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Original Article

Alzheimer's- and Multiple Sclerosis-Like Features on Key Brain Centres of Wistar Rats Exposed to Cypermethrin and Dichlorvos

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ABSTRACT

Insecticides are suspected environmental factors in the aetiology of Alzheimer's and multiple sclerosis diseases. Several studies have reported long-term exposure to insecticides in the development of plaques, neurofibrillary tangles, and demyelination. However, there is no literature on the impact of short-term combined insecticide exposure on specific neurological conditions. This investigation assessed the role of short-term exposure to the mixture of cypermethrin and dichlorvos on Alzheimer's- and multiple sclerosis-like features. Thirty-two male adult Wistar rats were divided into four groups: the control group was exposed to normal atmospheric air, and the test groups were exposed to a combined cypermethrin and dichlorvos at 2, 3, and 4 h/day [LC_{50} of 5 mm^{-1} (4.4 ppm) of dichlorvos and 10 mm^{-1} (8.7 ppm) of cypermethrin] for four weeks. In the last three days of exposure, neurobehavioural tests were conducted. Twenty-four hours after the last exposure, the animals were anesthetized using chloroform vapour. The brains were harvested by making an occipitofrontal incision for biochemical and histological assessment. The fixation was done in 10% neutral buffered formalin for 48 h following the brain mapping to isolate the brain tissue of interest. The insecticide-exposed rats showed significant ($p<=0.05$) progressive behavioural deficits and oxidative stress when compared to the control group. Neurofibrillary tangles, senile plaques, and demyelination of the neurons were observed in the brains of the insecticide-exposed rats. This study indicates that the mixture of cypermethrin and dichlorvos can induce the development of Alzheimer's and multiple sclerosis within a short time.

Keywords
Cypermethrin, Dichlorvos, Demyelination, Neurofibrillary Tangles, Senile Plaques

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INTRODUCTION

Cypermethrin and dichlorvos are parts of pyrethroid recommended in some African countries for curbing mosquito species that transmit pathogenic microorganisms to humans and animals (Costa 2015). These chemicals are widely used in different agricultural establishments, forestry, and households where the lives of humans and animals are at risk (Costa 2015; Hołyńska-Iwan and Szewczyk-Golec 2020). There is an increase in the use of pyrethroid insecticides among residents of low socioeconomic environments because of their uniqueness in combating mosquito spread (Mulla et al. 2001; Tusting et al. 2017). Cypermethrin acts by modifying the voltage-gated sodium channels, thereby delaying their closure (Hassouna 2020). Oxidative stress is implicated in cypermethrin-mediated neurotoxicity (Paravani et al. 2019; Udodi et al. 2022, 2023), which results in DNA damage by reducing mitotic and nuclear divisions (Kocaman and Topaktas 2009; Paravani et al. 2019). Potassium current is another target of cypermethrin, causing neurotoxic effects in many neurons by altering the activity of delayed-rectifier voltage-dependent potassium channels and potassium ion transport across synaptosomes (Tian et al. 2009). Dichlorvos also exerts its toxic effect by irreversibly inhibiting neural acetyl cholinesterase. The inhibition provokes the accumulation of acetylcholine in synapses, disrupting nerve function and ultimately resulting in persistent muscle contractions (Wang et al. 2004). The effective control of malaria vectors is weakened by the resistance of individual insecticides (Wondji et al. 2012; Suh et al. 2023), leading to the use of more than one type at a time. This explains

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why many African countries employ insecticide mixtures for effective control of malaria vectors.

Lifelong exposure to insecticides is implicated in Alzheimer's disease (AD) and multiple sclerosis (MS) (Yan et al. 2016; Graves et al. 2017; Mar et al. 2018). Although the aetiology of AD and MS is still to some extent unknown, it is believed to be multifactorial, ranging from genetic to environmental factors (Yaffe et al. 2000; Sadovnick 2019; Breijyeh and Karaman 2020). These conditions are characterized by an insidious onset of cognitive decline (Ringman 2015), poor emotional interpretations of the environment (da Silva et al. 2021), and motor deficits (Buchman and Bennett 2011; Tasaki et al. 2019), which ultimately lead to dysfunction in daily life and work abilities. Amyloid plaques and neurofibrillary tangles are major pathological features of AD (Palop and Mucke 2010; Apostolova 2015), while demyelination, axonal damage, and subsequent neurological dysfunctions following the formation of multiple plaques are major pathological features of MS (Huang et al. 2017). Amyloid plaques are forms of protein aggregates derived from the dimers and oligomers of amyloid protein, while neurofibrillary tangles are made up of compact helical filaments of hyperphosphorylated tau protein (Maccioni et al. 2001; Nizynski et al. 2017). However, both neuropathological changes are involved in the loss of synapses, signalling, and neuronal cell death, which leads to several functional deficits in the brain (Apostolova 2015; Wang 2015).

Several studies have implicated long-term cumulative exposure to insecticides in the development of several neurological conditions (Cannon and Greenamyre 2011). However, it is unknown what the impact of short-term exposure to a mixture of more than one insecticide is on specific neurological conditions. Thus, this research demonstrates Alzheimer's- and multiple sclerosis-like features in key brain centres of animals exposed to an insecticide mixture of cypermethrin and dichlorvos within four weeks.

MATERIALS AND METHODS

Pre-treatment of the Animals

A total of 32 sixty day-old male Wistar rats were used for this scientific investigation. The animals were made to acclimate to the new environment for a period of 14 days. The animals were monitored under a controlled room temperature of about 25–28 ℃, a relative humidity of about 60–80%, and a photoperiodicity of 12 h day and night. The animals were given purified water and fed guinea feed pellets *ad libitum.*

Chemical Procurement

Cypermethrin and dichlorvos insecticides were procured prepared and authenticated at the Department of Industrial Chemistry, Nnamdi Azikiwe University Awka, with the authentication number AU134.

Acute Toxicity Test (LC_{50}) of a Mixture of Cypermethrin and Dichlorvos

The liquid concentrations of 5% dichlorvos and 10% cypermethrin were generated into gaseous concentrations of 5 mm-1 (4.4 ppm) for dichlorvos, and 10 mm-1 (8.7 ppm) for cypermethrin at the Department of Biochemistry, Nnamdi Azikiwe University. This was done according to the method of the Organisation for Economic Co-operation and Development (2018).

Animal Groups and Experimental Procedures

The animals were randomly divided into four groups of eight animals each. The groups were denoted as Control, and Cypermethrin and Dichlorvos (C&D) for 2 h/day, 3 h/day, and 4 h/day (Table 1). All the animals were treated following the approval of the ethical committee with the number FBMS/EA/1015, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, in line with the "Guide for the Care and Use of Laboratory Animals" (NRC 2011). Rats in groups C&D 2 h/day, C&D 3 h/day, and C&D 4 h/day were exposed to an insecticide mixture of 5 mm-1 (4.4 ppm) for dichlorvos and 10 mm-1 (8.7 ppm) for cypermethrin for four weeks.

Table 1: The experimental design and the animal groups

Groups	Exposure	Number of rats	Dosage	References	Duration (weeks)
Control	Normal air	8			
C&D h/day	Gaseous cypermethrin and dichlorvos	8	2 h/day	(Udodi et al. 2023)	4
C&D h/day	Gaseous cypermethrin and dichlorvos	8	3 h/day	(Udodi et al. 2023)	4
C&D h/day	Gaseous cypermethrin and dichlorvos	8	4 h/day	(Udodi et al. 2023).	4

The Exposure Procedure

A dynamic inhalation exposure system was utilized as a whole-body inhalation chamber that allowed the rats to spend time inside a structured chamber. The rats' skin was entirely wrapped to prevent dermal absorption of pyrethroid, which is known to be minimal. The gaseous form of the insecticides was generated using two gas heater pumps for the two chemicals. The rats in each group were immersed in a regulated test atmosphere with the required amount of gas as obtained in the LC50. The levels of cypermethrin and dichlorvos in the air and the required time of exposure were maintained for each group as described by Cheng et al. (2010).

Neurobehavioural Test

Morris Water Maze Test

Spatial learning and memory were assessed using the Morris water maze on the last two days of exposure, and

24 h after exposure (three days). The procedure was performed as described by Joca et al. (2014). The apparatus consisted of a large circular pool (tank) about 15 cm in diameter and about 8 cm deep. The inside of the tank was painted white, and the outside was brown. It was filled up with tap water at a temperature of about 25 ℃ under bright lighting. The maze was divided into four virtual quadrants, and a platform 24 cm high and 10 cm in diameter was placed in one quadrant of the pool. During training (day 27), the platform was exposed 3 cm above the water. Rats that were unable to locate the platform during training were led there by the experimenter and allowed to remain on the platform for 3 s. The platform was submerged in the quadrant where it was during the training session (day 28), and the animals were retrained to locate the submerged platform hidden in one of the four quadrants. Each test session involved three 60 s trials with an approximately 10 s inter-trial interval. During the test session (day 29), the escape platform was placed 8 cm below the water, made opaque by adding non-toxic white tempera paint. The animal that failed to climb onto the platform within 60 s were guided onto the platform. The rats then remained on the platform for 10 s before beginning the new trial. At the end of the three trials, the animals were removed and placed under a lamp for warmth. The inter-session interval was an hour. A stopwatch was used to manually record the escape latency.

Elevated Plus Maze Test

The elevated plus maze (EPM) test assessed anxiety-related behaviour in the rats on day 27 of exposure. The EPM apparatus consists of a plus-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a centre area. This was a five-minute test that allowed the rats to freely explore the maze; their behaviours were recorded by means of a video camera mounted above the maze and analysed using a video tracking system. The entry in and time spent in the open arms were regarded as direct measures of anxiety (Frye 2007; Walf and Frye 2007).

Wire Suspension Test

The motor activities were assessed using the wire suspension test protocol on day 28 of exposure. The rat's forepaws were suspended on a two-millimetre diameter metal bar that was 30 cm above a soft, flat surface. The longest time until the rats lost their grip and fell on the soft surface in three consecutive trials was measured and used to assess their motor function. Complete acquisition of the reflex was assumed when the rat was able to hang on the bar for 30 s (Moran et al. 1995).

Animal Sacrifice

There was no mortality in the course of the study. Twenty-four hours after the last exposure, the animals were anaesthetized with chloroform vapour. The brains were harvested by making an occipito-frontal incision, and fixed in 10% neutral buffered formalin for 48 h. This was followed by brain mapping to isolate the brain tissues of interest for histological investigations.

Biochemical Analyses

The brain tissues were analysed for oxidative stress markers namely; malondialdehyde (MDA) for lipid peroxidation and superoxide dismutase (SOD) and reduced glutathione for antioxidants (GSH) at the Department of Biochemistry, Nnamdi Azikiwe University, Nnewi Campus. One gram of each animal brain tissue was added to 10 mL of 0.9% normal saline and homogenized at room temperature. After this, each of the samples was centrifuged at 3,000 rpm for 20 min at room temperature. The supernatants were separated and stored for further analyses.

Tissue Processing

The brain tissues were processed for histological staining following the protocol by Feldman and Wolfe (2014).

Bielschowsky's Silver Stain

This was utilized to assess the nerve fibres, neurofibrillary tangles, and senile plaque in brain tissues (Standring 2018). The procedure adopted was according to Intorcia et al. (2019) protocol for demonstration of nerve fibres, neurofibrillary tangles, and senile plaques with little or no artifactual staining by pre-treatment with oxidizing agents. The nerve fibres, neurofibrillary tangles, and senile plaque appeared dark brown or black in colour.

MBP and Olig2 staining method

Sections measuring 20 µm in thickness were cut onto coated slides, deparaffinised, and rehydrated. A citrate-based antigen retrieval solution (pH 6.0) was utilized for antigen retrieval, which lasted for 30 min. Sections were then treated using mouse and rabbit HRP/DAB IHC detection kits. Endogenous peroxidase blocking (10 min) was performed. This was followed by incubation with the primary antibodies, viz., mouse anti-MBP and anti-Olig2 (1:5,000 dilutions; 2 h) for separate tissues. Sections were then incubated for 30 min in horse radish peroxidase (HRP) micro-polymer goat anti-rabbit HRP (Abcam USA). The reaction was developed with DAB chromogen (Abcam, USA). Sections were rinsed in water and counterstained with Mayer's haematoxylin. This was followed by dehydration, clearing, and eventual mounting with dibutyl phthalate xylene (Dako).

RESULTS

The Effect of Insecticide Mixture of Cypermethrin and Dichlorvos on the Rats' Behaviours

Table 2 represents the behavioural activities of the animals. The mean time spent by the rats to locate the submerged platform in the Morris water maze significantly ($p < 0.05$) increased in groups C&D (3 h/day) and C&D (4 h/day), with a statistical non-significant ($p = 0.341$) increase in group C&D (2 h/day) when compared to the control.

The mean time spent by the rats in the open arm of the elevated plus maze in groups C&D 3 h/day and C&D 4 h/day significantly ($p < 0.05$) increased, while group C&D 2

h/day present statistical non-significant (p = 0.169) increase when compared to the control.
The time spent by the rats suspended on the wire in three consecutive trials of the wire suspension test for motor assessment was a statistical non-significant (p > 0.05) decrease in their means when compared to the control group.

Table 2: The effect of insecticide mixture of cypermethrin and dichlorvos on the rats behaviours

	Groups	Mean ± SEM	p-value	F-value
Morris water maze test- escape latency (seconds)	Control	16.00 ± 2.93		6.369
	C&D 2 h/day	31.83 ±4.54	0.341	
	C&D 3 h/day	42.33 ± 3.84	0.044*	
	C&D 4 h/day	54.83 ± 11.23	0.002*	
Elevated plus maze test- open arm (seconds)	Control	29.37 ± 8.07		12.411
	C&D 2 h/day	57.15 ± 9.09	0.169	
	C&D 3 h/day	77.60 ± 4.54	0.033*	
	C&D 4 h/day	88.21 ± 6.94	0.016*	
Wire suspension test (seconds)	Control	135.78 ± 30.45		0.293
	C&D 2 h/day	128.78 ± 32.64	0.998	
	C&D 3 h/day	117.78 ± 42.63	0.974	
	C&D 4 h/day	97.37 ± 5.05	0.817	

Data were analysed using One-way ANOVA, followed by post hoc Turkey Multiple Comparison test, and data was considered significant at P<0.05

The Effect of Insecticide Mixture of Cypermethrin and Dichlorvos on Oxidative Stress
Table 3 presents the oxidative stress markers of the rats exposed to a mixture of cypermethrin and dichlorvos. MDA showed a significant (p < 0.05) increase across the experimental groups, while the decreases in GSH and SOD were significant (p < 0.05) except the GSH for the C&D 2 h/day group, which showed a non-significant (p = 0.890) decrease.

The Effect of Insecticide Mixture on Bielschowsky's Silver Expression on the Amygdala
The micrographs in Figure 1 present the deviation of normal axonal fibres in the amygdala region of the brain. The test groups exposed to C&D mixture for 2 h/day, 3 h/day, and 4 h/day, present neurofibrillary tangles and senile plaques in a graded sequence when compared to the control group that presents normal dendritic fibres and axonal fibres. The C&D (2 h/day) group presents an early stage of tau fibrilization, while groups C&D 3 h/day and 4 h/day present neurofibrillary tangles (curly fibres) and deposits of senile plaques.

Table 3: The effect of insecticide mixture of cypermethrin and dichlorvos on oxidative stress

	Groups	Mean ± SEM	p-value	F-value
MDA (mm^{-1})	Control	3.22 ± 0.01		44.160
	C&D 2 h/day	3.47 ± 0.01	0.003*	
	C&D 3 h/day	3.66 ± 0.02	0.000*	
	C&D 4 h/day	3.72 ± 0.06	0.000*	
GSH (mm^{-1})	Control	1.46 ± 0.02		14.246
	C&D 2 h/day	1.44 ± 0.01	0.890	
	C&D 3 h/day	1.34 ± 0.03	0.031*	
	C&D 4 h/day	1.27 ± 0.02	0.002*	
SOD (mm^{-1})	Control	8.66 ± 0.02		1336.758
	C&D 2 h/day	8.25 ± 0.02	0.000*	
	C&D 3 h/day	7.32 ± 0.01	0.000*	
	C&D 4 h/day	7.24 ± 0.03	0.000*	

Data were analysed using One-way ANOVA, followed by post hoc Turkey Multiple Comparison test, and data was considered significant at P<0.05

The Effect of Insecticide Mixture on Bielschowsky's Silver Expression on the Hippocampus (CA3)
The micrographs in Figure 2 present the hippocampal CA3 region of the brain. The test groups exposed to C&D mixture for 2 h/day, 3 h/day, and 4 h/day present increasing neurofibrillary tangles and senile plaques in a graded sequence when compared to the control group that presents normal dendritic fibres and axonal fibres.

The Effect of Insecticide Mixture on BS Expression of the Substantia Nigra (pars compacta)
The micrographs in Figure 3 present the pars compacta of the substantia nigra. The test groups exposed to C&D mixture for 2 h/day, 3 h/day, and 4 h/day presents increased neurofibrillary fibres and senile plaques according to its insecticide exposure time when compared to the control group that presents normal dendritic fibres and axonal fibres.

Myelin Basic Protein (MBP) Staining Technique
Table 4 and Figures 4–6 present the expression of myelin basic protein in the amygdala, hippocampus, and substantia nigra of Wistar rats exposed to the C&D mixture. While there was an exposure time-dependent decrease in the expression of myelin basic proteins in the test groups, the variations were significant (p < 0.05) in the amygdala of the

3 h/day and 4 h/day groups and also significant in the hippocampus of the 4 h/day group, but were not significant in the other remaining test groups assessed.

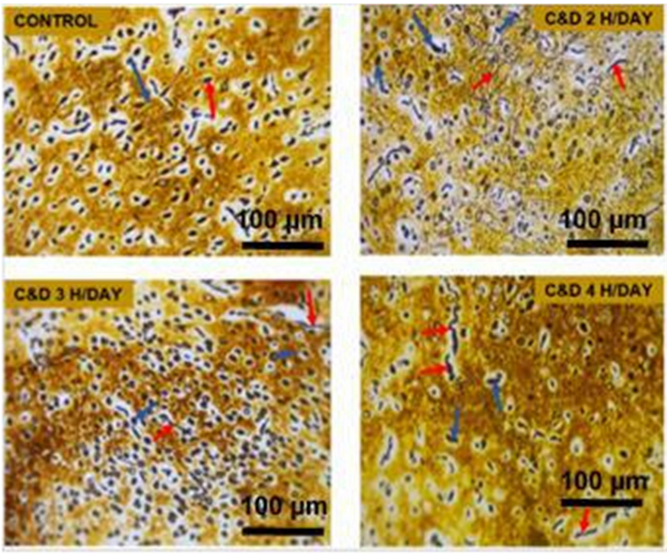

Fig. 1: Medial amygdala Bielschowsky's silver (BS) stain. Control group shows normal dendritic and axonal fibres indicated with red and blue arrows respectively, while the C&D 2 h/day group showed tau fibrilizing fibres and few senile plaques identified with red and blue arrows respectively. The C&D 3 h/day and C&D 4 h/day groups present established neurofibrillary fibres and senile plaques identified with red and blue arrows respectively. BS, ×400

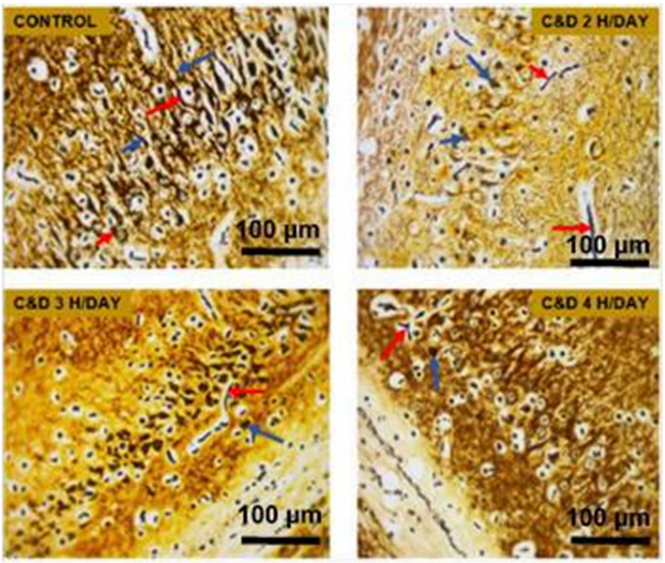

Fig. 2: Hippocampal CA3 region Bielschowsky's silver (BS) stain. Control group shows normal dendritic fibres and axonal fibres indicated with red and blue arrows respectively, while the C&D groups for 2 h/day, 3 h/day and 4 h/day present neurofibrillary fibres and senile plaques identified with red and blue arrows respectively. BS, ×400

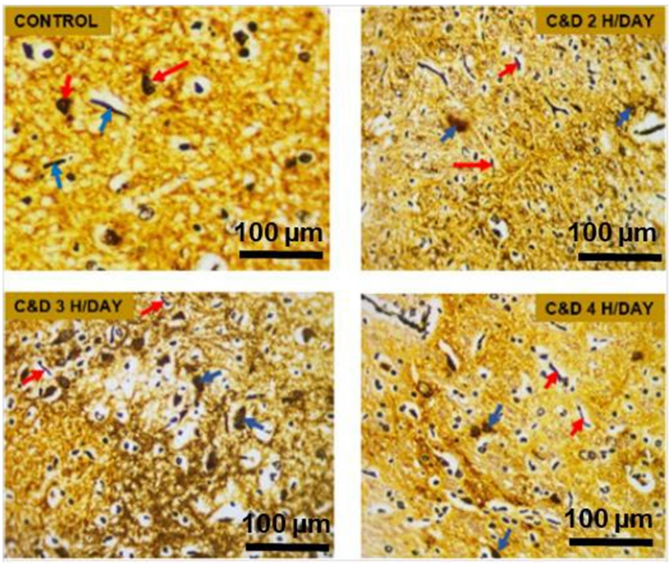

Fig. 3: Pars compacta of the substantia nigra Bielschowsky's silver (BS) stain. Control group shows normal dendritic fibres and nigrostriatal tract indicated with red and blue arrows respectively, while the C&D groups for 2 h/day, 3 h/day and 4 h/day present neurofibrillary fibres and senile plaques identified with red and blue arrows respectively. BS, ×400

Table 4: The effect of insecticide mixture of cypermethrin and dichlorvos on myelin basic protein expression

	Groups	Mean ± SEM	p-value	F-value
Amygdala	Control	6.52 ± 0.88		9.489
	C&D 2 h/day	3.76 ± 0.70	0.162	
	C&D 3 h/day	2.48 ± 0.71	0.023*	
	C&D 4 h/day	1.90 ± 0.26	0.008*	
Hippocampus (CA3)	Control	24.17 ± 1.06		6.751
	C&D 2 h/day	23.28 ±0.94	0.986	
	C&D 3 h/day	18.84 ±1.78	0.150	
	C&D 4 h/day	18.07 ± 1.01	0.006*	
Substantia Nigra	Control	50.89 ± 1.74		2.472
	C&D 2 h/day	48.03 ± 2.88	0.941	
	C&D 3 h/day	44.26 ± 2.12	0.132	
	C&D 4 h/day	40.48 ± 4.84	0.313	

Data were analysed using one-way ANOVA, followed by post hoc Turkey Multiple Comparison test, and data was considered significant at P<0.05

Oligodendrocyte Transcription Factor 2 (Olig 2) Staining Technique

Table 5 and its micrographs in Figure 7-9 present the expression of oligodendrocyte transcription factor 2 pro-

tein, which represents the volume of oligodendrocytes in the amygdala, hippocampus, and substantia nigra. While there were time-dependent decrease in the test groups across the three regions of the brain assessed, only the amygdala 3 h/day and 4 h/day groups and hippocampus 2 h/day, 3 h/day and 4 h/day groups were statistically significant. The amygdala 2 h/day group and the substantia nigra 2 h/day, 3 h/day and 4 h/day groups were non-significant.

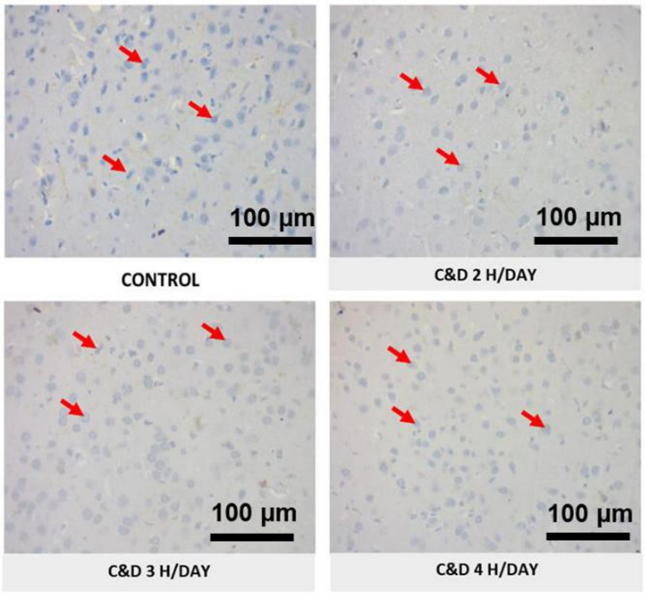

Fig. 4: Medial amygdala myelin basic protein (MBP) expression. The control group shows normal immunoreactivity and expression of myelin basic protein, while the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of myelin basic proteins in medial amygdala. MBP, ×100

DISCUSSION

Several studies have reported long-term exposure to insecticides in the development of plaques, neurofibrillary tangles, and demyelination. However, there is no literature on the impact of short-term combined insecticide exposure on specific neurological conditions. The results obtained in this study indicates that the mixture of more than one insecticide, cypermethrin and dichlorvos, extrapolated to the environmental human exposure levels, induced AD- and MS-like features in short-term exposure with the presentation of demyelination associated with amyloid plaques and neurofibrillary tangles in the amygdala, hippocampus, and substantia nigra of adult Wistar rats. Udodi et al. (2022 and 2023) established the short-term general toxic effect of pyrethroid insecticide mixtures on the brain of an adult Wistar rat. However, this present research work has further established the presence of pathological features associated with AD and MS in the cognitive, emotional, and motor centres of the brain. The development of neurological ailments associated with dementia has possible links to occupational exposure to insecticides (Cannon and Greenamyre 2011; Kamel et al. 2012), with the loss of synapses and dysfunctions of neurotransmission, which are more directly tied to the level of pathological changes (Rajmohan and Reddy 2017).

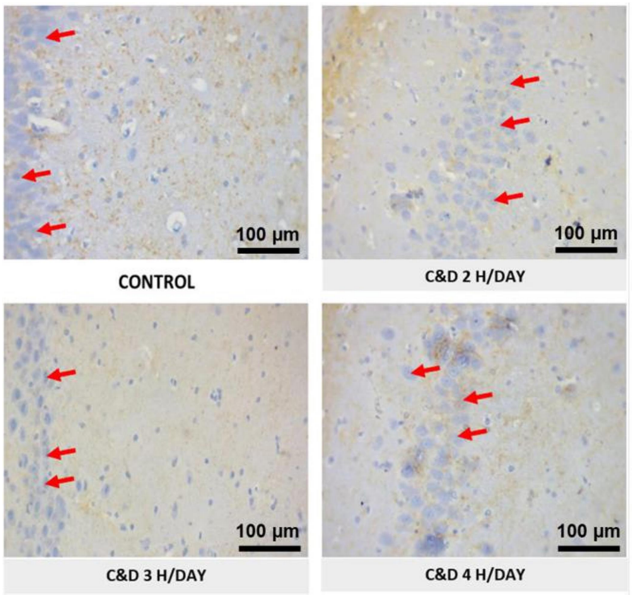

Fig. 5: CA3 region of hippocampus myelin basic protein (MBP) expression. Control group shows normal immunoreactivity and expression of myelin basic protein, while the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of myelin basic proteins in the CA3 region of hippocampus. MBP, ×100

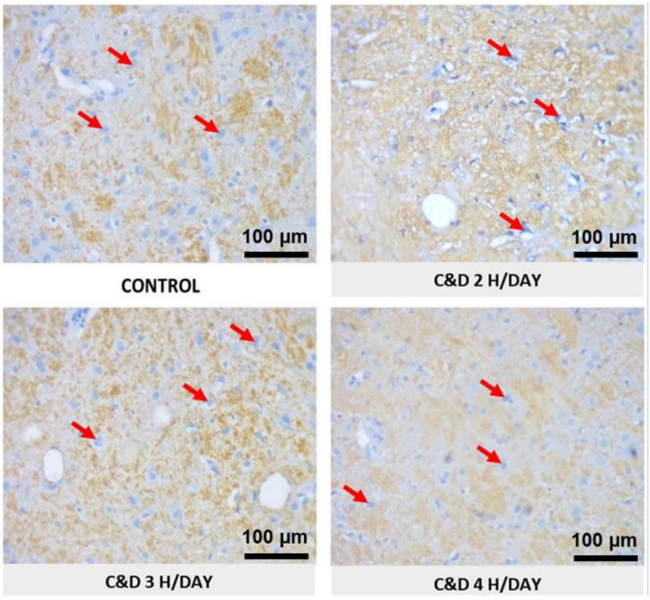

Fig. 6: The substantia nigra pars compacta myelin basic protein (MBP) expression. Control group shows normal immunoreactivity and expression of myelin basic protein while, the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of myelin basic proteins in substantia nigra. MBP, ×100

Table 5: The effect of insecticide mixture of cypermethrin and dichlorvos on olig2 expression

	Groups	Mean ± SEM	p-value	F-value
Amygdala	Control	13.56 ± 1.62		13.626
	C&D 2 h/day	13.30 ± 1.36	1.000	
	C&D 3 h/day	7.27 ± 1.19	0.034*	
	C&D 4 h/day	3.90 ± 0.87	0.001*	
Hippocampus (CA3)	Control	44.25 ± 2.76		8.865
	C&D 2 h/day	32.08 ± 2.66	0.011*	
	C&D 3 h/day	29.08 ± 3.23	0.001*	
	C&D 4 h/day	25.55 ± 1.51	0.000*	
Substantia Nigra	Control	18.50 ± 1.67		0.270
	C&D 2 h/day	17.06 ± `2.57	0.970	
	C&D 3 h/day	15.90 ± 1.21	0.890	
	C&D 4 h/day	15.75 ± 3.32	0.855	

Multiple Comparison test, and data was considered significant at P<0.05

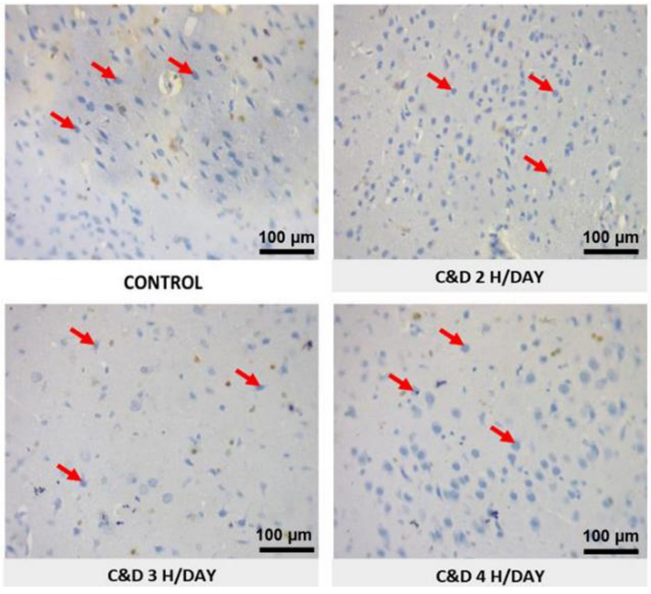

Fig. 7: Medial amygdala oligodendrocyte transcription factor 2 (Olig 2) expression. Control group shows normal immunoreactivity and expression of oligodendrocyte transcription factor 2, while the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of oligodendrocyte transcription factor 2 in medial amygdala. Olig 2, ×100

The decline in the cognitive, emotional, and motor behaviours of the rats was a sequel to several in vitro and in vivo studies that implicated long-term single insecticide exposure. This may result in neuronal damage in certain brain regions, which could result in subsequent cognitive impairment, decreased emotional memory (episodic memory) and attention, and loss of motor activities (Ullrich and Humpel 2009; Tartaglione et al. 2016).

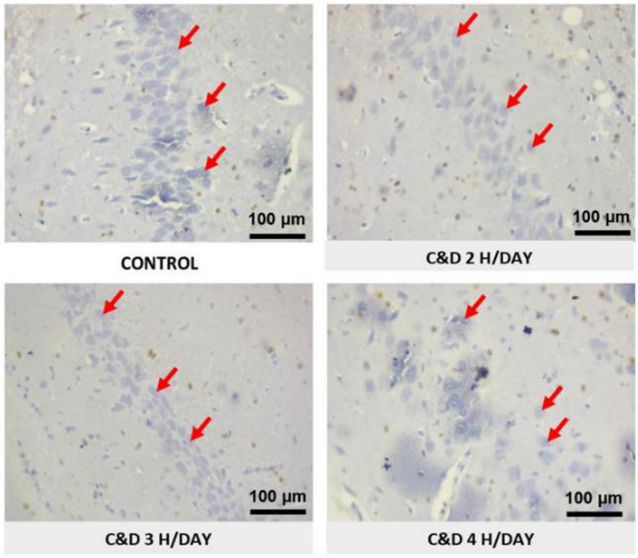

Fig. 8: CA3 region of hippocampus oligodendrocyte transcription factor 2 (Olig 2) expression. Control group shows normal immunoreactivity and expression of oligodendrocyte transcription factor 2, while the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of oligodendrocyte transcription factor 2 in the CA3 region of hippocampus. Olig 2, ×100

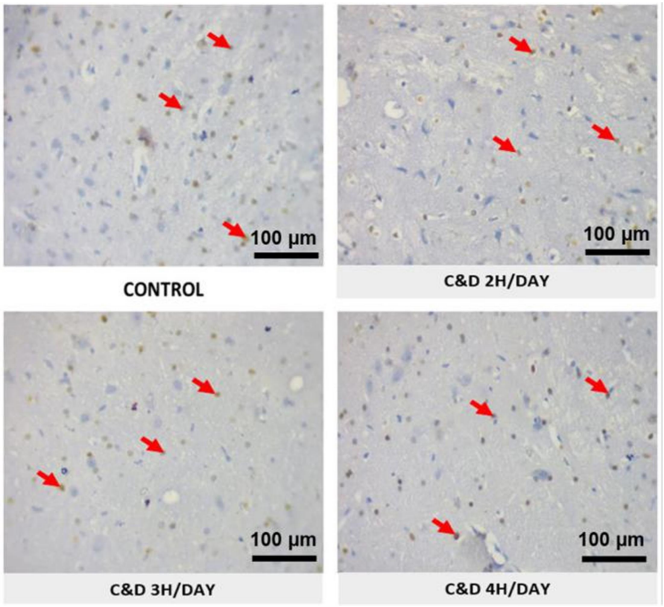

Fig. 9: The substantia nigra pars compacta oligodendrocyte transcription factor 2 (Olig 2) expression. Control group shows normal immunoreactivity and expression of oligodendrocyte transcription factor 2, while the groups treated with C&D for 2 h/day, 3 h/day and 4 h/day show progressively lower expression of oligodendrocyte transcription factor 2 in substantia nigra pars compacta. MBP, ×100

Increased lipid peroxidation and decreased antioxidant levels have been established as the mechanisms of pyrethroid insecticide action on the brain (Udodi et al. 2023). The oxidative stress and neurobehavioural deficits recorded in the present research may ultimately result in neurodegenerative forms of dementia later in the animal's life (Hayden 2010; Parron et al. 2011, Udodi et al. 2023).

Amyloid plaques, neurofibrillary tangles, and demyelination observed in the cognitive centre of this research work are responsible for the massive cognitive decline in AD or MS conditions. Neuropathological changes in the hippocampus could lead to deficits in learning and memory. The precise level of cognitive deficit reflects the distribution of pathological changes in AD and MS (Corey-Bloom 2002). The severity of the pathological changes in this research varies along the continuum of aetiological factors. For example, the level of pathological features in the C&D 4 h/day group was higher than that of the C&D 2 h/day and 3 h/day, which is because of their duration of exposure. Amyloid plaques and neurofibrillary tangles considered as biomarkers of AD, and demyelination that of MS, were observed in this research to have spread not only to the cognitive centres, but also to the emotional and motor areas of the brain in a stereotypical pattern (Thompson and Vinters 2012; Arab and Mostafalou 2022). These occurred through the induction of oxidative stress, as previously reported by Udodi et al. (2022 and 2023).

The amygdala complex assessed in the present study has been established as one of the brain centres affected in the early stages of AD and MS. Neuropsychiatric presentations that greatly contribute to the physical disorders observed, including demyelination associated with neuroaxonal damage that build up plaques and fibrillary tangles, have been ascribed to these conditions (Poulin et al. 2011; Filippi et al. 2018). The massive presentation of pathological features in the amygdala region when compared to the hippocampus and substantia nigra in the present study indicates neuropsychiatric symptoms, which may be common at the onset of AD and MS. According to Lyketsos et al. (2002), approximately 80% of AD patients presented neuropsychiatric features including agitation, anxiety, delusions, hallucinations, paranoia, and affective disturbances. The demyelination observed in the amygdala region of this study, together with the build-up of plaques and neurofibrillary tangles may subsequently result in the degeneration of cholinergic magnocellular neurons, thereby affecting the signalling pathway, which impairs the activity of this brain area (Mesulam 2004).

Together with the neuropsychiatric symptoms observed in our study, aberrant motor behaviour has been recorded in AD and MS conditions (Poulin et al. 2011). The pathological features observed in the substantia nigra of the present study can be compared to some established pathological features such as alpha-synuclein aggregation and hyperphosphorylated tau accumulation observed in AD and MS conditions with clinically detected extrapyramidal signs (Burns et al. 2005). However, findings in the present study implicated the pyrethroid mixture of cypermethrin and dichlorvos in the development of AD and MS, which are marked by demyelination associated with the build-up of plaques and neurofibrillary tangles in the amygdala, hippocampus, and substantia nigra.

Conclusion

This study indicates that the mixture of more than one insecticide, cypermethrin and dichlorvos, extrapolated to the environmental human exposure levels, induced AD- and MS-like features in short-term exposure with the presentation of demyelination associated with amyloid plaques and neurofibrillary tangles in the amygdala, hippocampus, and substantia nigra of adult Wistar rats.

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Conflict of Interest

None declared.

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Authors' Contribution

DNE - made a practical design and carried out the laboratory work: PSU - Prepared the manuscript, labelled the results, and interpreted them with a constructive discussion of the research findings.

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