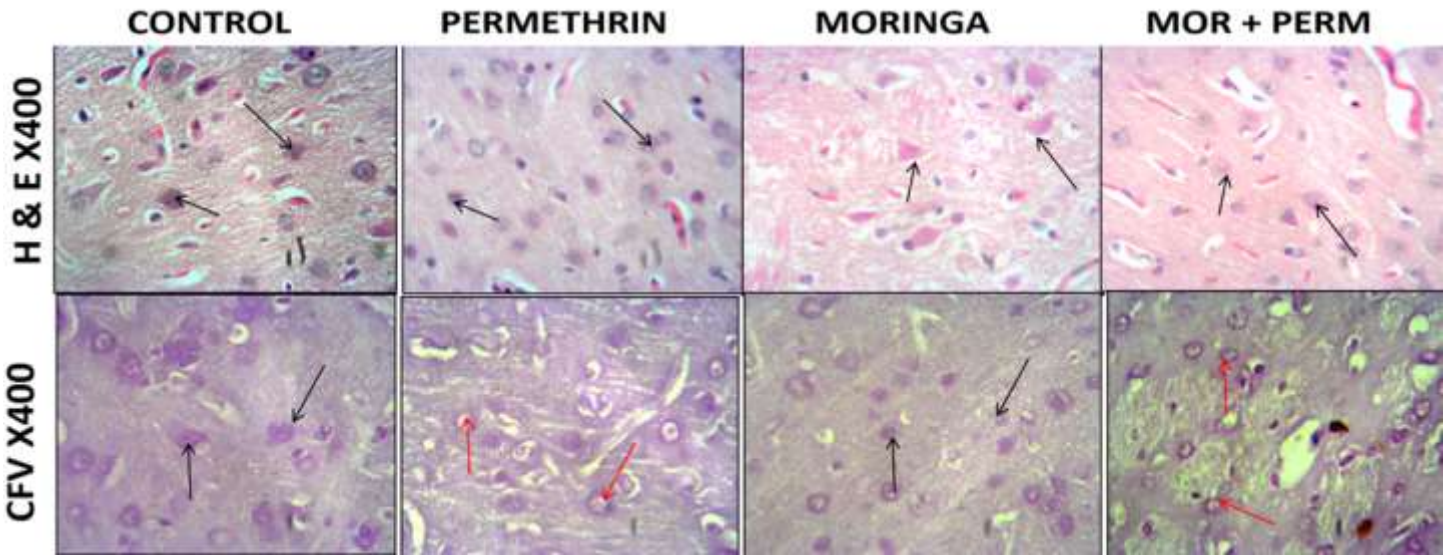

Figure 5: Representative photomicrograph of cytoarchitecture and Nissl profile of the pre-frontal cortex of experimental animals. The prefrontal cortex displays pyramidal layers with typical cellularity and characteristics histoarchitecture across all groups. The control group presented with typical Nissl staining intensity when compared to the permethrin group. The cells of the Moringa + permethrin group appeared deeply stained compared to the permethrin only group. However, vacuolation of cells were observed in the Moringa + permethrin group when compared to those seen in the permethrin group.

DISCUSSION

The widespread use of permethrin pesticides domestically and industrially is alarming. The tendency for humans to come in contact with permethrin increases with time. This poses a major threat to public health as many studies have revealed its health hazards ranging from lymphocyte toxicity to teratogenesis. Though few studies have been done to reveal it neurotoxic potential, a study conducted by Omotoso *et al*. (2020) characterized some neurotoxic effects of permethrin. In this study, the effect of permethrin on body weight changes, neurobehaviour, histomorphology of the prefrontal cortex, oxidative stress, neuroinflammation was characterized and *Moringa oleifera* oil extract was used as a ameliorative intervention on permethrin- induced neurotoxicity.

Oxidative stress is considered to be a key mechanism in pesticide toxicity (Mohammadi *et al*., 2019). It is a phenomenon characterized by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products (Pizzino *et al*., 2017). This reactive products (Reactive Oxygen Species (ROS) including Superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen), are normally generated as by-products of oxygen metabolism, but environmental stressors such as pollutants like pesticides contribute to greatly increase their (ROS) production. The imbalance that results from this usually leads to cascades of events that results in cell injury and death (Pizzino *et al*., 2017).

Rats exposed to permethrin showed increase in the production of ROS. This is because permethrin lowered the activity of SOD leading to the accumulation of ROS in the brain. This correlates with the observation made by omotoso et al., (2020). Since cells deploy antioxidant defensive system based on enzymatic components with SOD at the front line, to protect themselves from ROS-induced cellular damage (Pizzino *et al*., 2017), accumulation of the ROS causes a breach in this defence system resulting into serious neurological injuries evident in neurodegenrative diseases such as Parkinsonism, Alzheimer's disease (AD), amyotrophic lateral llsclerosis (ALS), multiple sclerosis, depression, and memory loss (Pizzino *et al*., 2017). In Alzheimer's disease, several experimental and clinical researches showed that oxidative damage plays a crucial role in loss of neurons and progression to dementia (Pizzino *et al*., 2017). β-amyloid, a toxic peptide often found present in AD patients' brain, is produced by free radical action and it is known to be at least in part responsible for neurodegeneration observed during AD onset and progression (Pizzino *et al*., 2017).

It was shown in this study that *Moringa oleifera* significantly restored the activity of SOD in the frontal cortex, after exposure to permethrin. This correlates with the observation made by Omotoso *et al*. (2018). *Moringa oleifera* treatment restored the level of SOD two proposed mechanism. Firstly, *Moringa oleifera* casuses a series of chemical reaction that helped in increasing the intrinsically produced superoxide thereby strengthening the antioxidant system to cater for the exacerbated ROS and nitrogen species. Secondly, it behaves as an antioxidant on its own, thereby supporting the intrinsic antioxidant system to mop up the excessively generated reactive species. Phytochemicals present in *Moringa oleifera* that give it its antioxidant potential include quercetin and luteoline (Gbadamosi *et al.,* 2016).

As earlier said, disruption in the biochemical processes involved in protection against oxidative stress can lead to alteration in cell morphology (cell injury) and death within the organ of concern (Pizzino *et al*., 2017). In this current study, Permethrin exposed experimental animals showed some dysmorphology in the histoarchitectural outline and morphology of cells of the prefrontal cortex including degenerative chromatolysis and karyolysis. This finding correlated with the observations made by omotoso et al, (2020). The principal function of PFC is to plan and execute behaviors. PFC collects information from cortex and subcortical structures, and arranges and controls this information; decides and executes the behavior (Akkoc & Ogeturk, 2017).

The neuronal cells of the PFC especially the pyramidal neurons, play a primary role in the execution of these functions. Pyramidal cells are the most common neuron in the cerebral cortex. They are the major source of intrinsic excitatory cortical synapses, and their dendritic spines are the main postsynaptic target of excitatory synapses. Moreover, they form most intra-areal projections and nearly all interareal projections, therefore are considered the principal neuronal building blocks of the cerebral cortex (Elton *et al*., 2011). Sequel to the aforementioned, structural damages and death of pyramidal cells occasioned by exposure to permethrin can result to the loss of important functions of the PFC. Stellate cells of the PFC, also play an important role in the execution of prefrontocortical functions. They function as interneurons (Alexander & Hasselmo, 2018), helping in the relay of cognitive impulses within the PFC. Loss and damages to this cell type within the PFC can result into reduction or loss of cognitive functions.

Moringa however ameliorated the distortions caused by permethrin. A study conducted by omotoso et al. (2018), reveal that mornings oleifera restored normal histoarchitecture of perturbed brain region. Owolabi and Ogunnaike (2014), also reported from their research the potential of *Moringa oleifera* as therapeutic intervention with no side effect on cerebral tissue morphology. This effect of *Moringa oleifera* is as a result of its richness in phytochemicals (phenols, flavinoids, quercetin etc.) with anti-oxidative activity. Oxidative stress is one of the main causes of alteration of histoarchitecture in the brain.

In furtherance, there is a connection between oxidative stress and cognitive decline (Liu *et al*., 2002; Omotoso *et al., 2020*). Compromise of the microscopic integrity of the PFC, brought about by permethrin-induced oxidative stress could be the underying cause of perturbation in certain neurobehaviour. Permethrin altered the normal working memory of exposed animals. These animals had low percentage alternation in Y maze test (Omotoso *et al.,* 2020). Locomotory activities of the permethrin-treated animals were not significantly altered, however, animals exposed permethrin had low locomotory index in the open field test. All these altered behavioral display indicated that the key brain regions involved in processing and execution may have been compromised neurochemically or structurally, with affectations on their normal function (Omotoso *et al.,* 2020). There is a striking evidence that permethrin induces neuroinflammation in brain areas involved in memory processing, formation and consolidation (Omotoso *et al.,* 2020).

Conclusively, *Moringa oleifera* showed remarkable ameliorative effect on oxidative stress, neurohistochemical alterations and neurobehavioural deficit associated with exposure to permethrin in rat prefrontal cortex.

REFERENCES

Agrawal S, Singh A, Tripathi P, Mishra M, Singh PK, Singh MP (2015). Cypermethrin-induced nigrostriatal dopaminergic neurodegeneration alters the mitochondrial function: a proteomics study. Molecular neurobiology 51:448-65.

Agrawal S, Dixit A, Singh A, Tripathi P, Singh D, Patel DK, Singh MP (2015). Cyclosporine A and MnTMPyP alleviate α-synuclein expression and aggregation in cypermethrin-induced Parkinsonism. Molecular neurobiology 52:1619-28.

Akkoc RF, Ogeturk M (2017). The Prefrontal Cortex: A Basic Embryological, Histological, Anatomical, and Functional Guideline. Journal of Human Anatomy and Physiology 1(4).

Alexander AS, Hasselmo ME (2018). Shedding light on stellate cells: The relationship between grid cells and two types of neurons found in the medial entorhinal cortex has been clarified. eLife. 7:e41041.

de Siqueira Patriota LL, de Lima BR, de Oliveira Marinho A, da Costa JA, de Lucena AL, Paiva PM, Napoleão DC, Cavalcanti JV, Pereira MC, Napoleão TH, da Rosa MM (2024). Moringa oleifera Lam. seed lectin (WSMoL) reduces chronic stress-induced anxiety and depression in mice by lessening inflammation and balancing brain chemicals. Behavioural Brain Research 477:115318.

Elton A, Faulkner ML, Robinson DL, Boettiger CA (2021). Acute depletion of dopamine precursors in the human brain: effects on functional connectivity and alcohol attentional bias. Neuropsychopharmacology 46(8):1421-1431.

Ezeamuzie IC, Ambakederemo AW, Shode FO, Ekwebelem SC (1996). Antiinflammatory effects of Moringa oleifera root extract. International Journal of Pharmacognosy 34(3):207-212.

Fischer, A.H.; Jacobson, K.A.; Rose, J.; Zeller, R. Hematoxylin and eosin staining of tissue and cell sections. In Basic Methods in Microscopy; Cold Spring Harbor Laboratory Press: New York, NY, USA, 2006; Chapter 4.

Gambo A, Moodley I, Babashani M, Babalola TK (2021). Impact of Moringa Oleifera leaves supplementation on quality of life of people living with HIV: A double-blind randomized controlled trial. Quality of Life Research 30:2563-2571.

Ganguly R, Hazra R, Ray K, Guha D (2010). Effect of Moringa oleifera in experimental model of Alzheimer's disease: Role of antioxidants. Annals of Neurosciences 12(3):33-36.

Gbadamosi IT, Omotoso GO, Olajide OJ, Dada-Habeeb SO, Arogundade TT, Lambe E, Obasi KK (2016). Moringa protects against nicotine-induced morphological and oxidative damage in the frontal cortex of Wistar rats. Anatomy 10(3):170-176.

Hadju V, Marks GC, Nontji W, Abdul Hafid R, Arundhana AI (2020). 'Moringa oleifera'leaf powder supplementation improved the maternal health and birth weight: A randomised controlled trial in pregnant women. Australian Journal of Herbal and Naturopathic Medicine 32(3):94-101.

Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, Cotman CW, Ames BN (2002). Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid. Proceedings of the National Academy of Sciences 99(4):2356-2361.

Ma ZF, Ahmad J, Zhang H, Khan I, Muhammad S (2020). Evaluation of phytochemical and medicinal properties of Moringa (Moringa oleifera) as a potential functional food. South African Journal of Botany 129:40-46.

Mohammadi H, Ghassemi-Barghi N, Malakshah O, Ashari S (2019). Pyrethroid exposure and neurotoxicity: a mechanistic approach. Archives of Industrial Hygiene and Toxicology 70(2):74-89.

Omotoso GO, Gbadamosi IT, Afolabi TT, Abdulwahab AB, Akinlolu AA (2018). Ameliorative effects of Moringa on cuprizone-induced memory decline in rat model of multiple sclerosis. Anatomy & cell biology 51(2):119-127.

Omotoso G, Oloyede O, Lawal S, Gbadamosi I, Mutholib N, Abdulsalam F, Bature A, Babalola A, Ayeni B, Amedu N (2020). Permethrin exposure affects neurobehavior and cellular characterization in rats' brain. Environmental analysis, health and toxicology 35(4), e 2020022-0

Owolabi JO, Ogunnaike PO (2014). Histological evaluation of the effects of Moringa leaf extract treatment on vital organs of murine models. Merit Research Journal of Medicine and Medical Sciences 2(10):245-257.

Pari L, Karamac M, Kosinska A, Rybarczyk A, Amarowicz R (2007). Antioxidant activity of the crude extracts of drumstick tree [Moringa oleifera Lam.] and sweet broomweed [Scoparia dulcis L.] leaves. Polish journal of food and nutrition sciences 57(2):203-208.

Pearse AG (1975). The role of histochemistry in increasing objectivity in histopathology. Postgraduate Medical Journal 51(600):708-710.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A (2017). Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity 2017(1):8416763.

Sunderkötter C, Wohlrab J, Hamm H (2021). Scabies: epidemiology, diagnosis, and treatment. Deutsches Ärzteblatt International 118(41):695.

Ummah AS, Muhammad F, Rahmawati YE, Ridwan I, Nurudhin A, Fauzi ER, Dewi RT, Werdiningsih Y (2023). Effect of Moringa oleifera Leaf Extracts on Depression in Rheumatoid Arthritis Patients. Age (years) 21(30):31-40.

Wang F, Bao Y, Zhang C, Zhan L, Khan W, Siddiqua S, Ahmad S, Capanoglu E, Skalicka-Woźniak K, Zou L, Simal-Gandara J (2022). Bioactive components and anti-diabetic properties of Moringa oleifera Lam. Critical Reviews in Food Science and Nutrition 62(14):3873-3897.