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Effect of inhalation exposure to dimeforce organophosphate pesticide on the histology of the brain of wistar rats

Udeme Jude Ogoloma and Piriye Jesse-Abraham

Department of Science Laboratory Technology, School of Science and Technology, Captain Elechi Amadi Polytechnic, Rumuola, Port Harcourt, Rivers State, Nigeria.
Corresponding author email: demyjay13@gmail.com

ABSTRACT

This study examined the impact of inhalation of dime force pesticide on histology of the brain of Wistar rats. A total of twenty (20) rats weighing (80-142g) were divided into four groups of five rats each. The control group received feed and water only, whereas groups (2,3,4) were exposed once daily for 30 minutes (low-dose), 45 minutes (medium-dose), and 1 hour (high-dose) respectively for 14 days. At exactly 24 hours following the last treatment, animals were anaesthetized using chloroform and the brain was extracted from each group, fixed and taken for histological analysis. The results on the morphology of the brain revealed distinct alterations in cerebellar tissue, Purkinje cell loss, mild to moderate vacuolation, and other structural disruptions across all the exposed groups as compared to the control. These findings suggest that Dimeforce exposure at certain concentrations or durations may induce mild to moderate pathological changes in the brain, however, the degree of brain damage may depend on exposure levels, route and duration, which underscores the importance of adoption of safety measures during applications in agriculture and for occupationally exposed populations.

Keywords: *Histology, inhalation, pesticide, vacuolation, dimeforce, brain*

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INTRODUCTION

Pesticides are the substances used to control, kill or repel undesirable biological organisms. These pests do not only compete with humans for food, but also transmit diseases. Now, the use of pesticides has become inevitable all over the world due to increased crop destruction caused by various pests and insect contamination in homes (Rajabu *et al.*, 2017). In most African countries where the economy is dependent on agriculture, the use of pesticides is necessary to ensure increased crop production (Holy *et al.*, 2015). Pests are of different types including insects, herbs, fungi and rodents. They usually lead to crop losses and spread of diseases to other non-targeted organisms. Since the 1950s the use of pesticides in agriculture has led to improvements in crop production and animal yields hence the economic growth (Rajabu *et al.*, 2017). Pesticides are of different categories depending on the types of target insects and pests they control (insecticides, fungicides, herbicides and rodenticides) and their chemical compositions such as organophosphates, organochlorines, carbamates and pyrethroids. Organophosphate insecticides are highly toxic and therefore very effective in controlling agricultural pests

(Holy *et al*., 2015). Dimeforce is one of the most widely used organophosphorus insecticides mainly used in agriculture for controlling chewing and sucking insects and also used for the control of flies, mosquitos and cockroaches in public health programs and/or indoor use. It is very effective in the control of aphids, spider mites, mushroom flies, caterpillars, white flies, trips, mosquitoes, termites and cockroaches. While the use of pesticides like Dimeforce is of great benefit in agriculture, their excessive or improper usage poses significant health risks to both humans and animals due to their toxic nature (Saoudi *et al.,* 2018). Exposure to these chemicals, especially through inhalation, can lead to systemic toxicity affecting multiple organs, including the heart.

In recent years, environmental contamination with pesticides represents one of the problems of global concern. Regardless of their importance in agricultural and public health programs, organophosphate pesticides are harmful chemicals to the environment, humans and other non-targeted organisms.

Studies have shown that the prolonged exposure to organophosphate insecticides may lead to adverse immunological, reproductive, developmental as well as neurological effects and changes in hematological parameters (Ige *et al*., 2021). The effects induced by organophosphate poisoning are more rapid compared to other pesticides and symptoms are rapidly recovered due to its quick degradation and removal from the organism's body (Celik *et al*., 2009). The exposure to Dimeforce insecticide may be by dermal absorption, inhalation or ingestion. Due to its volatility, Dimeforce can easily be inhaled by people using it at home, People with underlying health problems such as impaired liver functions, reduced pulmonary function and convulsive disorders may be at great risk when exposed. The adverse effects of Dimeforce exposure may be highly pronounced in developing countries where it is widely used due to its low cost and limited knowledge on its effects to human health.

The brain and the spinal cord together make up the system of nerve tissues in vertebrates called the central nervous system which control both voluntary movements such as those involved in walking and in speech and involuntary movements such as breathing and reflex actions (Figure 1). It is also the center of emotion and cognition. The biochemical Integrity of the brain is vital for normal functioning of the central nervous system (CNS). One of the factors contributing to the cerebral biochemical impairment is a chemical process called oxidation stress (Salim, 2017), and so many studies have implicated oxidative stress induction in the alteration of memory functions, cardiovascular diseases and development of degenerated diseases (Song *et al*., 2018).

Scientifically, the use of any chemical product must be preceded by a good knowledge of its adverse effects, toxicity in addition to its beneficial properties. In other to have safety information of any formulated pesticide, preliminary investigation has to be done to evaluate

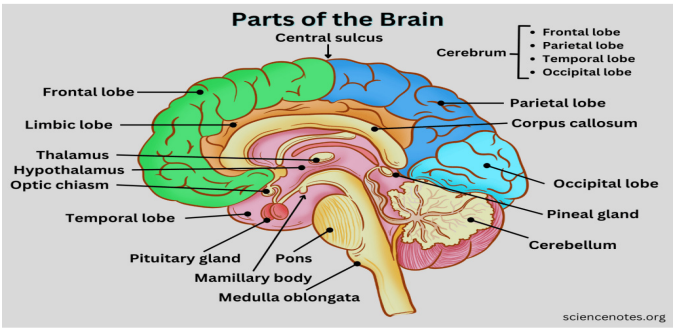

Figure 1: the part of human brain

possible risks, undesirable effects, lethal dose or exposure and some alterations in bodily functions in Wistar rats**.** Dimeforce a formulation of Organophosphorus has been generally used because of its efficiency in killing and controlling insects and it is economically cheap. However, studies have shown that the exposure and inhalation of organophosphorus pesticide has caused series of health-related issues such as changes in bodily functions, hematological alterations, short term and chronic health risk and environmental contamination.

The widespread use of pesticides, particularly Dimeforce, poses significant health risks due to the toxic nature of these chemicals. While pesticides are essential for agricultural productivity, their improper handling, excessive exposure, and inadequate regulation have led to rising concerns about their impact on human and animal health. Among the various routes of exposure, inhalation of pesticides is of serious concern, as it directly affects respiratory and cardiovascular system. There is lack of detailed information on how inhaled Dimeforce pesticide affects the structure and functions of the brain, hence, the need for histopathologic evaluation of this pesticide product.

MATERIALS AND METHOD

Materials

The following were the materials used for this research work:

- Wistar rats,
- Plastic cage with wedged cover,
- 1ml syringes,
- 2ml syringes,
- 5ml syringes,
- 20ml syringes,
- Lithium heparinized bottles,
- Plain bottles
- Sample bottles,
- Sensitive weighing scale,
- Dissecting set,
- Permanent markers

- Hand gloves,
- Nose masks,
- Beakers,
- Towel,
- Methylated spirit,
- Cotton wool
- Food trough and water drinker,
- Wood shavings, Liquid soap
- Rat pellets
- Dimeforce Organophosphate Pesticides.

REAGENTS

- Dichloromethane
- Distilled water
- Ethanol
- Formalin
- Normal saline
- Chloroform

Experimental Animals

The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and as approved by the Institute of Laboratory Animal Resources, National Research Council. Twenty (20) Wistar rats weighing between (80g -142g) were bought from Animal House of Anatomy Department, University of Port Harcourt. The Wistar rats had unrestricted access to standard rat food and water under well maintained environmental conditions and controlled humidity and temperature. The animal beddings were changed every other day to provide a hygienic environment free from diseases and infections.

Animal care and management

The animals were left for seven days to acclimatize, provided with food (Standard rat chow) and water Ad-libitum throughout the period of experimentation. They were placed in plastic cages with net covers for ventilation. The beddings of the animals were changed every two days to prevent toxic ammonia build-up. Wood shavings (sawdust) were used as base for the cage.

Animal grouping and treatment

After one week of acclimatization, the animals were randomly assigned to 4 groups, containing 5 Wistar rats each. 1ml of the Dimeforce pesticide was added to different pieces of cotton wool and placed at different point round and at center of the exposure chamber, thereafter, the wistar rats were introduced into the chamber. The experimental protocol is stated below:

- Group I was the control group fed with rat chow and distilled water only (No Exposure)
- Group II was allowed 30minutes inhalation exposure (low dose)
- Group III was allowed 45minutes inhalation exposure (medium dose)
- Group IV was allowed 1-hour inhalation exposure (high dose)

The exposure was once daily after which the Wistar rats were transferred out of the exposure chamber to the main cage where they stayed till another exposure time, maintained under standard environmental conditions of temperature, relative humidity, and 12-hour dark/light cycle. The inhalation exposure was for a period of 14days. All the rats had free access to food and water throughout the time of exposure and experiment and they were observed daily for symptom of toxicity and mortality. The weight of the rats in each group was taken before and after the experiment. After 14days exposure, the animals were anaesthetized with chloroform, dissected and the brain was collected for histopathological studies.

Histological procedure and analysis

The brain tissues were cut about 0.5cm thick and fixed in 10% normal saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and cleared in xylene. After clearing, the tissues were embedded in molten paraffin wax in the oven at 40ºC. The tissues were subjected to automated tissue processing machine, thin sections of 5-micrometres were made using microtome and later stained with hematoxylin and Eosin stain, after which they were passed through a mixture of equal concentration of xylene and alcohols, following clearance in xylene, the tissues were oven dried. Photomicrographs were taken using a JVC video camera mounted on an Olympus light microscope (Olympus UK Ltd Essex, Uk) ready for histological examination.

RESULTS

The findings in this study of histopathological assessment of the effect of inhalation exposure to Dimeforce pesticide on the brain of Wistar rats are presented in (Figures a-h). The photomicrographs from the control group (Figures a & b) showed normal appearance of the cerebellum with evenly distributed purkinje cells, thickened granular and molecular layer of the gray matter; figures (c & d) are photomicrographs of the cerebellum from the low dose exposed group showing granular and molecular with reduced Purkinje cells, minimal vacuolation of the cerebellum. Figures e and f showed decreased density of the granular layer with less Purkinje cells and moderate vacuolation in medium dose exposed rats. The high dose

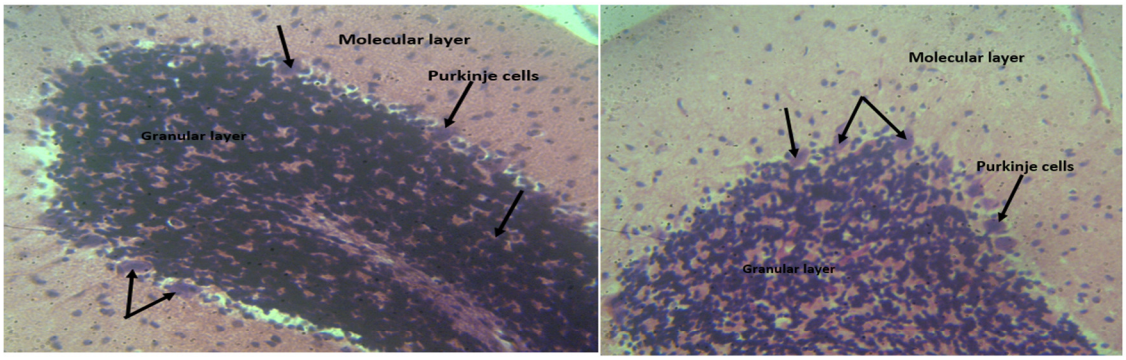

Fig a. **Fig b.**

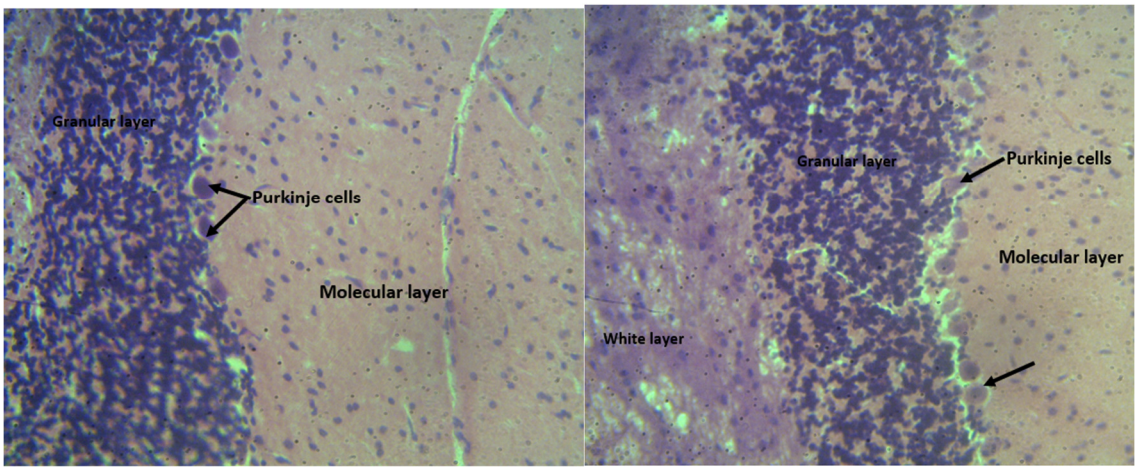

Fig c. **Fig d.**

Figure 2: Photo micrographs of the brain of wistar rat exposed to dimeforce pesticide

exposed group also showed reduced Purkinje cells, vacuolation and cerebellar distortion (Figures g and h). Photomicrograph sections of the brain (H&E X400). Figure a:Control group showing the cerebellum with evenly distributed purkinje cells, thickened granular and molecular layer of the Gray matter with normal appearance (arrows). Diagnosis: Normal appearance of the cerebellum. Figure b: Control group showing cerebellum with a well delineating purkinje cells: the decreased density of the granular layer and molecular layer (arrows). Diagnosis: Normal cerebellar tissue. Figure c: Low dose group showing the cerebellum: granular and molecular layer with reduced purkinje cells (arrows) Diagnosis: Purkinje cell loss of the cerebellum. Figure d: Low dose group showing the cerebellum: decreased density of granular layer with less purkinje cells and minimal cerebellar vacuolation (arrows). Diagnosis: minimal vacuolation and purkinje cell loss of the cerebellar tissue. Photomicrograph sections of the brain (H&E X400). Figure e: Medium dose group showing the cerebellum with increased density of granular cells with less purkinje cells (arrows). Diagnosis: Purkinje cell loss of the cerebellar tissue. Figure f: Medium dose group showing cerebellum with decreased density of granulation cells with less purkinje cells and minimal cerebellar vacuolation (arrows). Diagnosis: Moderate vacuolation of the cerebellar tissue. Figure g: High dose group showing the cerebellum with low density of granulation layer with reduced purkinje cells (arrows). Diagnosis: Moderate Purkinje cell loss and cerebellar distortion. Figure h: High dose group showing the cerebellum with high density of granular layer with irregular and reduced purkinje cells and vacuolation (arrows). Diagnosis: Purkinje cell loss of the cerebellar tissue.

DISCUSSION

Dimeforce pesticide is usually applied directly to control pests in both the indoor and outdoor environment and as such non-target victims of pesticide intoxication is inevitable. The mode of action of Dimeforceorgano-phosphate insecticide is by inhibiting the acetylcholinesterase enzyme whose function is to hydrolyze acetylcholine neurotransmitter in nerve

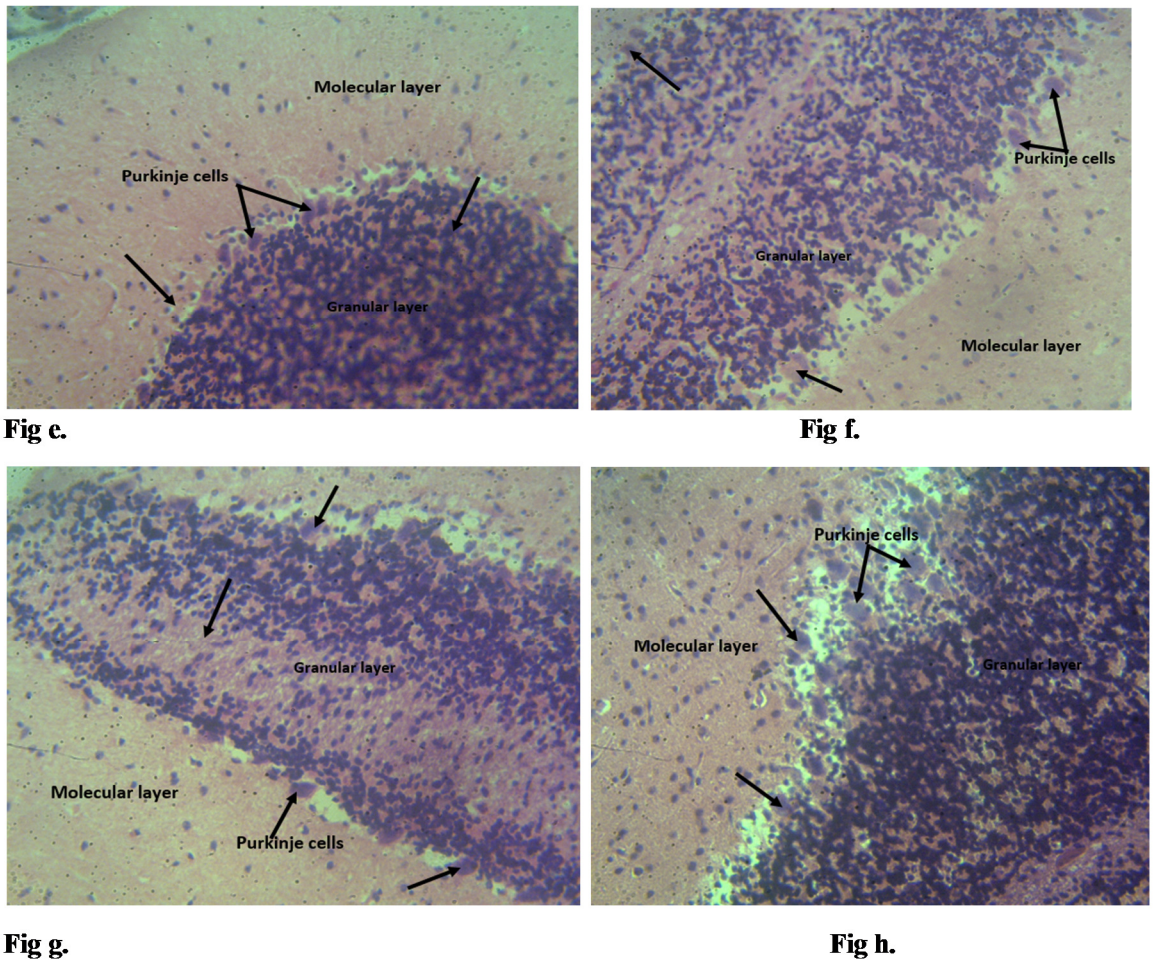

Figure 3: Photo micrographs of the brain of wistar rat exposed to dimeforce pesticide

synapses (Ige *et al*., 2021). The acetylcholinesterase enzyme blockage leads to accumulation of acetylcholine neurotransmitter in nerve presynaptic spaces that impedes nerve functions (Okoroiwu & Iwara 2018). Organophosphates have another mechanism of toxicity; when microglial cells of central nervous system (CNS) gets activated by cellular damage, they secrete neurotoxic particles such as nitric oxide (NO) that destroy cells and hence cause DNA alteration (Koutros *et al*., 2008). Organophosphate compounds may directly or indirectly modify the antioxidant defense capability of exposed subjects and thus affect their susceptibility to oxidative stress (Ige *et al*., 2021). Oxidative stress represents an imbalance production and clearance of reactive oxygen species or free radicals in biological system. Disturbance in the normal redox state of tissues can cause toxic outcomes through the production of peroxides and free radicals that damage certain components of the cell including protein, lipid, and DNA. Oxidative stress has been implicated as one of the factors in many diseases' proliferation. Excessive oxidative stress particularly at vascular linings and blood brain barrier can lead to toxic impact to blood cells and brain. (Anionye *et al*., 2015). The brain with its high oxygen consumption and lipid rich contents is highly susceptible to oxidative stress. Therefore, oxidative stress induced damage to the brain has a strong potential to negatively impact normal CNS functions. The cerebellum is a convenient model to study cell death for several reasons. Firstly, the long developmental schedule around six weeks in mice makes the cerebellum particularly vulnerable to both developmental and environmental insults. Secondly, cerebellar abnormalities are usually straightforward to recognize, cerebellar damage manifests itself as motor coordination problems, deficits and abnormalities in gait and posture that are easily recognized both clinically and in animal holding facilities. Biochemical Integrity of the brain is vital for normal functioning of the central nervous system (CNS). One of the factors contributing to the cerebral biochemical impairment is a chemical process called oxidation stress (Salim, 2017).This study investigated the histological changes in the brain morphology of Wistar rats following exposure to Dimeforce. The results revealed distinct alterations in cerebellar tissue architecture, Purkinje cell death, mild to moderate vacuolation, and other structural disruptions

across all the exposed groups (Figures c to h) as compared to the control, which showed normal appearance of the cerebellum with evenly distributed Purkinje cells, thickened granular and molecular layer of the gray matter having normal appearance (Figures a and b). These findings suggest that Dimeforce exposure at certain concentrations or durations may induce mild to moderate pathological changes in the brain tissues, possibly, due to induction of oxidative and pro-inflammatory stress effects in the animals (Arnal *et al*., 2019). However, the degree of brain damage may depend on exposure levels, route and duration, which underscores the importance of adoption of safety measures during applications in agriculture and for occupationally exposed populations. In this study, Dimeforce pesticide induced observable signs of toxicity such as nausea, shaking, vomiting, weakness, shortness of breath and death across all the exposed groups irrespective of the duration of exposure. These toxic effects could also be linked to induction of oxidative stress, Purkinje cell loss and toxic impact on the brain of the exposed rats (Armstrong, 2015).

Conclusion

This study investigated the histological changes in the brain tissues of wistar rats following exposure to Dimeforce pesticide. The results revealed distinct alterations in cerebellar tissue architecture, Purkinje cell death, mild to moderate vacuolation and structural disruptions across all the exposed groups. These findings are linked to depletion of antioxidant enzymes in the brain tissue as well as oxidative stress in the cardiovascular system of the exposed rats caused by dimethoate, an active ingredient in Dimeforce pesticide.

Recommendations

Based on the findings of this study, some recommendations are proposed:

1. Stringent Guidelines **and** Monitoring**:** Given the observed signs of brain injury at all levels of exposure, it is essential to implement stringent guidelines on Dimeforce usage, particularly in agricultural settings involving prolonged exposure and for occupationally exposed population.
2. Additional studies are recommended to explore potential protective agents, such as antioxidants, that may mitigate the oxidative stress and inflammatory effects associated with Dimeforce. Co-administration of protective compounds could reduce tissue damage.Contribution to Knowledge

This research work has established that prolonged inhalation exposure of Wistar rats to Dimeforce pesticide can cause significant damage to the brain.

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