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ORIGINAL ARTICLE

Exposure to Insecticide Mixture of Cypermethrin and Dichlorvos Induced Neurodegeneration by Reducing Antioxidant Capacity in Striatum

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KEYWORDS

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Chromatolysis;
Malondialdehyde

ABSTRACT: To evaluate the effect of cypermethrin (CP) and dichlorvos (2,2-dichlorovinyl dimethyl phosphate, DDVP) on the striatum of adult Wistar rats. Thirty-two animals were grouped into 4; group A (control) inhaled fresh air, and groups B, C, and D were exposed to a formulation of 5 mm^{-1} (4.4 ppm) of dichlorvos and 10 mm^{-1} (8.7 ppm) of cypermethrin insecticide for 2hrs/day, 3hrs/day and 4hrs/day respectively. We utilized the wire suspension test to demonstrate the neurobehavioral changes across the four groups of animals to identify the animal groups that have lost their motor function. Following the neurobehavioral test, the animals were weighed, anesthesized and dissected for brain tissue harvesting. Half of the brain tissue was frozen for biochemical analysis while the other part was fixed in 10% Neutral Buffered Formalin for two days and grossed to isolate the brain tissue of interest for histopathology. The results from the neurobehavioral studies show a significant decrease in motor function of the experimental groups. There was a significant elevation in the malondialdehyde and glucose levels of all the exposed groups, while their various antioxidant levels decreased significantly ($p<0.05$). Histopathological features were observed across the exposed groups ranging from the presence of vacuolated neuronal cells, neuronal cell shrinkage, and chromatolysis, which characterize the neurodegenerative effect of cypermethrin and dichlorvos on the striatum. This study indicates that a combined administration of cypermethrin and dichlorvos exerts a neurodegenerative effect on the striatum of adult Wistar rats.

INTRODUCTION

Increased cases of pyrethroid toxicity have recently been observed in animals, and few cases of poisoning have been recorded in man. Insecticides are common chemical agents used specifically for the elimination of vectors causing malaria, which is a prevailing disease that has become endemic in Africa [1]. Targeting the vectors, therefore, is among the most practical means against the malaria parasite, thereby leading to the production of more potent pesticides

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containing two or more pyrethroids, all in a bid to deal with this menace [2, 3].

A Pyrethroid is a preparation of crushed flowers called the Chrysanthemum cineraria folium, which contains the active insecticide pyrethrin. Pyrethroids are currently the most commonly used pesticides globally [4]. Ingestion, inhalation, or skin contact is the route of pyrethrin access into the body system, which rapidly metabolizes in mammals with only little accumulation; however, adverse health effects in acute poisoning may be due to chronic exposure [5]. Chronic exposure to pyrethroids in experimental animals produces several toxic effects like irritation of the nose, throat, skin allergy, asthma, and damage to the heart, lungs, liver, kidneys, and brain [6].

In China, 27% of about 2600 workers that were exposed to acute poisoning from pyrethroids, experienced severe headaches, loss of appetite, blurred vision, dizziness, paresthesia, and thoracic tightness [7]. Summarily, pyrethroids are neurotoxic to both insects and mammals (rats and humans) although the toxicity level is between low to moderate, due to inadequate absorption through the route of ingestion and rapid metabolism. However, the major toxicity of pyrethoids, which is observed via oral administration on the CNS, as claimed by Soderlund [8] is insignificant. There is an established pyrethroid association with poorer early social-emotional and language development, [9] and also motor activities [10]. The striatum is a neuronal circuit and one of the main input areas for the basal ganglia of the forebrain. It is made up of both ventral and dorsal parts, considered to be a vital component of the motor and reward systems. It is responsible for myriad aspects of cognition, action initiation, judgement, motor function, motivation, reward, and discernment. It is only safe to say therein, that lesions in the striatum will lead to certain motor and reward impairments, thus intrinsic to the investigation of pyrethroid insecticide [11-13].

Over the years, multiple experiments on the neurotoxicity of permethrin, cypermethrin, or other pyrethroid insecticides have been carried out, but no evidential knowledge on the neurotoxic impact of combined pyrethroid insecticides (as commonly used in the low economic environment), on the motor functions of the striatum, elucidating its neurodegenerative disorder.

MATERIALS AND METHODS

Chemicals

Certification of Cypermethrin and dichlorvos was done at the Industrial Chemistry Department, Nnamdi Azikiwe University, Awka (NAU) with the authentication number-AU125. The acute toxicity test of the mixture of $5mm^{-1}$ (4.4 ppm) of dichlorvos and $10mm^{-1}$ (8.7 ppm) of cypermethrin insecticide was obtained from Nnamdi Azikiwe University, Department of Biochemistry, according to the method employed by OECD [14].

Animal care and maintenance

Thirty-two (32) adult Wistar rats were procured from Ela's Animal Farm, Oko, Anambra state. Perspex cages comfortably housed a group of four (4) animals for the routine experiment. Each cage had a wire gauze top for cross ventilation. The animals were acclimatized for two weeks, weighed, and monitored under regulated room temperature, relative humidity of about 60-80%, and 12h photo-period. They were given water and fed guinea feed pallets and *Ad libitum*.

Experimental design

Following the weighing of the Wistar rats, we randomly grouped into A, B, C and D with eight animals each. All the animals received treatment following the approval of the ethical committee, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, concerning the "Guide for the Care and Use of Laboratory Animals" [15]. The acute toxicity test of Cypermethrin and Dichlorvos was done in the Department of Biochemistry, Nnamdi Azikiwe University with reference to the method employed by OECD [14]. Rats in groups B, C, and D got exposed to 5mm-1 (4.4 ppm) of dichlorvos and 10mm-1 (8.7 ppm) of cypermethrin mixture for five consecutive days with two days gap each week, throughout the four-week duration of exposure. The $5mm^{-1}$ (4.4 ppm) of dichlorvos and $10mm^{-1}$

(8.7 ppm) of cypermethrin were the LC_{50}. The exposure time increased along the groups

Control group (A) - received fresh air all through.

Group B - (Low-term exposed animals) - 2 hours/day.

Group C - (Medium-term exposed animals) - 3 hours/day.

Group D - (High-term exposed animals) - 4 hours/day.

A Dynamic inhalation system that uses whole body inhalation chambers was utilized in the exposure of the animals. The gaseous form of the insecticides was generated, the animals in each group were immersed in the test atmosphere regulated with the amount of gas required as obtained in the LC_{50} and the gaseous state of cypermethrin and dichlorvos in the air were measured for a specified length of time depending on the group exposed. This was necessary to ensure the accurate concentration of the insecticide within the experimental time as described by Cheng *et al.* [16]. The motor assessment was evaluated by a wire suspension test was. The principle of this test involved suspending the animals by their front paws on a 2mm diameter iron bar which was raised 30cm above a soft surface. The period it took the animals to lose grip and fall on the surface was calculated. Persisted reflex was deduced when the animal could remain on the bar for the 30 seconds period of suspension. The behavioral changes for the motor function were tested across the animal groups. About 24hours past the last exposure, the animals were weighed and then euthanized. The brain tissues were harvested and weighed. Some part of the brain tissues was taken for biochemical analysis while the rest were fixed for 48hrs and the tissue of interest isolated for histology.

Estimation of lipid peroxidation products

We trimmed exactly one gram per brain tissue of each animal and put in 10 ml of 0.9 % normal saline each and homogenized with homogenizer at room standard temperature. The homogenized tissue was centrifuged for 20mins at 3000rpm and the supernatant extracted and stored for biochemical analysis at a temperature of 2 degrees Celsius. Lipid peroxidation (LPO) was investigated as described by Ohkowa *et al.* [17]. 1Ml brain homogenate (10%) mixed with equal volume of cold (w/v) trichloroacetic acid (10%) was incubated for 10 minutes at 37◦C. Following room temperature centrifugation for 15minutes (2500 rpm), 1 mL of supernatant was mixed with 1mL of thiobarbituric acid (TBA) (0.67%). The mixture was then boiled for 10-15 min.

After cooling to room temperature, there was infusion of distilled water (1mL) to the mixture. 530 nm wavelength was set for the absorbance while nmol MDA/h/g tissue was the unit of expression for the results.

Reduced glutathione as antioxidant nonenzymatic assay:

Glutathione (GSH) was investigated [18]. One milliliter of 5% TCA (w/v) was put in 1 ml of 10% homogenate. Thirty minutes after observing the suspension, it was centrifuged at 2,500 rpm for 15 min. 0.5 ml of supernatant was added to 2.5 ml of 5'5'-dithionitrobenzoic acid (DTNB) and shaken vigorously. We read at 412 nm and reported the results in µmol g^{-1}.

Antioxidant enzyme assays

The activities of the antioxidant enzymes were performed using standard protocols: Superoxide dismutase (SOD) assay was according to the protocol of Kakkar *et al* [19]; Catalase (CAT) assessment was determined using the procedure described by Sinha [20]; Glutathione peroxidase (GPx) activity was calculated by the method of Rotruck *et al* [21]; Glutathione reductase (GR) was determined by the method of Carlberg and Mannervik [22]; Glutathione-s-transferase (GST) was estimated by the method of Habig *et al* [23].

Protein content

The investigation for protein content followed the protocol of Lowry *et al*. [24].

AChE assay

Acetylcholinesterase activity was estimated with the protocol earlier described [25]. Weight of the brain was determined before homogenization with phosphate buffer (0.1M, pH 8.0). A mixture of DTNB (100 µl) + phosphate

buffer (0.1M, pH 8.0, 2.6ml) in a cuvette had 0.4 ml homogenate aliquot added and thoroughly mixed through air bubbling. At 412nm, LKB spectrophotometer was used to determine the absorbance. Acetylthiocholine (20 µl) serving as the substrate was mixed with the cuvette content to document absorbance change/minute every two-minute interval for ten minutes nmol/min/g tissue was the expression unit for the results.

Glucose

Glucose was analyzed by the glucose oxidase-peroxidase (GOD-PAP) [26] method using the Trace company reagents. Absorbance rate was measured at 500nm wavelength against the blank and the standard.

Histopathology

Tissue samples remained in a 48-hour long fixation, we utilized 10% Neutral Buffered Formalin (Formaldehyde 37% 100.0ml, Sodium Phosphate Dibasic 6.5gm, Sodium Phosphate Monobasic 4.0gm, Distilled Water 900.0ml). Next, we washed under running water to remove surplus fixatives. We dehydrated in ascending grades (50%, 70%, and 95% and 100%) of ethanol. Dehydration was carried out two times in every grade with the tissue spending 60 minutes each time. We cleared in xylene and infiltrated with paraffin at 60◦C. The tissue block allowed an easy sectioning [27]. Histological procedure was in accordance with the description by [28]. We dewaxed sections in xylene for a minute and then passed through descending grades of ethyl alcohol for rehydration before washing in distilled water. Hematoxylene staining preceded by a 2-second acid alcohol (2%) differentiation was then carried out. We thereafter did counterstaining for thirty seconds using 1% aqueous eosin following regaining of the purple shade for 10 minutes in running water. Prior to DPX mounting, dehydration with increasing concentrations of ethanol and clearing with xylene was carried out. Light microscopy was utilized for photomicrography. The Cresyl echt violet staining procedure demonstrates the Nissl substance in tissues. The procedure involves; the removal of paraffin and hydration, and a 2-minute staining with 0.5% Cresyl violet. This is followed by washing in distilled water, dehydration, and xylene clearing. Nissl presence is indicated by dark blue to purple coloration.

Statistical analyses

Data analysis was done with the SPSS software (version 27.0.1). The mean and standard deviation were obtained and one-way analysis of variance (ANOVA) compared values between groups followed by post hoc Fisher's LSD multiple comparisons. Values shown are Mean ± S.D.* denotes $p < 0.05$.

RESULTS

Weight

Weight loss was significant in animals exposed to pyrethroid mixture of CP and DDVP ($p < 0.05$). The control group maintained an increasing weight gain.

Group D animals which were exposed daily for four hours, showed the least final mean of animal weight when compared to other groups.

Table 1. The impact of insecticide mixture of cypermethrin and dichlorvos on body weight

Groups	Weight (g)	Mean ± SEM	t-value	p-value
Group A	Initial	198.67±0.88	-2.874	
	Final	204.33±1.76		
Group B	Initial	198.33±0.67	13.266	0.001
	Final	183.67±0.88		
Group C	Initial	201.33±0.88	9.839	0.001
	Final	186.67±1.20		
Group D	Initial	195.33±0.88	9.177	0.001
	Final	182.00±2.00		

Data analysis utilized the Student Dependent T-test, ANOVA (one way), Statistical significant is P<0.05.

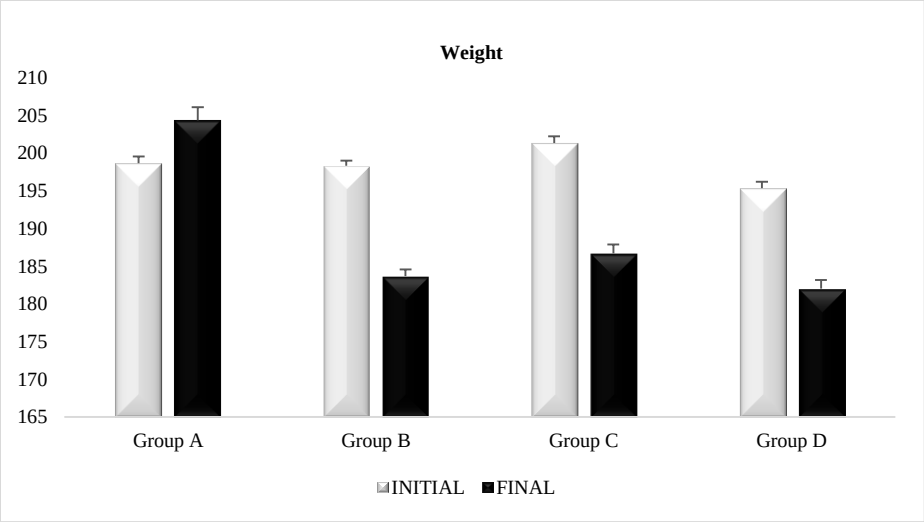

Figure 1. The impact of insecticide mixture of cypermethrin and dichlorvos on the body weight.

Locomotor capability: Wire sustenance experiment

The table compares meantime in the control group, spent by the animals hanging on a wire to that of the experimental groups. The experimental groups show a significant decrease in the duration of its sustenance on the wire ($p<0.05$).

From Figure 2, the neurobehavioural function test presents a significant decrease in the mean time spent by the exposed groups.

Table 2. Cypermethrin and dichlorvos mixture on motor function

	Groups	Mean ± SEM	P-value	F-value
	Group A	1.35±0.46		
Neurobehavioral Test (min)	Group B	1.28±0.27	0.999	0.280
	Group C	1.09±0.32	0.936	
	Group D	0.99±0.13	0.850	

Data were analyzed using one-way ANOVA followed by Post HOC Fisher's LSD multiple comparisons, and data were considered significant at $P<0.05$

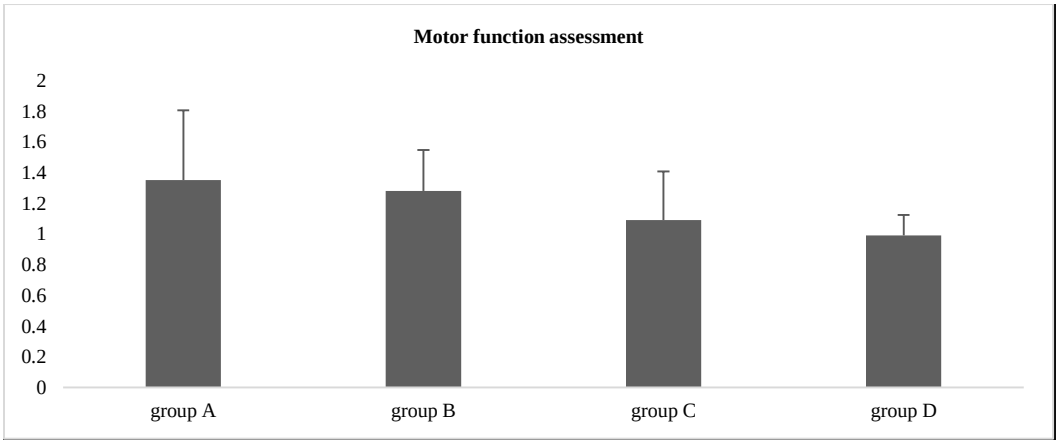

Figure 2. The effect on motor function.

Biomarkers for lipid peroxidation (MDA)

Significant increase in MDA levels ($p<0.05$) when compared with control.

Figure 3 represents malondialdehyde (MDA) levels of the various groups following exposure.

Table 3. Effect on malondialdehyde (MDA).

	Groups	Mean ± SEM	P-value	F-value
MDA (nmol mL^{-1})	Group A	3.31±0.01		
	Group B	3.59±0.01	0.000*	
	Group C	3.76±0.01	0.000*	853.833
	Group D	3.85±0.01	0.000*	

Data were analyzed using One-way ANOVA, followed by Post HOC Fisher's LSD multiple comparison, and data was considered significant at $P<0.05$.

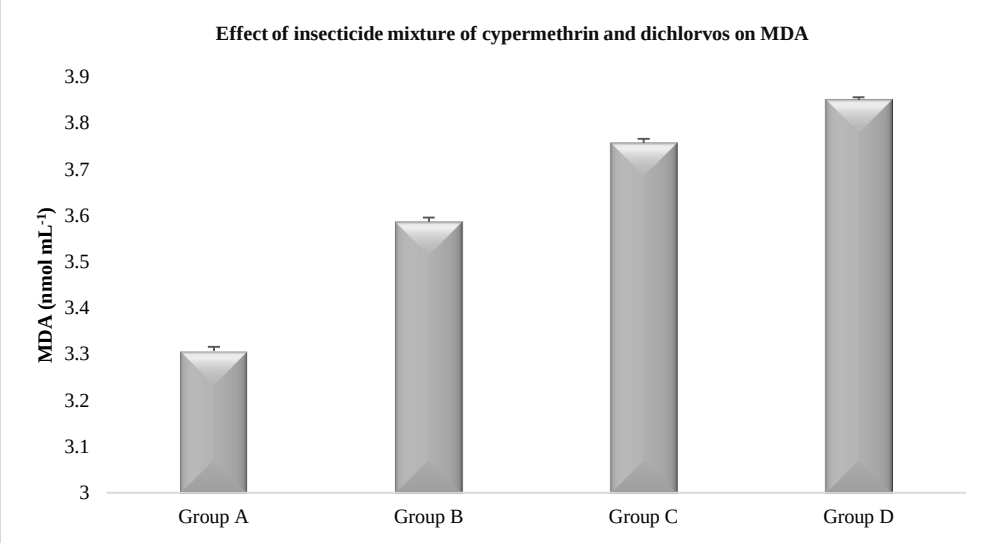

Figure 3. Malondialdehyde (MDA) levels across various groups.

Biomarkers for antioxidant (enzymatic and non-enzymatic antioxidant), protein and, AChE

The GSH level of the experimental animals was workably reduced relative to control ($p<0.05$). The GST, CAT, GPX, SOD and GR levels showed a decrement across all treated groups ($p<0.05$). In addition, the protein content was reduced in the experimental animal's acetylcholinesterase activity decreased in all exposed groups.

Table 4. GSH, CAT, SOD, GPX, GR, GST, Protein content and, AChE

	Groups	Mean ± SEM	p-value	F-value
GSH (µmol g^{-1} tissue))	Group A	1.57± 0.01		47.327
	Group B	1.53± 0.01	0.043	
	Group C	1.46± 0.01	0.000	
	Group D	1.39± 0.01	0.000	
CAT (µmol/min/mg protein)	Group A	59.75± 0.07		1079.351
	Group B	57.66± 0.06	0.000	
	Group C	56.44± 0.05	0.000	
	Group D	54.85± 0.06	0.000	
SOD (µmol/min/mg protein)	Group A	8.75± 0.05		159.708
	Group B	8.34± 0.06	0.001	
	Group C	7.46± 0.05	0.000	
	Group D	7.35± 0.04	0.000	
GPX (nmol/min/mg protein)	Group A	8.13± 0.02		258.206
	Group B	7.84±0.03	0.000	
	Group C	7.52± 0.02	0.000	
	Group D	7.22± 0.01	0.000	
GR (nmol/min/mg protein)	Group A	2.16± 0.02		77.987
	Group B	1.78± 0.01	0.000	
	Group C	1.71± 0.06	0.000	
	Group D	1.23± 0.05	0.000	
GST (µmol/min/mg protein)	Group A	0.17±0.00		10.032
	Group B	0.16±0.00	0.014	
	Group C	0.15±0.00	0.004	
	Group D	0.14±0.00	0.001	
Protein (mg ml^{-1})	Group A	4.17±0.04		336.226
	Group B	3.91±0.02	0.001	
	Group C	3.26±0.05	0.000	
	Group D	2.660.04	0.000	
AchE (nmol/min/g tissue)	Group A	4.09±0.01		122.334
	Group B	3.84±0.05	0.002	
	Group C	3.34±0.04	0.000	
	Group D	3.150.02	0.000	

Analysed using one-way ANOVA, followed by Post HOC Fisher's LSD multiple comparisons, and data were considered significant at $P<0.05$

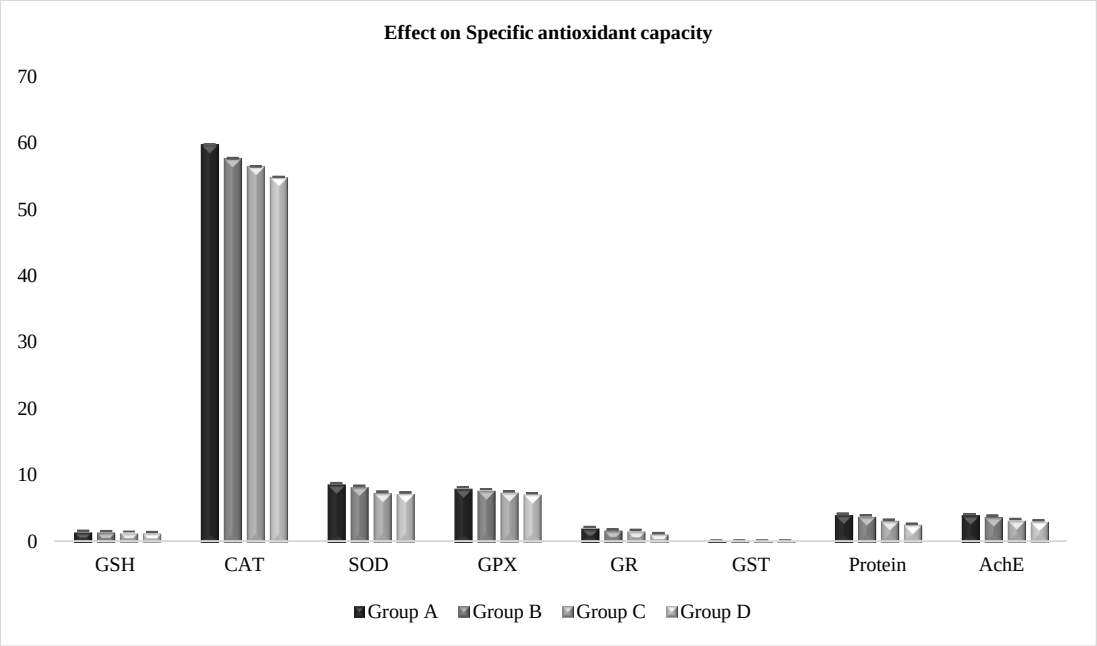

Figure 4. Specific antioxidant capacity.

The substrate for energy metabolism in the brain

In relation to Group A, group B (low term exposed show significantly higher levels of glucose ($p<0.05$) animals) shows a non-significant increase while C and D

Table 5. Effect on the brain glucose level.

	Groups	Mean ± SEM	p-value	F-value
Glucose (mg dl^{-1})	Group A	66.64±1.02		
	Group B	69.66±1.22	0.082	
	Group C	72.15±1.01	0.007	7.515
	Group D	73.28±1.03	0.002	

Values were considered significant at $p<0.05$.

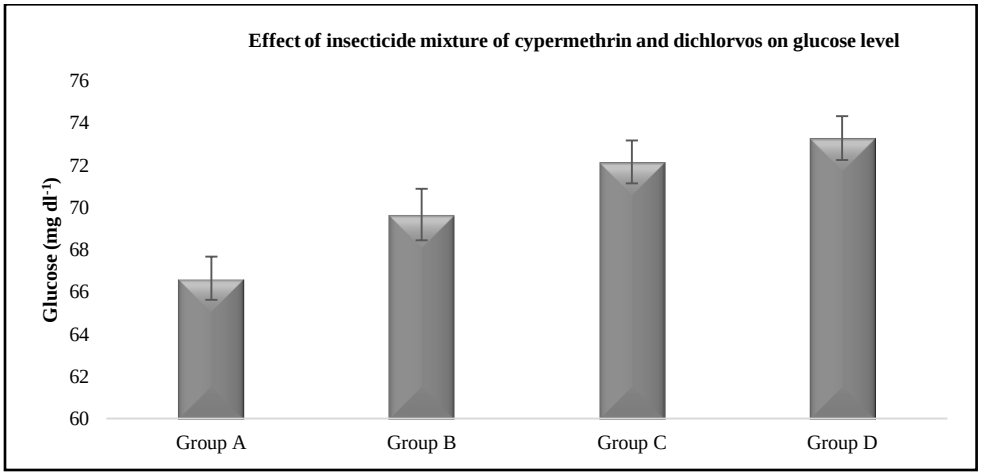

Figure 5. Brain glucose level

Histopathological analyses

In photomicrograph Figure 6, tissue presents normal cells of the nucleus accumbens arranged in clusters. The dense nucleus of the neuronal cells is elliptical and the entire tissue architecture is void of histopathological features.

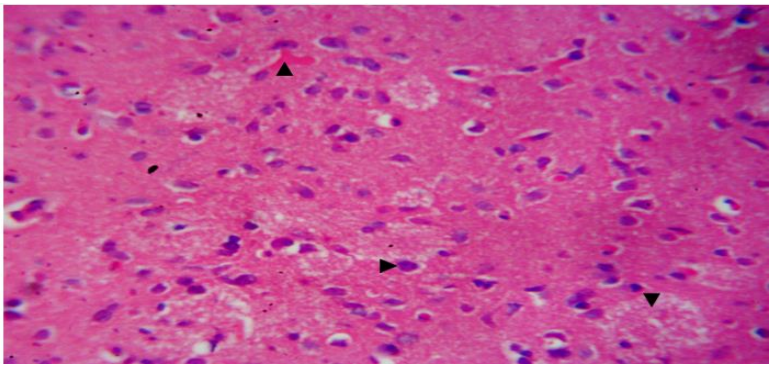

Figure 6. (Group A): A photomicrograph section of the nucleus accumbens of the striatum showing normal neuronal neurons (►), striosomes (▼), and blood vessels (▲). Stained by H&E technique (X400)

The photomicrograph Figure 7 presents mildly distorted tissue architecture of the nucleus accumbens with few histopathological features such as vacuolated neuronal cells and the presence of conspicuous pericellular spaces. The plate 2 photomicrograph represents the group B animals.

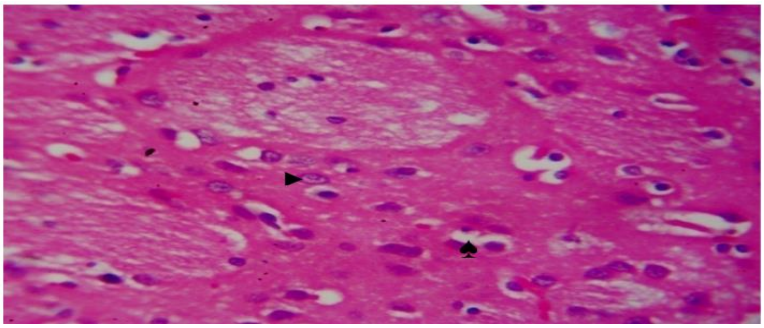

Figure 7. (Group B): A photomicrograph section of the striatum showing mildly distorted tissue architecture of the nucleus accumbens with vacuolated neuronal cells (►), presence of pericellular spaces around the cells (♠). Stained by H&E technique (X400).

The photomicrograph Figure 8 presents a mild distortion in the architectural pattern of group C animals' nucleus accumbens with degenerating (vacuolated) neuronal cells and increased conspicuous pericellular spaces.

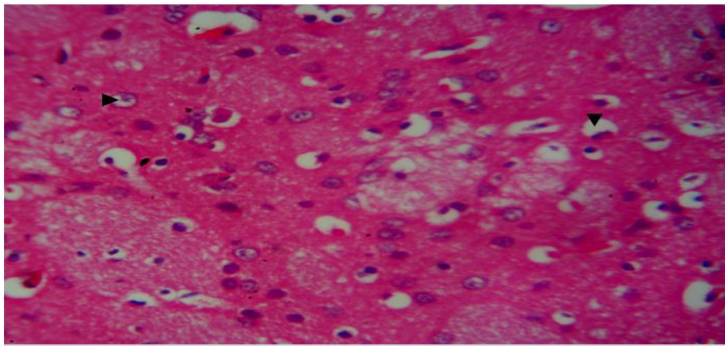

Figure 8. (Group C): A photomicrograph section of the striatum showing mildly distorted tissue architecture of the nucleus accumbens with degenerating (vacuolated) medium spiny neurons (►) and increased pericellular spaces (▼). Stained by H&E technique (X400).

The photomicrograph Figure 9 presents a severe distortion in the architectural pattern of the group D nucleus accumbens with increased conspicuous pericellular spaces as in neuronal cell atrophy, increased vacuolation and, the presence of hyperchromatic cells.

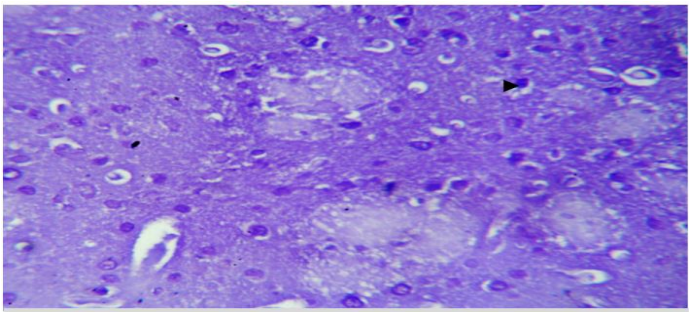

Figure 9. (Group D): Severe distortion of tissue architecture of nucleus accumbens with increased pericellular spaces as in neuronal cell atrophy (▼), increased vacuolation (►) and, hyperchromatic cell (▲). Stained by H&E technique (X400)

Striatum stained with cresyl violet

The photomicrograph Figure 10 presents the normal distribution of nissl substance in neuronal cells of the nucleus accumbens. The neuronal cells arranged in clusters show dark purple coloration, which indicates the normal presence of its substance in the neuronal cell bodies of group A nucleus accumbens.

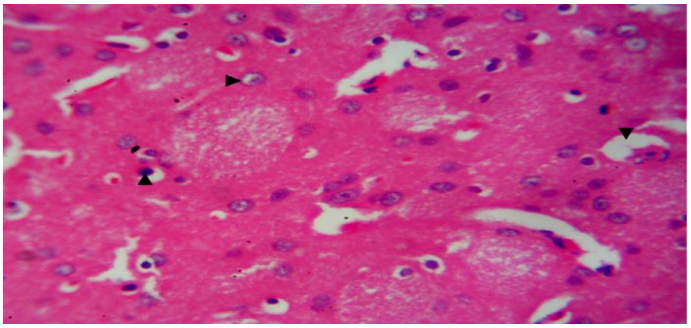

Figure 10. (Group A): Normal nucleus accumbens of striatum showing positive medium spiny neurons (►). Stained by Cresyl Violet technique (X400).

The photomicrograph Figure 11 presents a mild dissolution of the nissl substance in the nucleus accumbens of group B animals. The chromatolytic cells are seen, sparsely distributed around the nucleus accumbens.

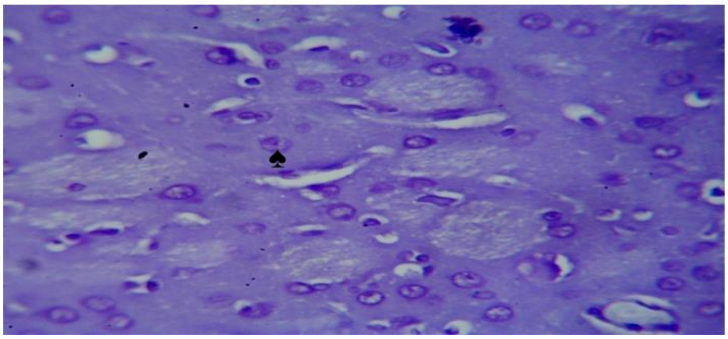

Figure 11. (Group B): A photomicrograph section of mildly distorted nucleus accumbens of striatum showing chromatolysis medium spiny neurons (♠). Cresyl Violet technique (X400)

The photomicrograph Figure 12 presents a severe disintegration of the nissl substance in the nucleus accumbens. The chromatolytic cells are sparsely distributed around the nucleus accumbens.

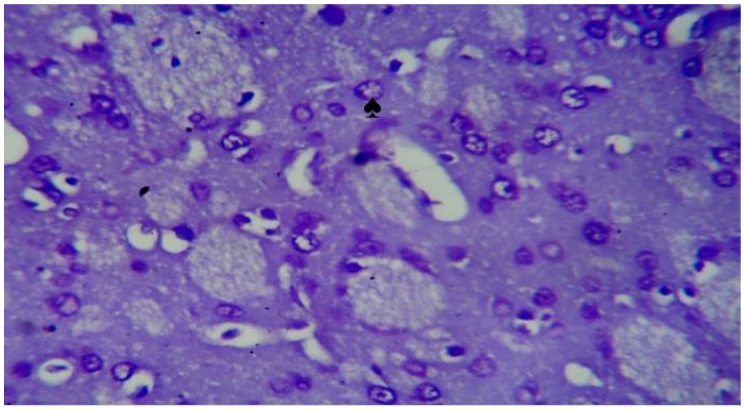

Figure 12. (Group C): A photomicrograph section of severely distorted nucleus accumbens of striatum showing barely stained chromatolysis medium spiny neurons (♠). Cresyl Violet technique (X400).

The photomicrograph Figure 13 presents a severely distorted staining pattern of its substance in the perikaryon of neurons. The chromatolytic cells are sparsely arranged in the nucleus accumbens of group D animals.

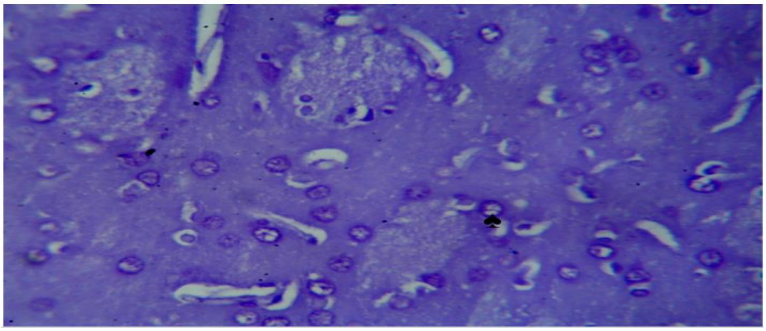

Figure 13. (Group D): A photomicrograph section of severely distorted nucleus accumbens of striatum showing sparsely stained chromatolysis medium spiny neurons (♠). Cresyl Violet technique (X400)

DISCUSSION

In cognizance of the toxic effects of insecticides on humans, animal organisms and, their environment, its application is governed by global organizations, including the WHO and, internal regulations by several countries. Pyrethroids are insecticides usually suggested for protection against insects transmitting pathogens to mammals. The use of pyrethroids is not just vast in agricultural practices but equally in medicine and veterinary practice [29-32]. The growing threat posed by Plasmodium parasites and Zicka viruses which are commonly transmitted by insect vectors has increased patronage of pyrethroids, for their importance to modern society [33-35], although the influence of this combination on the human brain is not well distinguished. However, findings from our scientific investigation present a notable decrease in the weight of exposed groups. Yadav *et al*. [36] also supported this observation where, in this case, subchronic exposure to pyrethroid-based mosquito vapouriser fumes adversely affected the body weight of exposed animal groups. The notable decline in body weight suggests that inhalation of pyrethroid-based insecticide has general toxic effects on the entire body system.

The effects of pyrethroids on motor activity have grown to be a matter of interest to neurotoxicologists and researchers. In the neurobehavioural test conducted by our team of researchers, we obtained a non-significant, progressive decline in movement abilities of experimental groups. This

non-significant motor deficit recorded in our investigation could be ascribed to the brain tissue's ability to utilize the remnant cells and sustain the brain function by increasing brain glucose level in one-month exposure to such toxicity as recorded by Udodi *et al.* [10] Some other findings recorded a visible motor symptom like reduced limb grip strengths with heightened tremor following a dose-dependent pattern when orally exposed to cypermethrin [37]. Another observation by our researchers was that the insignificant motor skill reduction in time spent on the suspension wire was dose-dependent as previously recorded in a report. Time spent on suspension with the hanging pole reduces as time of exposure increases. The implication of this is that rats exposed for a long period, in this case, group D stayed for the least interval on the hanging pole. Next are rats exposed for a medium-term (group C), followed by rats exposed a short term (group B). it was proposed that toxicity is typically time-dependent and this implies that greater effects are produced as time of exposure to toxic agents increase. [38, 39].

Oxidative stress is involved in pyrethroid-facilitated neurotoxicity. A disproportionate generation of reactive oxygen species (ROS) and reactive nitrogen species in pyrethroid exposed tissues are principal contributors to oxidative stress. Addition is a lowering in constituents of the antioxidant organization. Intraperitoneal or oral intake of cypermethrin brings about oxidative stress in the nervous system [40, 41]. Oxidative impairment basically presents by a generation of reactive oxygen species which leads to a breakdown of lipids, proteins and, DNA. It is then proper to attribute the reduced enzymatic activity and its structural organization as resulting factors of oxidative damage [42]. Pyrethroids exert their debilitating effects through alteration of oxidative stress in target organs [43]. In a recent assessment, a critical and time-propelled reduction in GSH content in pyrethroid exposed animals, was observed [44]; this corresponds to the discovery with our treated animals. A second line of defense is the non-enzymatic radical scavenger GSH which traps free radicals created from oxidative metabolism and escaping breakdown by the antioxidant enzymes [45]. During GSH metabolic activities, its sulfhydryl group forms a disulfide compound, GSSG (oxidized form) by the process of oxidation [46] in a time-dependent manner. Due to free radicals produced by insecticidal compounds, there could be a decrease in GSH levels. Other authors [47, 48] have previously studied these effects in vitro and in vivo. Additionally, GSH actively partakes in the clearing of xenobiotic materials as a base for the enzyme- GST. This enzyme GST have purifying abilities which activate the process of conjugation in various electrophilic substrates to the thiol group of GSH, which produces less harmful forms; and also lowers lipid peroxides [49]. Oxidative stress and genotoxicity could be a resulting factor of the exposure of mammals to Lambda-cyhalothrin, a type II pyrethroid, [50, 51]. The decreased GSH contents in lambda-cyhalothrin insecticide-treated rat tissues could be from a reduction in the activity of GST. In addition, GST inhibition has been documented to occur under other oxidative stress conditions [49-52]. The notable arrests in GST activity in pyrethroid-exposed tissues indicate deficiency in electrophilic conjugation and subsequent detoxification. The reduction in GST activity can also be attested to a lowering in glutathione content in various organs, in this case, the brain. And also cardinal defense actions against reactive effects of oxygen metabolism are SOD and CAT [44]. SOD induces the changing of superoxide radicals to hydrogen peroxide, while CAT promotes the decomposition of hydrogen peroxide into water. These antioxidant enzymes in turn, alleviate the debilitating effects of reactive oxygen species (ROS) [49]. The decline of SOD levels as described in our experiment, corroborated with earlier findings which demonstrated that animals treated with pyrethroids were observed to have a decreased brain activity level for SOD [53]. The evident reduction in CAT activity can be linked to the stream of superoxide radicals [54]. We equally observed from this study that, the levels of AChE were reduced in the exposed groups. From previous studies, Pyrethroids are observed to have been observed to establish a decrease in AChE erythrocytes and the brains of several organisms [55]. The decreased AChE might be a result of increased lipid peroxidation. Inhibition of acetylcholinestrase (AChE) activity reduces cellular activity, influnces distortions of cellular membrane, and interferes with body metabolism and nervous function [56]. The decline in acetylcholinesterase (AChE) activity causes a reflux of ions and differential permeability of the membrane [57].

Shaw and Panigrahi [58] in their assessment equally suggested the deactivation of the AChE enzyme due to an attachment of pollutants (insecticides) to its binding regions. Post-synaptic receptors which are part of the active sites of AChE are made up of protein molecules. Another assessment implies that the speed by which protein is synthesized was reduced in a concentration-dependent pattern, following insecticide exposure [59]. This also supports our experiment owing to the notable drop in protein levels across the exposed groups. This decrease is an indication of excessive tissue protein breakdown [60]. GPx is a protein enzyme that also plays a major role in minimizing oxidative damage, it was investigated by Afolabi *et al.* [61] and was observed in line with our findings, to reduce significantly when exposed to pyrethroid. GR which was significantly reduced in our research is another antioxidant enzyme which sped up the reduction of glutathione disulfide (GSSG) to glutathione (GSH) and plays a role in protecting cells from oxidative stress [62].

Malondialdehyde (MDA) is one of the final products of lipid peroxidation [63]. In the process of lipid peroxidation, highly reactive products such as malonaldehyde (MDA) and 4-hydroxynonenal are produced [64]. Lipid peroxidation modifies the structures of cell membrane and results to damage –this is a pointer to intracellular oxidative stress [65]. In corroboration with Afolabi *et al.* [61], cypermethrin exposure significantly elevated the levels of MDA in the exposed groups.

Elevation in brain glucose levels was seen across the exposed animal groups. Hyperglycaemia, though not specific, is quick feedback of the effects of the toxic doses of type II pyrethroids [66]. Over excitation of the nervous system magnifies glucose needs and distorts most organ function, leading to a disorderly energy homeostasis. This could modify glucose and lipid metabolisms [67]. Reports have shown a reduced brain glycogen content with increased activity of glycogen phosphorylase by organophosphorus insecticide and dichlorvos [68] thereby making available more glucose to negate the complete shutdown of brain function in short duration exposure to toxicity [10]. Some studies on humans and animals have found that exposure to pyrethroids can distort glucose and lipid metabolisms which could be a risk factor for type II diabetes as glucose homeostatic change is expected to sustain brain function [69,70].

The histopathological findings revealed variable degrees of lesions and distortions of the tissue architecture in the striatum of the exposed groups. A one month xposure to pyrethroids induced certain morphologic alterations in brain tissues, as well as biochemical and functional changes [10]. Dose-dependent striatal lesions evident by the presence of pericellular spaces, vacuolated neuronal cells, chromatolysis cells and, other pathological features were seen in the research. Pericellular spaces were a result of autophagy, vacuolated neuronal cells occurred due to cellular degeneration leading to loss of cellular contents while chromatolysis was a resultant effect of nissl substance destruction in the neuronal cell body which characterizes Huntington's disease [71]. The striatal cell loss typically involves the medium-sized calbindin positive GABAergic efferent neurons that are extending to the globus pallidus and substantia nigra as well as striatal interneurons [72]. The pathological effects demonstrated in this experiment were time-dependent which suggests severe pathological presentation in longer exposure to pyrethroid-based insecticide mixture of cypermethrin and dichlorvos [73]. However, other supposed action mechanism of pyrethroids on the striatum was not established thus, further studies to demonstrate the ultra-structural features of the striatum exposed to pyrethroid insecticide could help to identify other mechanisms of action of this toxicant which will specifically unveil effective therapeutic windows for mitigation of pyrethroid insecticide induced motor deficit.

CONCLUSIONS

From the final results stipulated in this work, it can be indicated that exposure to an insecticide mixture of dichlorvos and, cypermethrin exerts morphologic, functional and biochemical modifications in the striatum of exposed adult Wistar rats. Some of these presentations are common in certain degenerative conditions of the nervous system such as Parkinson's and Huntington's diseases among residents of low

socioeconomic environment, fond of using pyrethroid-based insecticide mixture as a potent insect repellant.

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ETHICAL CONSIDERATION

Ethical approval was sought and received from the ethical committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University with the ethical approval number; FBMS/EA/1011

Conflict of Interest

The authors unanimously declare that this manuscript has no conflicting interest nor there is any rancor among the authors.

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