Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

The Neurotoxic Impact of Formulated Pyrethroid Insecticide on the *Substantia nigra* of Adult Wistar Rat

Princewill Sopuluchukwu Udodi[*1], Edwin Ifeanyichukwu Nnadi[1], Damian Nnabuihe Ezejindu[1], Emeka Christian Okafor[1], Ifechukwu Justicia Obiesie[1], Charles A. Oyinbo[2], Darlington Cyprain Akukwu[3], Godwin Chinedu Uloneme[3]

[1]*Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nigeria*

[2]*Department of Anatomy, College of Health Sciences, Niger Delta University, Nigeria*

[3]*Department of Human Anatomy, Imo State University, Owerri, Imo State, Nigeria*

(Received: 20 August 2021 Accepted: 8 January 2022)

KEYWORDS

Formulated pyrethroid;
Substantia nigra;
Neurodegeneration;
Insecticide;
Histopathology;
Oxidative stress

ABSTRACT: Pyrethroid toxicity has been reported in animal models and some cases of poisoning have been reported in humans. This research was aimed at evaluating the effects of formulated pyrethroid insecticide on the *substantia nigra* of adult Wistar rat. Thirty two (32) adult Wistar rats were divided into 4 groups, group A (control) inhaled fresh air, group B (short-term), group C (medium-term) and group D (high-term) were exposed to 10% formulated pyrethroid for 2hrs/day, 3hrs/day and 4hrs/day respectively for five times a week in four weeks. Wire suspension test was utilized to demonstrate the motor function. The brain tissues were homogenized for biochemical analysis while some tissues were fixed in 10% Neutral Buffered Formalin for 48hrs and grossed to isolate the brain tissue of interest for histological investigations. The neurobehavioral test presents statistical non significant decrease in the motor function of the exposed groups when compared to the control group ($p<0.05$). The malondialdehyde and glucose level of the exposed groups were significantly higher, while the total antioxidant capacity in the exposed groups were significantly lower. Mild to severe histopathological features were observed in the exposed groups which suggests *substantia nigra*. Neuronal degeneration on exposure to formulated pyrethroid. This study indicates acute exposure of formulated pyrethroid in rats exacts neurodegenerative changes in the *substantia nigra* via elevation of oxidative stress and depletion of antioxidant capcity. Thus, further studies should be carried out to demonstrate the long-term effect as well as the mechanism of action of formulated pyrethroid insecticide induced degeneration on the *substantia nigra*.

INTRODUCTION

Insecticides are the first line of defense in control of disease vectors and plant pests. Four commonly used insecticide classes are pyrethroids, organochlorines, carbamates and organophosphates. According to the World Health Organization (WHO) global report on insecticide resistance in malaria vectors, resistance to these insecticide classes is widespread in all major malaria vectors across Africa, America, South-East Asia, the Eastern Mediterranean and the Western Pacific. This increase in resistance is followed by an increase in the intensity of use of insecticides; as a result, scientific data, estimates that roughly three million cases of pesticide poisonings occur annually and an excess of 250,000 deaths worldwide had been reported [1].

Pyrethrins are insecticides obtained from the flowers of Chrysanthemum cinerariifolium whose primary toxicodynamic mode of action is neurotoxic through delayed closure of voltage-gated sodium channels in the central nervous system (CNS) of exposed organisms [2]. Pyrethroids are class of synthetic compounds with insecticidal activity based on the structure of naturally occurring pyrethrins. Although natural pyrethrins do

*Corresponding author: udodip@gmail.com (P. S. Udodi)
DOI: 10.22034/jchr.2022.1938288.1392

have insecticidal activity, they also are inherently unstable when exposed to light hence the need for the modification the pyrethrin structure to produce pyrethroids, which are more stable compounds that retained the desirable insecticidal and toxicologic properties. The efficacy of pyrethroid group of insecticide has made them more preferable to their predecessors like organophosphates; concomitantly, human exposure to pyrethroids has increased since they replaced the organophosphorus insecticides [3].

The substantia nigra, popularized by its role in Parkinsonism, is the pigmented part of the midbrain just behind the crus cerebri. Its main projections are the nigrostrial dopaminergic fibers, which play crucial roles in the control of motor functions. Therefore, lesions to the *substantia nigra* leads to a decrease in dopamine levels and these results in various motor dsyfunctions [4], and recent has provided an evidence that pyrethroids exposure at certain levels and duration in animal models results to damage to vital organs and also increases oxidative stress in the central nervous system [5].

Although, there are lots of studies in the scientific open literature that documents the neurotoxicity of cypermethrin or other pyrethroid insecticide, no study has reported the neurotoxic effect of a formulated insecticide containing two or more pyrethroids (as commonly used in Nigeria and other African countries), exposure on motor functions in animals. Its persistence in toxic stress-related brain, and the mechanisms by which pyrethroid insecticides elucidates neurodegeneration in the basal ganglia is yet to be unraveled. This study hence assessed the neurotoxicity of formulated pyrethroid insecticide in the *substantia nigra* and its relationship to motor functions deficits, as well as pattern of tissue degenerations and oxidative stress.

MATERIALS AND METHODS

Collection of experimental substances

10% Formulated-Pyrethroid insecticide (Cypermethrin and Dichlorvos) were procured and authenticated at the department of Industrial Chemistry, Nnamdi Azikiwe University, 5ml of absolute Cypermethrin and Dichlorvos each was diluted in 45ml of distilled water, to obtain 10% Formulated-Pyrethroid insecticide which were exposed to the experimental animals by inhalation.

Experimental design and protocols

The 32 animals were weighed and allocated into four groups of eight animals each. The groups were designated as group A, B, C, and D. Rats in groups B, C and D were exposed to 10% Formulated-Pyrethroid insecticide (Cypermethrin and Dichlorvos) for five days a week in four weeks. *Group A*- Control animals were exposed to fresh air in four weeks; *Group B*- Low-term exposed animals were exposed to 10% concentration of Formulated-Pyrethroid insecticide 2 hour/day, for five times a week in four weeks; *Group C*- Medium-term exposed animals were exposed to 10% concentration of Formulated- Pyrethroid insecticide 3 hours/day, for five times a week in four weeks; *Group D*- High-term exposed animals were exposed to 10% concentration of Formulated- Pyrethroid insecticide 4 hours/day, for five times in four weeks.

Mode of exposure

The exposure of the experimental animals were carried out by soaking cotton wool in 10% Formulated-Pyrethroid insecticide with a pH of 9.2 at room temperature and were placed in enclosed (designed) wire gauze within the animal cages, thus exposing the animals to the vapour according to the duration for each group.

Wire suspension test

Wire suspension test was used to analyze the motor function across different groups of the animals. The animals were suspended by their forepaws on a 2mm diameter metal bar that was elevated 30cm above a soft surface. The time until the pups lost their grip and fell on the soft surface was measured. Complete acquisition of the reflex was assumed when the animal was able to hang on the bar for 30s.

Animal euthanasia

Twenty-four hours after the last exposure, following the behavioral test, the animals were weighed, then anaesthetized under chloroform vapour, and the animal skull dissected by occipitofrontal incision. The brain

tissues were harvested and weighed. Brain tissues of some of the animals were prepared for biochemical analysis through the process of homogenization while the remaining tissues of some of the animals were fixed in 10% neutral buffered formalin for 48 hours and grossed to isolate the brain tissue of interest for histological investigations in the histology laboratory of Anatomy Department, Nnamdi Azikiwe University, Nnewi campus, Nigeria.

Biochemical analysis

The brain tissues were taken to Biochemistry Department for the analysis of malondialdehyde (MDA) for lipid peroxidation, total antioxidant capacity (TAC) and glucose as a significant brain tissue substrate for energy metabolism.

Method of homogenization

One gram of the respective organs were weighed and put into 10ml of 0.9%normal saline each. Each of the brain tissues were homogenized with homogenizer at room temperature. After this, each of the samples was centrifuged at 3000rpm for 20min at room temperature. The supernatant was separated and stored in a refrigerator at 2 degrees Celsius for further analysis.

Tissue processing

For the histological analysis of the tissues under the microscope, the tissues were passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% Neutral Buffered Formalin (Sodium Phosphate Monobasic 4.0gm, Sodium Phosphate Dibasic 6.5gm, Formaldehyde 37% 100.0ml, Distilled Water 900.0ml). The tissues remained in the fluid for 48 hours. After fixation, the tissues were washed overnight under a stream tap water so as to remove any surplus fixatives. Dehydration of the fixed tissues was done so as to remove water and other substances. This was carried out in different percentages of alcohol 50%, 70% and 95% absolute. In each grade of alcohol, tissues were changed twice for two (2) hours, and one (1) hour for each change. After dehydration, tissues were cleared in xylene for two (2) hours after which infiltration was done in molten paraffin wax at a temperature of 60oc for two (2) hours, each in two changes. When the paraffin wax cools, it sets as a hard block which allow for easy sectioning of the tissues.

Heamatoxylin and eosin stain

Histological protocol by [6] was adopted. Sections were dewaxed using xylene for one (1) minute and then rehydrated by alcohol through descending grades of ethyl alcohol and thereafter washed in distilled water. The sections were stained with heamatoxylene for twenty (20) minutes and differentiated with 2% acid alcohol for two (2) seconds. The acid alcohol was washed off with tap water and the sections passed through running tap water for ten (10) minutes to regain the blue colour. The sections were counterstained in 1% aqueous eosin for thirty (30) seconds and were dehydrated through ascending grades of ethyl alcohol, cleared in xylene and mounted using DPX. After which, the sections were viewed under light microscope.

Cresyl echt violet stain

This was utilized to demonstrate nissl substance in tissue sections. Nissl substance is lost after cell injury and if the axon degenerates, the myelin covering also breaks down. Neurons contain Nissl substance, which is primarily composed of rough endoplasmic reticulum, with the amount, form, and distribution varying in different types of neurons. Because of the RNA content, Nissl substance is very basophilic and will be very sharply stained with basic aniline dyes. By varying the pH and the degree of differentiation, both Nissl substance and nuclei or only Nissl substance may be demonstrated.

The procedure involves; Deparaffinize and hydrate with distilled water, then put in 0.5% Cresyl violet for two minutes, wash in distilled water then, dehydrate, clear in xylene, and cover with coverslip. Nissl substance presents dark blue to purple colour.

Data analysis

Data was analyzed using SPSS version 27.0.1 software package. Mean and standard deviation was obtained and one way analysis of variance (ANOVA) was used to

compare values between groups. Data was expressed as Mean ± Standard Deviation (SD) and was considered statistically significant when $P \leq 0.05$.

RESULTS

Effect of formulated pyrethroid insecticides on the animal body weight

As shown in Table 1, the animals in group A (control) had increased body weights at the end of experiments. However, the animals that were exposed to pyrethroid presents weight loss. Furthermore, the weight loss in group C was significant (p=0.05).

Table 1. Shows the effect of formulated pyrethroid on the animal body weight.

Groups	Weight	Mean±SEM	t-value	p-value
Group A	Initial weight	198±3	-2.50	0.13
	Final weight	207±2		
Group B	Initial weight	199±1	2.75	0.11
	Final weight	185±5		
Group C	Initial weight	202.5±2.5	4.23	0.05
	Final weight	186±3		
Group D	Initial weight	195±5	2.21	0.16
	Final weight	181.5±3.5		

Data was analyzed using independent sample t-test. Results were significant at p<0.05.

In comparison to the control group, Figure 1 above presents experimental animals that have lost weight after exposure to formulated pyrethroids. Group B (short-term), group C (medium-term) and group D (high–term) showed greater weight loss when compared to control

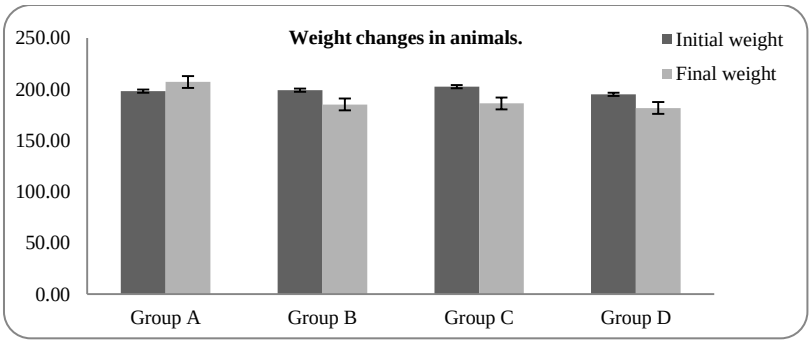

Figure 1. A bar chart illustrating the comparison between the initial and final body weight of animals in various groups.

Wire suspension test for assessment of motor function

Table 2 above presents the result of neurobehavioural function test which was carried out after four (4) weeks of exposing the animals to formulated pyrethroid insecticides. The table compares the mean time spent by the control group (A) on wire suspension to the mean time spent by the experimental groups (B, C, D). The group B (p=0.68), group C (p=0.44), and group D (p=0.26), shows non-significant decrease in the time spent by the animals suspending on the wire when compared to group A.

Table 2. Effect of pyrethroid Insecticide on the motor function.

	Groups	Mean ± SEM	p-value	F-value
Neurobehavioral test (sec)	Group A	1.4±0.16		
	Group B	1.31±0.18	0.68	0.63
	Group C	1.22±0.18	0.44	
	Group D	1.12±0.04	0.26	

Data was analyzed using One Way Anova and PostHoc LSD. Results were significant at p<0.05.

The bar chart Figure 2 above presents the result of neurobehavioural function test which was carried out after four (4) weeks of exposing the animals to formulated pyrethroid insecticides. The bar chart above illustrates the mean time spent by the control group (A) of animals on wire suspension to the mean time spent by the experimental groups (B, C, D). The group B (1.31±0.18), group C (1.22 ± 0.18), and group D (1.12±0.04), shows non-significant decrease in the time spent by the animals suspending on the wire when compared to group A (1.4±0.16).

Effect of formulated pyrethroid insecticides on lipid peroxidation levels, oxidative stress markers and glucose levels

Table 3 shows the MDA, TAC and glucose levels across all groups.

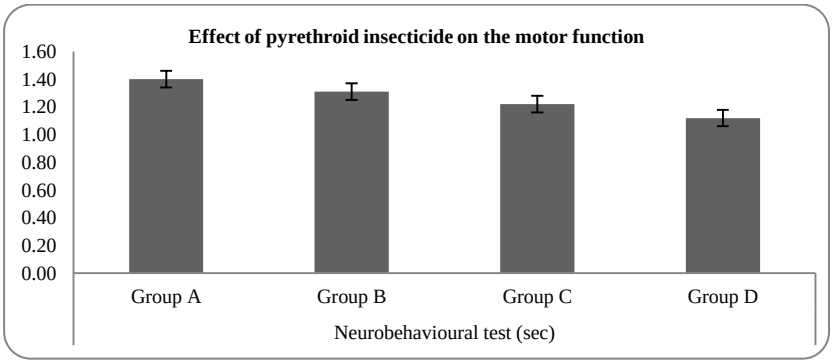

Figure 2.A bar chart illustrating the neurobehavioural test outcomes of animals in various groups.

Table 3. The MDA, TAC and glucose levels across all groups.

		Mean±SEM	p-value	F-value
MDA (m m^{-1})	Group A	3.31±0.08		
	Group B	3.57±0.03	0.02	21.86
	Group C	3.76±0.04	0.00	
	Group D	3.85±0.05	0.00	
TAC (m m^{-1})	Group A	258.67±1.33		
	Group B	250.11±2.11	0.05	7.37
	Group C	248.05±2.05	0.03	
	Group D	244.91±2.91	0.01	
Brain glucose (m m^{-1})	Group A	66.92±0.92		
	Group B	69.66±0.35	0.07	15.07
	Group C	72.26±0.74	0.01	
	Group D	73.99±1.01	0.00	

Data was analyzed using One Way Anova and PostHoc LSD. Results were significant at $p<0.05$.

The MDA level of the animals that were treated with pyrethroid were significantly higher than the control ($p<0.05$). These negative alterations in the products of lipid peroxidation for groups B, C, and D, results from the destruction of lipid component of olfactory bulb following formulated pyrethroid exposure for 2, 3 and 4 hours respectively. The TAC level was significantly lower in the pyrethroid exposed groups compared to the control. Finally, the brain glucose level was increased in the pyrethroid exposed groups and the increase was significant in groups C and D ($p>0.05$).

Figure 3 shows MDA levels in animals following exposure to pyrethroid. The test groups B, C, and D shows higher levels of lipid peroxidation (MDA) when compared to the control group which portends an increased level of lipid peroxidation in substantia nigra.

Figure 4 shows the TAC in animals following exposure to pyrethroid. The test groups B, C, and D presents lower

levels of total antioxidant capacity (TAC) when compared to the control group which indicates low ability of the tissue to combat the activities of free radicals. Figure 5 illustrates glucose levels in animals following exposure to pyrethroid. The test groups B, C, and D shows higher levels of glucosewhen compared to the control group, which portends a decreased glycolytic activities which results to hyperglycemia.

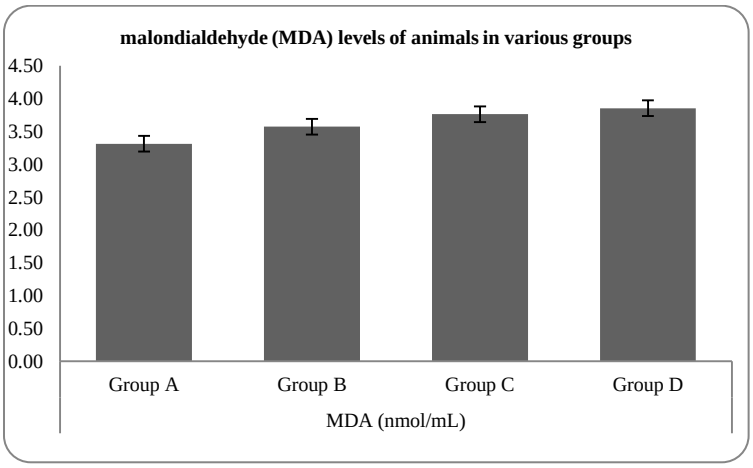

Figure 3. A bar chart illustrating the malondialdehyde (MDA) levels of animals in various groups.

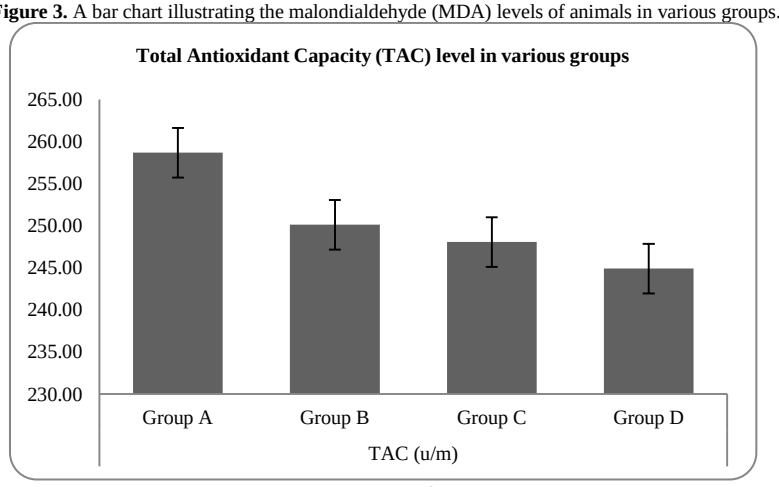

Figure 4. A bar chart illustrating the level of total antioxidant capacity (TAC)

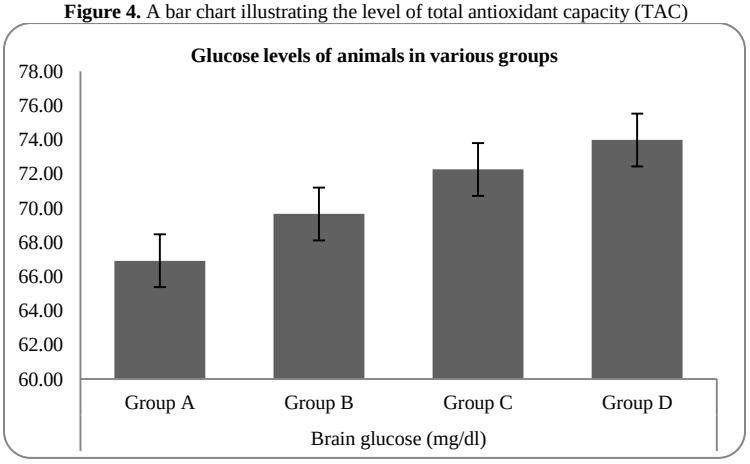

Figure 5. a bar chart illustrating the glucose levels of animals in various groups.

Histology of Substantia nigra stained with H and E staining technique

The micrographs in Figure 6a presents a normal histoarchitecture of an entire substantia nigra, comprising of pars compacta and reticulata. While the micrograph plate 6b presents a normal cytoarchitecture

of pars compacta comprising of dopaminergic nerve cells and progenitor cells of neurogenic potentials. The dopaminergic nerve cells are arranged in cords and sheaths with densely stained large centrally located nucleus.

The Micrograph in Figure 7 presents a mild distorted tissue of pars compacta with mild population of vacuolated progenitor cells, lewis body, pigment-laden macrophage, pericellular spaces, hyperchromatic cell and focal area of haemorrhagic necrosis in adult wistar rat exposed to 10% concentration of formulated pyrethoid insecticide for 2 hours/day, 5 times a week in 4 weeks.

Micrograph in Figure 8 shows severe distortion of pars compacta with evidence of autophagy, ranging from the presence of lewis body, pigment-laden macrophage, abundant neuronal cell shrinkage, pericellular spaces, vacuolated progenitor cells, hyperchromatic cell and coagulative necrosis in animals exposed to 10% concentration of formulated pyrethoid insecticide for 3 hours/day, 5 times in a week for 4 weeks.

Micrograph in Figure 9 shows severe distortion of pars compacta with evidence of autophagy, ranging from the presence of lewis body, pigment-laden macrophage, abundant neuronal cell shrinkage, pericellular spaces, vacuolated progenitor cells, hyperchromatic cell and coagulative necrosis in animals exposed to 10% concentration of formulated pyrethoid insecticide for 4 hours/day, 5 times in a week for 4 weeks.

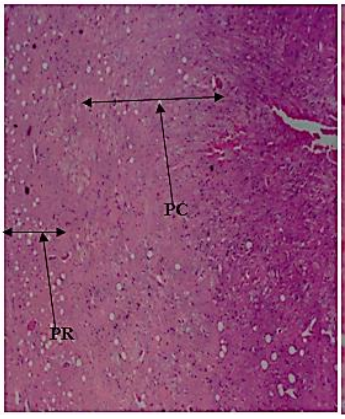

Figure 6a. (Control) A photomicrograph section of substantia nigra showing normal pars compacta (PC) and pars reticulate (PR) stained by H&E (X40)

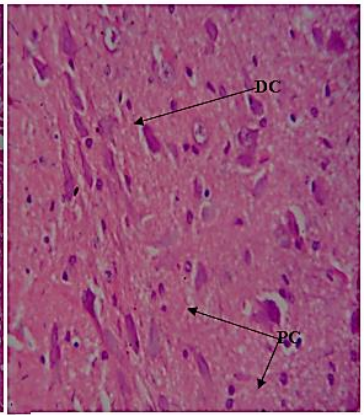

Figure 6b. (Control) A photomicrograph section of substantia nigra showing normal dopaminergic cells (DC) and progenitor cells (PC) stained by H&E (X400)

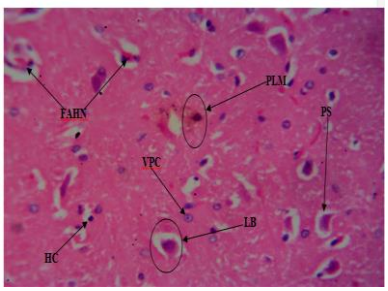

Figure 7. (Short-term) - A photomicrograph section of *substantia nigra* showing mild distortion of pars compacta with the presence of lewis body (LB), pigment-laden macrophage (PLM), pericellular spaces (PS), vacuolated progenitor cells (VPC), hyperchromatic cell (HC) and focal area of haemorrhagic necrosis (FAHN). Stained by H & E (X 400)

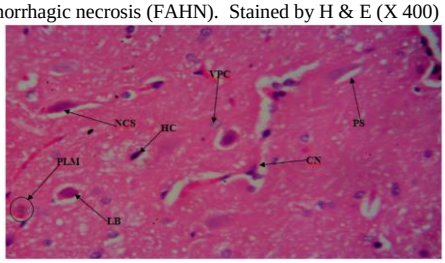

Figure 8. (Medium-term) - A photomicrograph section of *substantia nigra* showing severe distortion of pars compacta with the presence of lewis body (LB), pigment-laden macrophage (PLM), neuronal cell shrinkage (NCS), pericellular spaces (PS), vacuolated progenitor cells (VPC), hyperchromatic cell (HC) and coagulative necrosis (CN). Stained by H & E (X 400)

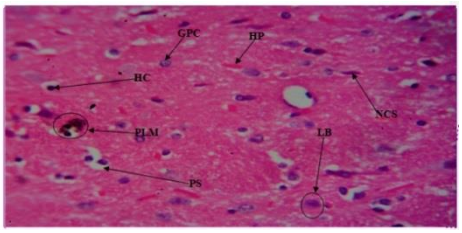

Figure 9. (High-term) - A photomicrograph section of *substantia nigra* showing severe distortion of pars compacta with the evidence of lewis body (LB), pigment-laden macrophage (PLM), neuronal cell shrinkage (NCS), pericellular spaces (PS), hyperchromatic cell (HC), haemoglobin pigmentation (HP) and granulated progenitor cells (GPC). Stained by H & E (X 400)

Histology of substantia nigra of animals stained with

crysl violet staining technique

The micrograph in Figure 10 presents densely stained cell cytoplasm of active non condensed nissl bodies of dopaminergic neurons, and neuronal precursor cells. This indicates normal distribution of nissl bodies in the neuronal cell bodies of wistar rat exposed to fresh air for four (4) weeks.

Micrograph in Figure 11 presents severely distorted pars compacta of substantianigra with condensed inactive dopaminergic neurons and positive progenitor cells of animals exposed to10% concentration of formulated pyrethoid insecticide for 2 hours/day, 5 times in a week for 4 weeks.

Micrograph in Figure 12 presents severely distorted pars compacta of substantianigra with condensed inactive dopaminergic neurons and positive progenitor cells of animals exposed to 10% concentration of formulated pyrethoid insecticide for 3 hours/day, 5 times in a week for 4 weeks.

Micrograph in Figure 13 presents severely distorted pars compacta with aberrant condensed inactive dopaminergic neurons and positive progenitor cells of animals exposed to 10% concentration of formulated pyrethoid insecticide for 4 hours/day, 5 times in a week for 4 weeks.

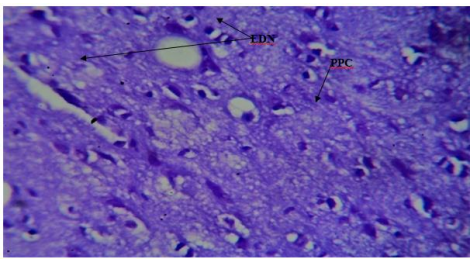

Figure 10. (Control)- A photomicrograph section of normal pars compacta of substantia nigra, showing positive euchromatin dopaminergic neurons (EDN) and positive progenitor cells (PPC). Stained by Crysl Violet technique (X400)

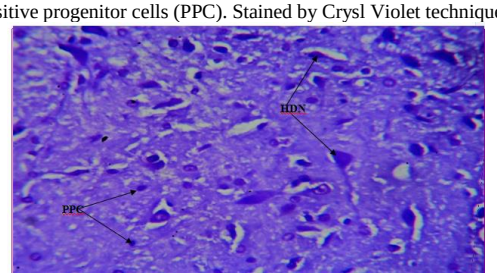

Figure. 11 (Short-term)- A photomicrograph section of milddistorted pars compacta of substantia nigra, showing positive heterochromatin dopaminergic neurons (HDN) and positive progenitor cells (PPC). Stained by Crysl Violet technique (X400)

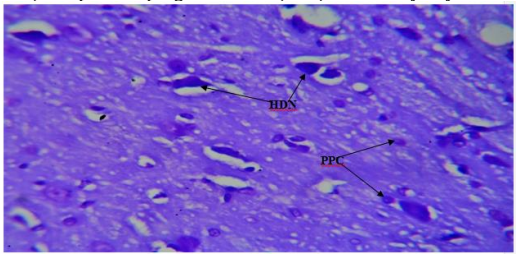

Figure 12. (Medium-term)- A photomicrograph section of severelydistorted pars compacta of substantia nigra, showing positive heterochromatin dopaminergic neurons (HDN) and positive progenitor cells (PPC). Stained by Crysl Violet technique (X400)

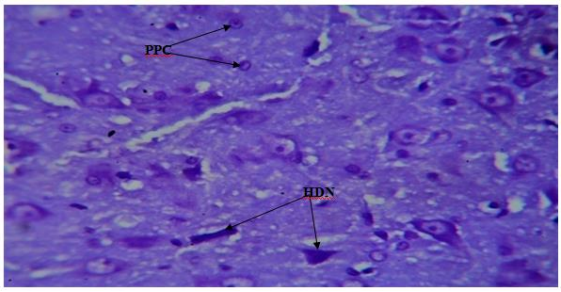

Figure 13. (High dose)- A photomicrograph section of severelydistorted pars compacta of substantia nigra, showing sparsely stained heterochromatin dopaminergic neurons (HDN) and positive progenitor cells (PPC). Stained by Crysl Violet technique (X400).

DISCUSSION

A scientific global investigation by world health organization reported that indiscriminate use of insecticides could increased the resistance of malaria vectors to insecticides thus leading to an increase in the use of pyrethroid based insecticide presumed to be effective against mosquitoes[1]. This indiscriminate use of insecticide was estimated to cause about three million pesticide poisonings across various regions [1]. The toxic effect reported in this research indicates that formulated pyrethroid insecticide transverses the blood brain barrier to the substantia nigra. Similar findings made by [7] shows that chemical in the nasal cavity directly reach the brain. This suggests that certain toxins can penetrate and gain entry into the central nervous system by means of axonal transport [8].

An assessment done by [9] suggested that inhalation of pyrethroid based mosquito vaporisers have toxic effect reflected as reduced weight gain following subchronic exposure which was similar to our findings on the effect of formulated pyrethroid insecticide on the body weight of the animals across various groups. Early weight loss was observed in the exposed groups similar to the finding demonstrated in neurodegenerative conditions, such as Alzheimer disease [11], amyotrophic lateral sclerosis [10] and Huntington disease [12]. The statistical significant decrease in group C and non significant decrease in group B and D, corroborates with the findings from [13] who reported a non significant decrease in the body weight and [14] who postulates an increased brain temperature in patients with neurodegenerative diseases which subsequently increases energy requirements and raises body metabolism, resulting in significant weight loss.

The neurobehavioural test for motor function presents debilitating motor activity of *substantia nigra* across the exposed group which denotes reduced coordination due to mild lesion of the *substantia nigra* [15]. The animals in the exposed groups spent less time when suspended on the wire which reveals the role of pyrethroid in inducing nigro-striatal dopaminergic neurodegeneration and behavioural deficits [16]. The time spent suspending on the suspension wire decreases with high exposure time, which implies that the high-term exposed animals spent the shortest time on the suspension wire and was followed by the medium-term exposed animals, then the short-term exposed animals as suggested by [17-18], who proposed the time dependency as a typical feature of toxicity: the longer the exposure to a toxic substance, the greater the effects. Adverse health effects with more severe presentations may result from longer term (above one month) exposure to this insecticides [19-20].

Generally, insecticides intoxication produces oxidative stress through overproduction of free radicals and induction of tissue lipid peroxidase in mammals and other organisms [21]. Malondialdehyde (MDA) is one of the end products of lipid peroxidation [22] while TAC is a biomarker of oxidative stress [23]. Biochemical alterations in biological tissues explain the vulnerability of the tissues to certain diseases of the body [24]. The statistical significant increase observed in the product of lipid peroxidation (MDA levels) is as a result of destruction of the lipid component of *substantia nigra* [25]. There was a decrease in the total antioxidant capacity of *substantia nigra* across groups B, C and D which explains the vulnerability of body tissues to metabolites [24-25]. The brain as a complex structure has some existing mechanisms for sustaining its function in short-term exposure to toxic materials; an impairment of glycolytic capacity which results to high glucose level, sensitizes macrophages to cytokine stimulation which

reduces phagocytosis and nitric oxide production, thereby serving as a brain mechanism to sustain brain function in mild short-term exposure to toxicity [26].

The histopathological results revealed various degrees of lesion in the *substantia nigra* of the exposed animals. Among the pathological features was Lewis body which is an inclusion body of abnormal aggregations of protein that develop inside nerve cells affected by Parkinson's disease [27]. Substantia nigra, which functions as a coordinating and regulating system for movement, can be damaged after exposure to pyrethroid as seen in this research.Pigment-laden macrophages present in the tissues were sensitized by the necrosis of dopaminergic neurons as they clear dead cell debris [28]. Heterochromatic nerve cells and the aberrant distribution of nissl bodies in the tissue sections of exposed animal groups were due to DNA damage [29]. The pathological pattern obtained in this study indicates that formulated pyrethroid insecticides causes DNA damage and reduces mitotic and nuclear divisions [30]. The pathological effect demonstrated in this study were time dependent which indicates a possible sever tissue degeneration when exposed above one month [31].

CONCLUSIONS

The results presented in this study indicate that exposure to formulated pyrethroid insecticides causes morphologic, biochemical and functional alterations in the *substantia nigra* of adult wistar rat exposed for short-term, medium-term and high-term. Some of the features observed could be seen in some neurodegenerative diseases such as Parkinson and Alzheimer diseases.

Contribution to Knowledge

This scientific investigation expounds our understanding on the toxicity of formulated pyrethroid insecticides on the *substantia nigra* of animals. This insecticide is mostly used by residents of low socioeconomic environment. This would also serve as a guide in sensitizing the necessity of formulating animal friendly insecticides and caution its application in various homes to prevent certain brain disease conditions such as Parkinson and Alzheimer diseases. The chemical alterations and neurodegenerative effects presented by the formulated pyrethroid insecticides are critical for developing effective modalities not only for the treatment of vulnerable population but also for understanding the etiology of neurodegenerative diseases where brain chemical imbalances are involved.

ACKNOWLEDGEMENTS

My sincere and greatest gratitude is to God Almighty for His infinite mercy, protection, love, unlimited favour and provision, in the course of this study. I sincerely appreciate the team of scientist from different universities who contributed immensely to this scientific investigation. My sincere gratitude and love also go to my parents Mr. and Mrs. E.O UDODI and my siblings, for their love, care and unlimited support in ensuring that my dreams and aspirations become reality.

Conflict of interests

No conflict.

REFERENCES

1. World Health Organization Report on Impact of Malaria, 2004, Available at: https://www.who. int/malaria, accessed June, 2017.

2. Soderlund D.M., 2010. State-dependent modification of voltage-gated sodium channels by pyrethroids. Pestic Biochem Phys. 97, 78–86

3. Khan M.J., Drochner W., Steingass H., Islam K.M.S., 2008. Nutritive evaluation of some tree leaves from Bangladesh for feeding ruminant animals. Indian J Anim Sci. 78(11), 1273-1277

4. Sonne J., Reddy V., Beato M.R., 2020. Neuroanatomy, Substantia Nigra. Stat Pearls Publishing. https://www. ncbi.nlm.nih.gov/books/NBK536995/

5. Sharifi-Rad M., Anil Kumar N.V., Zucca P., Varoni E.M., Dini L., Panzarini E. et al. 2020. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front Physiol. 11, 694.

6. Drury R., Wallington B., 1973. Carleton's histological technique. 4th ed. Toronto, NY: Oxford University Press

7. Sasajima H., Miyazono S., Noguchi T., Kashiwayanagi M., 2015. Intranasal administration of rotenone in mice attenuated olfactory functions through

the lesion of dopaminergic neurons in the olfactory bulb. Neurotoxicology. https://DOI: 10.1016/j. neuro. 2015.10.006

8. Dando S.J., Mackay-Sim A., Norton R., Currie B.J., John J.A.S., Ekberg J.A. et al., 2014. Pathogens penetrating the central nervous system: infection pathways and the cellular and molecular mechanisms of invasion. Clin Microbiol Rev. 27, 691–726

9. Yadav M., Jhunjhunwala S., Phung Q.T., Lupardus P., Tanguay J., Bumbaca S.,2014. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature. 27,515(7528), 572-6

10. Besser L.M., Gill D.P., Monsell S.E., Brenowitz W., Meranus D.H., Kukull W.,2014. Body mass index, weight change and clinical progression in mild cognitive impairment and Alzheimer disease. Alzheimer Dis. Assoc. Disord. 28, 36-43

11.Cumming S., Lloyd R.S., Oliver J.L., Eisenmann J., Malina R., 2017. Bio-banding in sport: applications to competition, talent identification, and strength and conditioning of youth athletes. Strength Cond J. 39, 34–47

12. Mariosa D., Hammar N., Malmstrom H., Ingre C., Jungner I., Ye W., 2017. Blood biomarkers of carbohydrate, lipid, and apolipoprotein metabolisms and risk of amyotrophic lateral sclerosis: a more than 20-year follow-up of the Swedish AMORIS cohort. Ann Neurol. 81, 718–28

13. Garba S.H., Adelaiye A.B., Mshelia L.Y., 2007. Histopathological and Biochemical Changes in the Rats Kidney Following Exposure to a Pyrethroid Based Mosquito Coil. Journal of Applied Sciences Research. 3, 1788-1793

14. Marinelli L., Quartarone A., Hallett M., Frazzitta G., Ghilardi M.F., 2017. The many facets of motor learning and their relevance for Parkinson's disease. Clin Neurophysiol. 128(7), 1127-1141

15. Singh S., Nag S.K., Kundu S.S., Maity S.B., 2010. Relative intake, eating pattern, nutrient digestibility, nitrogen metabolism, fermentation pattern and growth performance of lambs fed organically and inorganically produced cowpea hay-barley grain diets. TropGrassl. 44, 55-61

16. Tiwari A.K., Viswanadh V., Gowri P.M., Ali A.Z., Radhakrishnan S.V.S., 2010. Oleanolic acid, an α-glucosidase inhibitory and antihyperglycemic active compound from the fruits of Sonneratia caseolaris. Open Access JMed Aromatic Plants. 1, 19-23.

17. Tennekes H.A., 2010. The significance of the Druckrey-Küpfmüller equation for risk assessment--the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. Toxicology. 30; 276(1), 1-4.

18. Verma P., Yadav A.N., Kazy S.K.., Saxena A.K.., Suman A., 2014. Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (Triticum aestivum) growing in central zone of India. Int J Curr Microbiol. Appl Sci. 3, 432-447

19. Casida J.E., Quistad G.B., 1995. Pyrethrum flowers: production, chemistry, toxicology, and uses. New York: Oxford University Press. p. 356

20. Schoenig G.P., 1995. Mammalian Toxicology of Pyrethrum Extract. In: Pyrethrum Flowers: Production, Chemistry, Toxicology and Uses, Casida, J.E and G.B. Quistad (Eds.). Oxford University Press, New York. p. 249-257

21. Saxena D.K., Sharma S.K., Sambi S.S., 2011. Comparative extraction of cottonseed oil by n-Hexane and ethanol. ARPN J Eng Appl Sci. 6(1), 84-89

22. Bouwstra H., Smit E.B., Wattel E.M., van der Wouden J.C., Hertog C.M., Terluin B., Terwee C.B., 2019. Measurement Properties of the Barthel Index in Geriatric Rehabilitation. J Am Med Dir Assoc. 20(4), 420-425.

23. Sharma A., Halder S., Felix M., Nisaa K., Deshpande G., Prasad M., 2018. Insulin signaling modulates border cell movement in Drosophila oogenesis. Development. 145(14), dev166165.

24. Saygili E., Konukoglu D., Papila C., Akcay T., 2003. Levels of plasma vitamin E, vitamin C, TBARS, and cholesterol in male patients with colorectal tumors. Biochem Mosc. 68, 325–328.

25. Tsvetkov N., Bochev P., 1996. Free Radical–Induced Damage and Perspectives of Antioxidant Prophylaxis and Therapy. Centre for Medical Information.46- 48

26. Pavlou G., Biesaga M., Touquet B., Lagal V., Balland M., Dufour A., Hakimi M.A., Tardieux I., 2018. Toxoplasma Parasite Twisting Motion Mechanically Induces Host Cell Membrane Fission to Complete

Invasion within a Protective Vacuole. Cell Host Microbe. 11; 24(1), 81-96

27. Jellinger K.A., 2007. Morphological substrates of parkinsonism with and without dementia: A retrospective clinico-pathological study. Journal of Neural Transmission-Supplement. 72, 91-104.

28. Begum G., Vijayaraghavan S., 1999. Effect of acute exposure of the organophosphate insecticide Rogor on some biochemical aspects of Clarias batrachus (Linnaeus). Environ Res. 1999; 80(1), 80-3.

29. Fortuny A., Polo S.E., 2018. The response to DNA damage in heterochromatin domains. Chromosoma. 127(3), 291-300.

30. Kocaman G., 2009. Condition of Graduate Nursing Education in Turkey. 12th International Participation Proceedings of the National Nursing Congress. 20-24

31. Javed S., Petropoulos I.N., Alam U., Malik R.A., 2015. Treatment of painful diabetic neuropathy. Ther Adv Chronic Dis.6(1), 15-28.